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CHARACTERIZATION OF ENDOGENOUS PEPTIDES AS ALARMINS

Joost Oppenheim, De Yang

Recent studies have identified a group of structurally diverse multifunctional host proteins that are rapidly released following pathogen challenge and/or cell death and, most importantly, are able to both chemotactically recruit and activate dendritic antigen-presenting cells. These potent immunostimulants, including defensins, cathelicidin (LL37) eosinophil-derived neurotoxin (EDN), and high-mobility group box protein 1 (HMGB1), serve as early warning signals to activate innate and adaptive immune systems. They interact with pertussis toxin sensitive chemokine and other receptors on host cells. For example, defensins, LL37, HMGB1 and EDN mimic chemokine and cytokine activities by interacting with CCR6, FPRL-1 and Toll-like receptors (TLR2) respectively. These antimicrobial peptides are constitutively produced and released by leukocytes, keratinocytes and epithelial cells lining the GI, GU and tracheobronchial tree. In addition, they are induced by injurious stimulants and cytokines. These peptides all have in vivo immunoadjuvant effects. We propose to highlight these proteins unique activities by grouping them under the novel term "alarmins", in recognition of their role in rapidly mobilizing the immune system. (This work is support by the Intramural Program of the NIH, NCI.)

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THE ROLE OF CATHELICIDIN AND DEFENSINS IN PULMONARY INFLAMMATORY DISEASES

Robert Bals

The initial contact of pathogenic microorganisms with the host usually takes place at internal or external body surfaces. Animals of various phyla have developed first line defense mechanisms to inhibit the growth and invasion of microorganisms. The first line of defense against pathogenic insult is called the innate immune system, which in mammals is followed by acquired immune responses associated with activation of T and B cells aimed against specific antigens. One principle of innate immunity is the production of endogenous antibiotic peptides. Defensins and cathelicidins are the prototypical antimicrobial peptides found in humans. Cathelicidins are antimicrobial peptides that are characterized by the conserved amino-terminal sequence of the peptide's propiece, whereas the carboxy terminal domains containing antimicrobial activities are remarkably variable. The prosequence is termed "cathelin" after the function of this domain to inhibit the activity of cathepsin L (cathepsin L inhibitor) and is between 99 and 114 amino acids long. Molecules with a cathelin-like pro peptide sequences have been isolated from multiple species including cow, pig, rabbit, sheep, human, mouse, monkey, and horse. The carboxyterminal antimicrobial peptide varies considerably between individual molecules and is highly variable in sequence, length (12-100 residues), and function. The cathelicidin peptides are expressed in epithelial and myeloid cells. Data from knock out animals overexpression studies showed that the peptide is active as endogenous antibiotic. Recent findings indicated that the peptide plays a larger role in innate immunity as an antimicrobial peptide. It has long been known that cathelicidin blocks the biological effect of endotoxin. Several groups showed that the peptide blocks the activation of immune cells by Toll like receptor ligands. Studies applying various models of inflammation and shock indeed showed that the cathelicidins have a profound anti-inflammatory activity and serve as regulator of the innate inflammatory system. Defensins are short peptides characterized by the presence of six cysteins and are classified in alpha- or beta-defensins based on the arrangement of these cysteins. Beta-defensins are expressed in airway epithelial cells and have direct antimicrobial activity. Several studies indicate that defensins are involved in the regulation of inflammatory diseases.

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A 3

ANTI-INFLAMMATORY ACTIVITY OF SLPI

Cliff Taggart

Secretory Leucoprotease Inhibitor (SLPI) is a serine antiprotease produced at various mucosal sites including the upper respiratory tract. It functions as an inhibitor of neutrophil serine proteases such as neutrophil elastase and cathepsin G and was traditionally thought to protect lung tissue and other soluble factors from excessive protease activity. However, in recent years SLPI has been shown to have other properties including antibacterial, anti-viral (to HIV) and anti-inflammatory/immunomodulatory activities. We have shown that SLPI can inhibit lipopolysaccharide (LPS)-induced activation of the pro-inflammatory transcription factor, NF-kB in monocytic cells. SLPI can enter these cells becoming localised to the cytoplasm and the nucleus. We have shown that upon entering the nucleus SLPI can bind to NF-kB binding sites thus inhibiting p65 binding and subsequent activation of pro-inflammatory genes. Analysis of the nuclear fractions of alveolar macrophages from healthy volunteers and patients with sepsis has revealed substantial quantities of SLPI present in the nucleus of these cells. In conclusion, our findings indicate that SLPI can act to inhibit activation of NF-kB by directly binding to DNA binding sites normally occupied by NF-kB during LPS activation of monocytes.

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A 4

GRANULYSIN: A NOVEL HOST DEFENSE MOLECULE AND ALARMIN

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Granulysin is a cationic antimicrobial and tumorcidal peptide expressed by human cytolytic T lymphocytes (CTL) and natural killer (NK) cells. We first identified granulysin using subtractive hybridization in a search for genes expressed by T lymphocytes "late" (3-5 days) after activation. Granulysin has broad antimicrobial activity against both Gram positive and Gram negative bacteria, Mycobacteria tuberculosis, fungi, and parasites. Expression of granulysin in peripheral NK cells of patients with cancer has been associated with good clinical outcomes. Granulysin is a member of the saposin-like protein family of lipid binding proteins and colocalizes in cytolytic granules with perforin and granzymes. Recombinant 9kD granulysin disrupts artificial lipisomes and cell membranes, damages mitochondria, and activates caspase 9 to induce apoptosis in nucleated cells. Recently, we found that this cytolytic molecule also functions as a chemoattractant and proinflammatory activator. Although granulysin mediates cytotoxicity at micromolar concentrations, it mediates chemoattraction at nanomolar concentrations. Granulysin induces chemotaxis of monocytes, both CD4+ and CD8+ memory (CD45RO) but not naive (CD45RA) T cells, NK cells, and mature (but not immature) monocyte-derived dendritic cells. G protein coupled receptor(s) are involved in chemotaxis since pertussis toxin partially abrogates the activity. 10 nM granulysin induces a ten-fold increase in chemokine (MCP-1 and RANTES) expression by monocytes and U937 cells and micromolar concentrations induce a two-fold increase in TNF-alpha production by LPS-stimulated monocytes. Taken together, these data indicate that granulysin functions both as a cytolytic molecule at higher concentrations and chemkine and proinflammatory activator at lower concentrations. Since mice do not express a granulysin homologue, we recently engineered a mouse transgenic for human granulysin and are in the process of evaluating the effect of granulysin in vivo in selected tumor and infectious disease models. To date, granulysin transgenic animals reject a syngeneic T cell lymphoma tumor C6VL more rapidly than wild type control littermates and allospecific cell lines show enhanced killing but no effects on Mycobacteria tuberculosis or Cryptococcus neoformans have been demonstrated in this in vivo model. The long term goal is to cross granulysin transgenic animals with granulysin and perforin knock-out animals in an attempt to show larger differences in vivo.

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A 5

PSORIASIN AND THE S100-FAMILIY OF PROTEINS: THEIR ROLE AS DEFENSINS AND ALARMINS

Jens-Michael Schroeder

S100 proteins comprise a multigene family of low molecular wight calcium-binding proteins that share common structural motifs. Inside cells, S100 proteins exist as antiparallel homo- and heterodimers that are held together by non-covalent interaction. S100 proteins are thought to participate as regulators of a variety of intracellular processes, and some S100 proteins are also released into the extracellular environment. Many S100 proteins are produced by epithelial cells and some, like S100A8 and S100A9, are produced by neutrophils.

Some S100 proteins, in particular S100A7 (psoriasin), can function as chemotactic factor, although yet no receptor has been identified.

S100A7 (psoriasin) is produced by skin keratinocytes and many epithelial cells. Healthy skin shows a focal expression of psoriasin, which suggests that it is locally induced. Indeed, its expression correlates with the density of bacterial colonisation. In vitro experiments with cultured skin keratinocytes and in vivo application of bacteria or its culture filtrates induce psoriasin gene expression and, surprisingly, the release of psoriasin protein. When psoriasin concentration were estimated in washing fluid collected at different body sites, there was found a high variability. In support with psoriasin transcription, highest psoriasin amounts were found at places, where highest bacterial densities were documented. This observation suggests that psoriasin acts as "alarmin", possibly indicating the presence of elevated bacterial densities at the skin surface.

Psoriasin represents the principle antimicrobial protein present in healthy skin washing fluid. In vivo- and in vitro-experiments have shown, that it is active preferentially against E. coli. Thus, psoriasin acts as alarmin signalling the presence of increased bacteria at skin surface and it kills the priciple gut commensal E. coli, that could contaminate skin surfaces.

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A 6

ROLE OF LUNG EPITHELIAL CELLS IN FAS MEDIATED INFLAMMATION DURING EXTRAPULMONARY ACUTE LUNG INJURY

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Our previous studies indicated that pulmonary inflammation during acute lung injury (ALI) following hemorrhage and sepsis (HEM+CLP) is mediated through Fas. However, it remains unclear whether this inflammation is

induced via activation of Fas on myeloid (alveolar macrophages) or non-myeloid (epithelial) cells in the lung. To study this, a method to silence Fas expression (verified by RT-PCR) in lung epithelial cells in mice in vivo using a double-stranded, 19 nucleotide small-interfering RNA (siRNA) construct instilled into the animals lungs was established. The uptake of this construct into pulmonary epithelial cells was verified by co-localization of fluorescent anti-Cytokeratin-18 antibody and Cy-5-tagged-siRNA construct in frozen lung tissue sections. No co-localization with alveolar macrophages (CD115) was observed. Based upon this, mice were subjected to hemorrhagic shock (30mmHg for 90min), followed by intratracheal instillation of Fas-siRNA and underwent sepsis (cecal ligation & puncture) 24hrs thereafter. Lungs were harvested 24hrs after sepsis and the concentration of inflammatory mediators (IL-6, TNF-α, IL-12, IL-10, IFN-γ, MCP-1, KC, MIP-2 by cytometric bead array and ELISA) was quantified along with the degree of ALI (H&E and myeloperoxidase staining). Silencing of Fas in lung epithelial cells markedly attenuated ALI by improving pulmonary architecture and reducing neutrophil influx following HEM+CLP. Furthermore lung inflammatory mediators were markedly reduced following Fas silencing in epithelial cells. To further dissect the role of non-myeloid and myeloid cells during Fas driven inflammation, Fas activating antibody (Jo-2) or isotype control were instilled into mice deficient in monocytes/macrophages (Csf1op) and their controls. Lungs were then analyzed for inflammatory mediators (MCP-1, KC, MIP-2) 4hrs thereafter. In response to Fas activation lung MCP-1, KC and MIP-2 were all markedly elevated to a similar degree in Csf1op and in background animals. In addition, murine lung epithelial cell lines when cultured and then stimulated with Jo-2 for 4hrs also showed markedly increased production of MIP-2, KC and MCP-1 in cell supernatants when compared to isotype stimulated controls. These data indicate that upon activation of the Fas receptor lung epithelial cells produce and release inflammatory mediators. Furthermore, silencing Fas in lung epithelial cells in vivo using siRNA might be of therapeutic value in the development of extrapulmonary ALI (NIH-HL73525).

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A 7

TUMOR NECROSIS FACTOR RECEPTOR 1 (TNFR1) AND MEMBERS OF THE DEATH INDUCING SIGNALING COMPLEX LOCALIZE TO THE MITOCHONDRIA FOLLOWING TNF TREATMENT IN RAT HEPATOCYTES

Raghuveer Vallabhaneni, Yinna Wang, Xiaoying Zhang, Kevin Mollen, David Kaczorowski, Timothy Billiar

Objective: Tumor Necrosis Factor (TNF) is an important cytokine that can induce cell death in severe shock, sepsis and fulminant hepatic failure. TNF receptor-mediated apoptosis has been studied extensively, however questions on the mechanisms of its cell signaling and localization still remain. The Death Inducing Signaling Complex (DISC), including Fas-Associated Death Domain (FADD) protein and Caspase-8 has been shown to form following TNF binding to the Tumor Necrosis Factor Receptor 1 (TNFR1). The localization of this DISC has been thought to occur in the cytosolic compartment. Following DISC formation, hepatocytes undergo type II apoptosis in which they are dependent on mitochondria to amplify the initial apoptotic signal. Given the importance of the mitochondria in hepatocyte apoptosis, we tested the hypothesis that the components of the DISC would be found in the mitochondria in rat hepatocytes following TNF stimulation.

Material and Methods: Primary hepatocytes were isolated, purified and cultured from Sprague-Dawley rats. Apoptosis was induced by treatment with TNF (2000 units/ml) +/- Actinomycin D (Act D, 0.2 ug/ml). Hepatocyte mitochondria were isolated using serial centrifugations and running through a sucrose gradient at 39,000 X g. Western blotting of TNFR1, FADD, Caspase-8, cFLIP, and TRADD on these mitochondrial fractions was performed. Immunoprecipitation (IP) of TNFR1 with blotting of caspase-8, FADD, and cFLIP was performed on hepatocyte mitochondrial and cytosolic fractions. Hepatocyte cells were also labeled with FADD and TNFR1 antibody, then labeled with a gold linked secondary antibody for imaging with immunoelectron microscopy (IEM, 40000X). To examine in vivo significance, Sprague Dawley Rats were injected with TNF (10 ug/kg) and mitochondrial and cytosolic fractions were obtained. All experiments were performed two to three individual times.

Data: Rat hepatocytes demonstrated colocalization of TNFR1 and caspase-8 at 1 hour following induction of apoptosis with TNF/Act D on confocal microscopy (n=3). TNFR1, FADD, Caspase-8, and cFLIP were found in the mitochondrial fraction on western blot at one hour. Immunoprecipitation of TNFR1 with both caspase-8 and FADD were found in the mitochondrial fraction. (n=3). Surprisingly, this IP was able to occur in the absence of Act D, the apoptosis sensitizing agent. Using IEM, TNFR1 was located to the mitochondria in one hour following TNF treatment alone compared to controls. FADD also localized to the mitochondria under IEM. TNFR1 and Caspase-8 were also found in the mitochondrial fractions of TNF in vivo treated hepatocytes. (n=2)

Conclusion: For the first time, we have shown the localization and colocaluzation of the TNFR1 and certain DISC components in the mitochondria. These novel findings occurred in vitro and in vivo, even in the absence of a sensitizing agent. This data suggests the mitochondria may play an important part in the early TNF signaling cascade. Further elucidation of the mechanisms by which TNFR1 and the DISC components are shuttled to and from the mitochondria may provide insight on how to prevent hepatocyte death in severe inflammatory states.

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A 8

APOPTOSIS IN SEPSIS IS REGULATED BY INHIBITOR OF APOPTOSIS PROTEINS

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Background: Inhibitor of Apoptosis Proteins (IAP) are endogenous inhibitors of caspase activity (1). During sepsis lymphocytes undergo accelerated apoptosis via activation of caspase cascades, which contributes to immunosuppression (2). The role of IAP's in this process so far is unknown.

Aim: The aim of the study was to investigate the expression of IAP's and their regulator smac/diablo in patients with severe sepsis.

Methods: With approval by the local ethics committee 16 patients with severe sepsis were included and blood was obtained as soon as the criteria of severe sepsis were fulfilled. 10 patients with mild systemic inflammatory response syndrome (SIRS) and 11 healthy volunteers served as two separate controls. Caspase activity and antigen expression in lymphocytes were measured by flowcytometry, and mRNA expression in whole blood was quantified by light-cycler rtPCR.

Results: Patients with severe sepsis were characterized by high SAPS II and Apache scores as well as elevated serum levels of procalcitonin as a marker for infection. In severe sepsis but not in SIRS phosphatidyl serin externalisation increased and the central executioner caspase 3 was found to be active in an expanded subpopulation of T- and B-cells. In healthy controls, the IAP's cIAP1, cIAP2 and xIAP were expressed at relatively low levels. While all three may inhibit caspase 3, xIAP is also known to inhibit caspase 9. In patients suffering from severe sepsis, the transcription of cIAP1, cIAP2 and xIAP was increased significantly ($p<0.001$) 2.4-fold, 4.8-fold and 9.5-fold. Surprisingly, in SIRS the respective transcripts were elevated even more (8.5, 8.8 and 20.5-fold, $p<0.001$). IAP's themselves are inhibited by smac/diablo which is stored in the mitochondrial intermembrane space and is released upon mitochondrial pore opening. Bcl-2 controls the mitochondrial integrity and was found to be decreased substantially in lymphocytes ($p<0.05$). The mRNA expression of smac/diablo was not changed in SIRS or sepsis.

Conclusion: The transcription of IAP's is increased during sepsis and SIRS, probably to counter increased caspase-3 activation. We hypothesize that elevated levels of IAP's in SIRS aide to abrogate caspase-3 activation. In sepsis, the compensatory increase in IAP transcripts may not be strong enough to efficiently block caspase-3 activation, thereby rendering lymphocytes susceptible to cell death.

The study was in part funded by grants from BONFOR and Fraunhofer Society.

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A 9

DISSECTING THE ROLE OF PROTEIN-GLYCAN INTERACTIONS IN NEGATIVE REGULATION OF TH1 RESPONSES

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Galectin-1, a carbohydrate-binding protein found at sites of T-cell activation, has immunosuppressive effects **in vivo**. Administration of recombinant galectin-1 or its genetic delivery in experimental models of chronic inflammation and autoimmunity results in selective elimination of antigen-activated T cells and a T-helper (T_H)1 to T_H2 shift associated with remission of inflammatory disease. In addition, selective blockade of the inhibitory effects of galectin-1 within the tumor microenvironment results in heightened T-cell-mediated tumor rejection and increased survival of T_H1 effector cells. The aim of the present study is to dissect the mechanisms involved in the galectin-1-mediated regulation of Th1 and Th2 responses. We demonstrate here that T_H1- and T_H2-promoting stimuli can differentially regulate the glycosylation pattern of human and murine T-helper cells and modulate their susceptibility to galectin-1. While T_H1-differentiated cells express the repertoire of cell surface glycans that are critical for galectin-1 binding and cell death, T_H2 cells are protected from galectin-1 through differential sialylation of N- and O-glycans on cell surface glycoproteins. Consistently, galectin-1-deficient mice develop hyper-T_H1 responses following specific antigenic challenge **in vivo**, characterized by significantly higher levels of interferon-g-producing cells ($\mathbf{P}<0.05$, Student's **t** test) and enhanced T-bet expression compared to wild-type mice. To evaluate the pathophysiological relevance of our findings, we examined the effects of **gal-1** deletion on the development and progression of experimental autoimmune encephalomyelitis (EAE), a prototypic Th1-mediated central nervous system (CNS) disease that is used as an animal model of human multiple sclerosis. Gal-1-deficient mice developed EAE with increased severity compared to wild-type mice ($\mathbf{P}<0.05$ Man Whittney test). Worsening of the disease was accompanied by a heightened autoimmune response, more diffuse CNS inflammatory infiltrates, decreased levels of $CD4^+$ T-cell apoptosis and a profound skewing toward Th1 responses **in vivo**. Our findings identify a novel mechanism, based on differential glycosylation of T_H1 and T_H2 cell subsets, by which galectin-1 may preferentially eliminate antigen-specific T_H1-effector cells with critical implications for immunotherapy.

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ENHANCED PHAGOCYTOSIS OF APOPTOTIC IMMUNE CELLS BY SPLENIC MARGINAL ZONE MACROPHAGES DURING POLYMICROBIAL SEPSIS

Ryan Swan, Chun-Shiang Chung, Jorge Albina, William Cioffi, Mario Perl, Alfred Ayala

Introduction: It is hypothesized that immune cell apoptosis (Ao) contributes to the immunosuppression of sepsis via multiple mechanisms. The most obvious is the loss of competent immune cells; however, another hypothesis implicates the mechanisms for disposing of Ao material. In vitro studies have shown that uptake of Ao cells by macrophages (MØ) leads to an anti-inflammatory MØ phenotype. It is unknown whether the capacity of MØ to clear this expanded population of Ao immune cells is altered during sepsis; therefore, we hypothesize that in a mouse model of sepsis the phagocytosis of Ao immune cells by MØ will be enhanced.

Methods:

Sepsis: Sepsis was induced by cecal ligation and puncture (CLP). Sham celiotomy was performed in controls. Phagocytosis Assay: Splenocytes were isolated at multiple time points following CLP and incubated in culture wells on glass coverslips to allow for MØ adherence. Thymocytes from donor mice were incubated in medium with dexamethasone to induce Ao, then co-incubated with the plated MØ for 90 minutes. Coverslips were then washed, fixed, and Giemsa stained. Light Microscopy: 300 MØ/sample were counted. The percent of MØ ingesting at least one thymocyte X the average number of thymocytes/ active MØ equals the phagocytic index (PI). Fluorescence Microscopy: Splenocytes from CLP/Sham mice at 48hrs were stained with the red fluorescent dye PKH26 and incubated on glass coverslips for MØ adherence. Ao was induces in thymocytes as above followed by staining with the green fluorescent dye CFSE. Co-incubation was followed by fixation and mounting with the nucleic acid dye DAPI. The PI was calculated as above. Confocal images with 5um sections of selected MØ were obtained. **In Vivo** Co-localization: 48 hrs after CLP/Sham, Ao donor thymocytes were stained with CFSE as above, then 10^7 cells were injected IV into CLP/Sham mice. After 90 minutes, the spleen was harvested for frozen sectioning and immunofluorescent staining of specific MØ sub-populations, including CD169 (marginal zone metallophilic MØ) and MARCO (marginal zone MØ). Flow Cytometry: 48hrs after CLP/ Sham, splenocytes were harvested and stained with MARCO-RPE, then analyzed by flow cytometry.

Data: PI: Both groups had a similarly low PI at 4 and 12 hrs; however, MØ from septic mice had a significantly higher PI by 24 hrs (13.41+/-2.38 vs 6.97+/-1.30, $P<0.05$), and this continued to increase by 48 hrs (36.25+/-5.19 vs 7.69+/-1.13, $P<0.05$). The difference at 48 hrs was confirmed with fluorescence microscopy (20.8+/-4.42 vs 1.8+/-0.37, $P<0.05$). Confocal imaging confirmed that the phagocytosed thymocytes were intracellular. **In Vivo** co-localization: CFSE-labeled Ao thymocytes co-localized with the MARCO-positive MØ sub-population, implicating the marginal zone MØ in the phagocytosis of Ao immune cells. MARCO Expression: 48 hrs after CLP/ Sham, MARCO expression on splenic MØ was significantly higher in septic mice (7.7% vs 1.2%, $P<0.05$).

Conclusions: We demonstrate in a mouse model of sepsis that the splenic MØ capacity to clear Ao immune cells is enhanced as sepsis progresses. Administration of Ao immune cells to septic and sham mice demonstrates uptake in the marginal zone of the spleen, over-lapping with MARCO expression. MARCO expression, in turn, is increased in late sepsis. This data appears to implicate the marginal zone MØ population in this enhanced clearance of Ao immune cells during sepsis. (NIH RO1-GM53209 & T32-GM65085)

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TAT-BH4 DECREASES INTESTINAL EPITHELIAL CELL DEATH AFTER SEPTIC INSULT IN VITRO

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Objective: The intestinal epithelium undergoes increased apoptosis in sepsis. Transgenic animals that overexpress Bcl-2, an anti-apoptotic protein, have decreased apoptosis and improved survival in sepsis. Unfortunately, transgenic therapies for human sepsis are impractical at present. In this experiment, we evaluated a novel agent to deliver an anti-apoptotic peptide across the cell membrane. The HIV-derived protein transduction domain, Tat, allows very large peptides to easily traverse the cell membrane and offers a unique opportunity to therapeutically administer peptides that were previously available only in transgenic animals.

Materials & Methods: T84 intestinal epithelial cells were grown in culture, aliquoted at a density of 106 cells/ml and incubated for 36 hours. The experimental groups were incubated with BH4 conjugated to Tat. BH4 is the active subunit of multiple anti-apoptotic proteins (e.g. BCL2, BCL-XL). One hour after treatment with Tat-BH4, ATCC 25922 strain E Coli was added to the wells after which the cells were incubated for six hours. A 20 nm porous filter separated bacteria from the T84 cells; control cells were not exposed to bacteria. After incubation, the cells were trypsinized, washed and centrifuged before undergoing FACS assay for TUNEL.

Data: Results from FACS TUNEL assay are presented below. Cells exposed to E coli showed a level of apoptosis roughly twice baseline, 7.8% versus 16.1%, $p < 0.001$. Cells exposed to E coli and 1000nM Tat-BH4 had levels of TUNEL positivity that were statistically similar to control. There was a trend toward a dose-response effect with 1000nM Tat-BH4 reducing apoptosis more than a 500nM dose.

Table

Treatment Group (n=3 wells/group)	Percent TUNEL Positive (* = p<0.001 compared to E coli alone)
Control (no bacteria, no Tat-BH4)	7.8 *
E Coli Alone	16.1
E Coli + 500nM Tat-BH4	11.0
E Coli + 1000nM Tat-BH4	8.0 * (p=ns vs. control)

Conclusion: Tat-BH4 treatment of intestinal epithelial cells in vitro prevents E coli induced apoptosis. Increasing the concentration of Tat-BH4 shows a trend toward increased protection from apoptosis. Further in vivo studies can assess if this compound protects experimental animals from septic morbidity and mortality.

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EPITHELIAL GUT APOPTOSIS IS INCREASED IN A MOUSE MODEL OF TROPICAL ENTEROPATHY

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Chronic bowel inflammation (i.e., tropical enteropathy) is a common condition in rural areas of the third world where Western diseases (e.g. diabetes, hypertension and atherosclerosis) are rare. We have recently suggested that tropical enteropathy might provide a protective effect against Western diseases in environments of poor sanitation (Bickler, 2006). The objective of this study was to develop a murine model of tropical enteropathy that could be used to test this hypothesis and to investigate whether increased apoptosis was associated with the inflammation.

Our proposed tropical enteropathy model is a T-cell receptor transgenic mouse (DO11.10 strain) that has been engineered to recognize the 323-329 peptide fragment of ovalbumin. When this transgenic mouse is fed a diet containing egg white protein it develops enteropathy (Murphy et al, 1990; Kikuchi, 2004). Seven week-old male Balb/cJ-[Tg]CARdelta1-[Tg]TCR-DO11.10 mice (Taconic Labs) were fed a 20% egg white protein diet. Balb/c mice, the background strain of the transgenic animals, were used as controls. Animals were sacrificed at 14 or 28 days, and their small bowel examined for histology and evidence of apoptosis by the TUNEL assay.

Transgenic mice fed an egg-white protein lost weight compared to controls. Histological features of the jejunum of transgenic mice fed an egg-white diet included villous atrophy, crypt hyperplasia, goblet cell hyperplasia and infiltration of inflammatory cells. Compared to control animals, transgenic mice fed an egg-white diet showed a marked increase in apoptosis at the villous tips. The enteropathy that develops in DO11.10 transgenic mice fed an egg-white diet appears to closely simulate the small bowel changes that occur in human populations with tropical enteropathy. Given the similarity of these changes, this model may be of value for investigating the systemic consequences of living in an environment of poor sanitation and mechanisms of apoptosis that occur during chronic small bowel inflammation.

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THE USPA1 PROTEIN OF MORAXELLA CATARRHALIS INDUCES CEACAM-1 DEPENDENT APOPTOSIS IN ALVEOLAR EPITHELIAL CELLS

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Moraxella catarrhalis is a major cause of exacerbations of chronic obstructive lung disease (COPD) and emphysema. Apoptosis of pulmonary epithelial cells possibly plays a critical role in the pathogenesis. The study presented demonstrates that M. catarrhalis induced apoptosis in pulmonary epithelial cells. M. catarrhalis-specific UspA1 and the epithelial carcinoembryonic antigen-related cell adhesion molecule (CEACAM1) were required for this process. M. catarrhalis-induced apoptosis was greatly increased in HeLa cells stably transfected with CEACAM1 as compared to HeLa cells which do not express CEACAM1. Apoptotic cells showed increased activity of caspases-3, -6, and -9 but not of caspase-8. Reduced levels of Bcl-2, translocation of bax into the mitochondrium and cytosolic increase of apoptosis-inducing factor (AIF) in M. catarrhalis-infected cells implicated involvement of mitochondrial death pathways. In conclusion, M. catarrhalis induced apoptosis in pulmonary epithelial cells, a process that was triggered by CEACAM1 - UspA1 interaction. M. catarrhalis-induced apoptosis of pulmonary epithelial cells may contribute to the development of COPD/emphysema.

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INTERACTIONS OF ALVEOLAR TYPE 2 EPITHELIAL CELLS IN THE ALVEOLAR COMPARTMENT AFTER BLUNT CHEST TRAUMA

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Alveolar type 2 epithelial (AT2) cells are involved in maintenance of the alveolar epithelium and in host defense. Previous studies from our laboratory have shown that AT2 cells are significantly reduced at 48 hours after blunt chest trauma. AT2 cells are reported to undergo apoptosis during inflammation. The present study was performed to elucidate which components of the alveolar milieu are responsible for apoptosis induction in AT2 cells.

To study this Male Sprague Dawley rats were subjected to either sham procedure or blunt chest trauma induced by a single blast wave. At different time points after injury (0.5-48h) alveolar macrophages (AM) were isolated from the lungs and cultured unstimulated. Bronchoalveolar lavage (BAL) fluids were also collected from the lungs. Additionally polymorphonuclear granulocytes (PMN) were isolated from whole blood and cultured under stimulation with PMA. Unstimulated AT2 cell cultures, isolated from untreated rats, were then coincubated with AM supernatants, BAL fluids or PMN supernatants, in the presence or absence of H_2O_2. Apoptosis detection in AT2 cells was performed by Annexin V stain.

In AT2 cell cultures, significantly increased numbers of apoptotic cells were detected after addition of AM supernatants obtained 48h after trauma, compared to AT2 cell cultures incubated with AM supernatants from sham animals, when oxidative stress was induced by H_2O_2. Coincubation with BAL fluids from rats receiving blunt chest trauma caused no difference in AT2 cell apoptosis when compared to sham. Slightly more AT2 cells stained positive for Annexin V after coincubation with supernatants of PMN, isolated at 24h after chest trauma than PMN supernatants from corresponding shams.

These results suggest that apoptosis induction in AT2 cells after blunt chest trauma is most likely induced by mediators released from alveolar macrophages. Although these mediators are present in the bronchoalveolar lavage fluids, their proapoptotic activity might be lost due to dilutionary effects. Further studies should delineate whether transmigrated PMNs differ from blood PMNs in their potential to induce apoptosis. (DFG KN 475/3-1)

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CAMP-MEDIATED REGULATION OF ADAPTIVE IMMUNE FUNCTION

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Activation of an inhibitory cAMP signal pathway insideT lymphocytes inhibits the function of the immune system. This pathway acts a gate-keeper for T cell activation and serves in normal cells as a brake or thermostat to prevent inappropriate immune activation and autoimmunity. We have mapped all the components of this pathway, which involves the signal molecule cAMP and the protein kinases PKA and Csk. Further studies are focused on the proteins that recruit and anchor the pathway to these membrane domains. A crucial point is also to understand the mechanisms and dynamics of how this inhibitory pathway is temporarily shut off to allow T cell activation to occur in order to initiate an immune response. We show that cAMP inhibits Src familie kinase signalling by PKA-mediated phosphorylation and activation of C-terminal Src-kinase (Csk). The PKA type I - Csk-pathway is assembled and localized in membrane microdomains (lipid rafts) and regulates immune responses activated through the T cell receptor (TCR). We have identified Ezrin as the dual-specificity AKAP responsible for targeting PKA type I to the TCR-CD3 complex during T cell activation. Moreover, Ezrin also binds EBP50, a linker protein that binds to Csk binding protein (Cbp/PAG). Thus, Ezrin, EBP50 and Cbp/PAG act as a scaffold that assembles the cAMP-PKA/Csk-pathway in lipid rafts of the plasma membrane during T-cell activation. Ezrin knockdown prevents cAMP/PKA type I mediated inhibition of T-cell activation. These findings provide functional evidence that PKA type I regulation of T cell responses is dependent on AKAP anchoring by Ezrin. Furthermore, we show that upon TCR/CD28 co-ligation, beta-arrestin in complex with phosphodiesterase 4 (PDE4) is recruited to lipid rafts. The CD28-mediated recruitment of PDE4 to lipid rafts potentiates T cell immune responses and which counteracts the local, TCR-induced production of cAMP that produces negative feed-back in the absence of a co-receptor stimulus. The specific recruitment of PDE4 thus serves to abrogate the negative feed-back by cAMP which is elicited in the absence of a co-receptor stimulus. During sepsis, the activity of this inhibitory pathway is highly increased due to elevated levels protaglandin E2 that triggers cAMP and the function of the patient's immune cells becomes strongly reduced. Immunostimulatory drug candidates that either interfere with the action or the production of the signal molecule cAMP are being developed. Such drugs will reduce perturbation of the inhibitory pathway and restore the immune function of T lymphocytes.

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A 16

THE INFLAMMASOME: A DANGER SIGNAL SENSING COMPLEX INVOLVED IN AUTOINFLAMMATORY DISEASES

Fabio Martinon

Pathogen-recognition receptors (PRRs) are key components of immune systems and are involved in innate effector mechanisms and activation of adaptive immunity. Since their discovery in vertebrates, Toll-like receptors (TLRs) have become the focus of extensive research that has revealed their significance in the regulation of many facets of our immune system. Recently a new family of intracellular PRRs, the NOD-like receptors (NLRs), which include both NODs and NALPs have been described. Here we will discuss the function and the role of NLRs mainly NALPs in the formation of multiprotein complexes termed inflammasomes, which are required for activation of inflammatory caspases and the maturation of interleukin-1. We will emphasis on the ability of these protein complexes to detect ædanger signals ' and to promote the development of autoinflammatory syndromes such as Gout and Pseudogout.

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A 17

DANGER, NLRS AND DELAYED TYPE HYPERSENSITIVITY IN THE SKIN

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The inflammasome is a cytosolic protein-complex regulating the activation of caspase-1, which cleaves the pro-inflammatory cytokines interleukin(IL)-1beta and IL-18 into their active form. The inflammasome is composed of a NALP family member that belongs to the NLR family, which acts as a sensor for danger signals such as bacterial components. It is also composed of the adaptor protein ASC, which is essential for the recruitment of caspase-1 in the complex. In the skin, exposure to trinitro-1-chlorobenzene (TNCB) causes an immune response called contact hypersensitivity (CHS) or eczema. In this delayed-type hypersensitivity response, efficient priming of the adaptive immunity is known to depend on the concomitant activation of the innate immune system, including IL-1beta activation in the skin. Here, we will discuss the function and the role of the inflammasome in CHS, showing that essential components of the inflammasome are present and functional in human and mouse skin, that contact sensitizers induce ASC / caspase-1 - dependent IL-1beta and IL-18 processing and secretion and that ASC- and NALP3-deficient mice display an impaired response to contact sensitizers. Moreover, we will provide further evidence that contact sensitizers provide danger signals at the skin level, by means of the inflammasome, which are essential to the development of an effective immune response.

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A 18

URIC ACID AS A DANGER SIGNAL WITH ADJUVANT PROPERTIES

Kenneth Rock, Yan Shi, Chun-Jen Chen, Arron Hearn

In all tissues there are sentinel cells of bone marrow origin whose role is to collect antigens in the tissues and report their presence to T lymphocytes. These sentinels, the most important of which are dendritic cells, play an essential role in the generation of immune responses. In their absence, the immune system is not able to respond to or even detect most cancers or viral infections. Dendritic cells have evolved specialized mechanisms that allow them to acquire tissue antigens and present them to T cells. However, while this process of acquiring and displaying antigen is necessary for immune surveillance, it is not sufficient. The sentinel cells must also become activated, which occurs when they sense that the antigens they are collecting are associated with a situation that is dangerous for the host. Some danger signals are components of pathogens while others are generated by host cells. We initially identified uric acid as an endogenous danger signal that is released by dying cells and which provides an adjuvant (immunostimulatory) effect on the generation of T cell responses. It can also stimulate acute inflammation as is seen in the disease of gout. We will discuss how uric acid may mediate these effects and specifically the role of TLR signaling pathways in these processes.

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A 19

GENETIC VARIATION IN NLR GENES: ROLE IN IMMUNITY AND INFLAMMATORY DISEASE

Gabriel Nunez, Luigi Franchi, Thirumama-Devi Kanneganti, Mohamed Lamkanfi, Jong-Hwan Park, Naohiro Inohara

NOD-like receptors (NLRs) are members of a family of intracytoplasmic proteins with structural homology to the apoptosis activator Apaf-1 and plant disease resistance (R) gene products. NLRs contain variable N-terminal effector domains, a centrally located nucleotide-binding oligomerization domain (NOD) and C-terminal leucine-rich repeats (LRRs). NLRs mediate recognition of conserved microbial structures through their LRRs and upon activation induce multiple signaling pathways. Nod1 and Nod2 sense conserved, but distinct structural motifs, in bacterial peptidoglycan while Ipaf and Cryopyrin sense cytosolic flagellin and microbial RNA, respectively. Cryopyrin/Nalp3 and Ipaf are critical for the activation of inflammasomes, molecular platforms that mediate the activation of caspase-1 and processing of pro- IL-1b/IL-18

into mature IL-1b and IL-18 in response to intracellular bacteria. Mutations in Nod2 are associated with Crohn's disease whereas Cryopyrin/Nalp3 are linked to several autoinflammatory syndromes that are characterized by inappropriate secretion of IL-1b. Genetic and biochemical analyses revealed that cytosolic NLR proteins activate host signaling pathways independently of TLR signaling, although both NLRs and TLRs cooperate for optimal immune responses to bacterial pathogens. The results available so far suggest that NLRs are critical mediators of innate immune responses by linking intracellular recognition of bacteria to host defense pathways and their deregulation play an important role in inflammatory disease.

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A 21

THE INFLAMMATORY CASPASES: KEY PLAYERS IN THE HOST RESPONSE TO PATHOGENIC INVASION AND SEPSIS

Maya Saleh

The inflammatory caspases: key players in the host response to pathogenic invasion and sepsis Maya SalehÂ Â Department of Medicine, McGill University, Montreal, Canada H3A 1A1 Abstract Caspases are cysteinyl-aspartate specific proteinases known for their role in apoptosis (cell death or apoptotic caspases) and pro-inflammatory cytokine maturation (inflammatory or group I caspases). The inflammatory caspases were among the first to be discovered, but only recently did the mechanisms leading to their activation and inhibition have started to be elucidated. Activation of the inflammatory caspases is a prerequisite for pathogen clearance, and their tight regulation is necessary to control the magnitude of the innate immune response and protect the organism from possible damaging effects such as sepsis. We have recently uncovered from population studies and animal models of infectious diseases and sepsis a role for caspase-12 in blocking the inflammatory response initiated by caspase-1, thus predisposing the organism to severe sepsis and sepsis-related lethality. In my talk, I will discuss the function of these caspases in inflammation and bacterial clearance, with an emphasis on a novel role for caspase-12 as a key factor during the first steps of innate immunity at the mucosal interface. I will also present a newly identified caspase-1 substrate essential in both bacterial clearance and apoptosis.

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A 22

HEPARAN SULFATE AND THE INITIATION OF SYSTEMIC INFLAMMATION

Jeffrey Platt

Trauma and various systemic diseases are associated with loss of tissue function and with widespread activation of inflammatory responses and the triggering of adaptive immunity. While inflammation and adaptive immunity are promoted by endotoxin and other exogenous substances, profound inflammatory and immune responses are seen in the absence of extrinsic agonists (e.g., the immune response to transplantation). We sought to identify a substance the metabolism of which could explain rapid changes in tissue physiology and activation of inflammation and immunity. We focused on heparan sulfate proteoglycan. Tethered to cell surfaces and integral to extracellular matrices, heparan sulfate proteoglycan plays a vital role iun manyphysiological functions and yet it is potentially digested under a variety of conditions of inflammation and immunity. For example, we found that heparan sulfate is shed from cells when the cells are acted upon by complement, macrophages, neutrophils, activated T cells and platelets. The shedding of heparan sulfate proteoglycan results from cleavage by proteases and/or heparanase. We found that heparan sulfate is rapidly shed in such biological conditions as ischemia-reperfusion, both in clinical and experimental models. Loss of heparan sulfate deprives many cells of normal functions. Moreover, the heparan sulfate shed from cells can among other things excite activation of TLR4, thus accounting for inflammation and adaptive immune reactions observed in diverse settings.

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A 23

RECEPTOR CROSS-TALK MECHANISMS OF NEUTROPHIL PRIMING

Jie Fan

Severe trauma and hemorrhage renders the patient more susceptible to a second, seemingly trivial, inflammatory stimulus, the so-called "two-hit" hypothesis. The post-trauma sepsis, which involves activation of innate immunity, can lead to severe multi-organ failure (MOF) or systemic inflammatory response syndrome (SIRS), and death. Studies have suggested that cell priming caused by a first hit is the mechanism for enhanced response of the cell to a second hit. Polymorphonuclear neutrophils (PMN) are essential effector cells of the innate immune system. The accumulation of PMN in tissue is considered a critical event in organ inflammation and injury, and has been the target of preventative strategies. PMN migration is a result of a cascade of cellular events, in which PMN, endothelial cells (EC), epithelia, and macrophages (MΦ) act in concert. Studies from our lab explored interrelated novel findings indicating that receptor cross-talk mecha-

nisms occurring in PMN, EC, and MΦ are important determinants for augmenting PMN migration in a sepsis setting. In PMN, LPS through Toll-like receptor (TLR)4 and phosphoinositide 3-kinase g signaling down-regulates the expression of G protein-coupled receptor kinases (GRK)2 and GRK5 in response to chemokine, and the reduced expression of GRKs decreases chemokine receptor desensitization and markedly augments the PMN migration response. However, in EC and MΦ, LPS/TLR4 signaling up-regulates TLR2, and oxidant signaling derived from PMN NADPH oxidase enhances the TLR2 upregulation through PMN-EC or PMN-MΦ interaction, and results in an amplified expression of adhesion molecules in the EC and release of cytokines and chemokines from the MΦ in response to TLR2 ligands, thereby promotes PMN migration. Furthermore, we elucidated that hemorrhagic shock is potent to activate PMN NADPH oxidase through high-mobility group box 1 (HMGB1) -TLR4 - p38 MAPK signaling, and therefore initiates the mechanisms of cell priming. Taken together, receptor cross-talk mechanisms are critical determinants for cell priming and subsequent augmented PMN migration in sepsis.

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A 24

ALTERED DENDRITIC CELL PHENOTYPE AND THE CHRONICITY OF THE SEPTIC RESPONSE

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An understudied consequence of patients who survive life-threatening sepsis is the subsequent susceptibility to various diseases due to the complication of immunosuppression. Interestingly, these immune based alterations may manifest themselves years after the septic event. In order to study the chronic effect of acute, severe sepsis, we investigated a number of alterations in mice that survived life-threatening peritonitis, induced by cecal ligation and puncture (CLP). This model was established such that 60% of the cohort were long-term survivors. Longitudinal studies of these survivors demonstrated that a usually innocuous lung challenge with Aspergullus fumigatus two and three weeks after CLP recovery was lethal to these animals, while the sham controls were unremarkable. To gain mechanistic insight into these studies we assessed the cytokine phenotype from both bone marrow-derived dendritic cells (DC) and lung DC, and found that both populations had a significant altered response to TLR2, TLR4 and TLR9 agonists, Pam3Cys, LPS, and CpG, respectively. The challenged DC isolated from the recovered CLP mice produced 15 fold less IL-12 and 6 fold more IL-10, as compared to their sham counterparts. Epigenetic analysis using chromatin precipitation analysis (ChIP) demonstrated that alterations in chromatin histone acetylation might serve as the mechanism driving this response. Splenic DC isolated from recovered septic animals at 2 weeks exhibited an impaired capacity to both present antigen and induce proliferation of sensitized normal T cells. This may be due to the altered expression of MHCII and co-stimulatory molecules on the DC recovered from mice post sepsis. Flow cytometric analysis found that MHCII expression was significantly reduced on these cells, while CD40, CD80 and CD86 expression were increased. Furthermore, microarray data of the DC recovered from post-septic mice demonstrated a significant increase in the expression of a cysteine protease inhibitor named Stefin A1 (Stfa1). This protease inhibitor could play a role in regulating DC-T cell communication via altering the enzymatic activity required for normal antigen processing and presentation. Collectively, these data strongly suggest that the normal phenotype of DC are profoundly altered, as a consequence of severe sepsis, and identifies specific mechanisms that could be restored as a strategy to reverse sepsis-induced immunosuppression.

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A 25

FLT-3 LIGAND AND DENDRITIC CELL FUNCTION IN BURN INJURY

Tracy Toliver-Kinsky

Dendritic cells are central in activation of immune responses to microorganisms, through the functions of immune surveillance and antigen recognition, antigen presentation, co-stimulation of effector cells, and cytokine production. While little is known of the direct effects that severe burn injury has on the functions and interactions of skin, circulating, and lymphoid tissue- dendritic cells, many immune responses that are regulated by dendritic cells, such as T cell proliferation and polarization, antigen-specific antibody production and pathogen-elicited cytokine production, are altered after severe burns. Therefore, global enhancement of dendritic cell numbers and functions may be beneficial for severe burn patients by increasing the number of cells that can recognize microorganisms and stimulate appropriate immune responses. The hemopoietic cytokine fms-like tyrosine kinase-3 ligand (Flt3L) enhances dendritic cell production from bone marrow-derived progenitor cells and has been shown in various models to enhance various immune functions. Using a mouse model of burn wound infection with P. aeruginosa, we have found that Flt3L treatments after burn injury significantly increase dendritic cell numbers, improve survival after wound infection, and decrease systemic dissemination of bacteria. Given the numerous functions of dendritic cells in activation of immune responses, it is important to determine which dendritic cell functions are enhanced by Flt3L and are beneficial in clearing a wound infection. Examination of dendritic cell properties after Flt3L treatment has revealed that surface expression of class II major histocompatibility complex and CD11c is increased, as is uptake of ovalbumin, suggesting that Flt3L treatments enhance antigen acquisition and presentation. The numbers of neutrophils, B cells, and T cells expressing the activation marker CD69 in the spleen and wound-draining lymph nodes after a burn wound infection are significantly increased by Flt3L treatments, as are

the number of splenic lymphocytes producing gamma interferon and Th1-associated cytokines after a systemic challenge. However, systemic inflammation, indicated by circulating levels of interleukin-6 after a burn wound infection, is reduced in Flt3L-treated mice, which could be attributed to the decrease in systemic dissemination of bacteria, or to modulation of cytokine-producing cells. Current efforts are focusing on dissecting the contributions of different dendritic cell populations to various immune responses to burn and wound infection, and modulation of various dendritic cells subtypes and immune functions by Flt3L treatments.

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TREATMENT OF EXPERIMENTAL COLITIS WITH VASOACTIVE INTESTINAL PEPTIDE-INDUCED TOLEROGENIC DCS

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Background: Crohn's disease (CD) is a chronic debilitating disease characterized by severe Th1-driven inflammation of the colon partially caused by a loss of immune tolerance against mucosal antigens. Available therapies for CD based on immunosuppressive agents are not entirely effective, nonspecific, and with multiple adverse side effects. Restoration of immune tolerance by reestablishment of regulatory T cells (Tr) repertory has been recently proposed as a new therapeutic approach for CD and other autoimmune diseases. Dendritic cells (DCs) are a heterogeneous population of antigen-presenting cells (APCs) that contribute to innate immunity and initiate the adaptive immune response, and also play an important role in immune homeostasis by inducing and maintaining tolerance. The use of tolerogenic DCs with capacity to induce regulatory T cells (Tr) has been recently proposed for the treatment of CD in a way to recover immune tolerance. Vasoactive intestinal peptide (VIP) is an immunomodulatory neuropeptide released in inflammatory/autoimmune conditions, which is able to generate regulatory/tolerogenic DCs (DC_{VIP}) with capacity to induce CD4 Tr. Objective: To exploit a novel strategy involving the use of tolerogenic DCs for the treatment of IBD, we investigated the therapeutic effect of the administration of VIP-induced tolerogenic DCs in TNBS-induced colitis and the mechanisms involved. Material and Methods: We examined the therapeutic action of DC_{VIP} in the colitis induced by intracolonic administration of trinitrobenzene sulfonic acid (TNBS). Diverse clinical signs of the disease were evaluated, including weight loss, diarrhea, colitis and histopathology. We also investigated the mechanisms involved in the potential therapeutic effect of DC_{VIP}, such as inflammatory cytokines and chemokines, Th1-type response and generation of Tr. Results: DC_{VIP} injection ameliorated significantly the clinical and histopathological severity of the TNBS-induced colitis, abrogating body weight loss, diarrhea, and inflammation, and increasing survival. The therapeutic effect was associated with downregulation of both inflammatory and Th1-driven autoimmune response, by regulating a wide spectrum of inflammatory mediators directly through activated macrophages, and by generating IL-10/TGFb1-secreting Tr with suppressive capacity on effector autoreactive T cells. Conclusions: The possibility to generate/expand ex vivo tolerogenic DC_{VIP} opens new therapeutic perspectives for the treatment of autoimmune/inflammatory diseases, including CD in humans. In vitro pulsing of tolerogenic DC_{VIP} with self-antigens, followed by in vivo injection leads to inhibition of inflammatory response and the differentiation of antigen-specific Tr cells. Therefore, the inclusion of tolerogenic DC_{VIP} in future therapeutic regimens may minimize the dependence on non-specific immunosuppressive drugs used currently for CD

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ALTERED GENE EXPRESSION PATTERNS IN DENDRITIC CELLS AFTER SEVERE TRAUMA: IMPLICATION FOR SYSTEMIC INFLAMMATION AND ORGAN INJURY

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Objective: Dendritic cells (DC) represent an important linkage between the innate and adoptive immunity within the systemic inflammatory response to trauma and sepsis.In this study, the gene expression pattern of DC from multiple trauma patients were compared to the signatures obtained from healthy volunteers.

Material and Method: Messenger RNA was isolated from highly purified peripheral DC (>95% of vital PBMC) and whole blood, respectively. Samples were obtained from 10 multiple trauma patients (ISS 30±9,7, on day of admission) and 5 healthy volunteers (control) after approval by the local ethics committee. Aliquots of target cDNAs and reference samples (cDNA derived from the monocytic cell line SIGM5) were cohybridized on a thematic medium-density microarray assessing 780 inflammation-related transcripts.

Data: In DC of multiple trauma patients 20 transcripts were upregulated compared to control. This transcriptomic profile was not observed in whole blood of either group. This specific cluster of DC include genes encoding for central effector molecules like 5-lipoxygenase (5-LO) and the corresponding LTB4 receptor, regulating DC migration and airway inflammation in asthma. Also BCL2L2 was upregulated, indicating an antiapoptotic effect as well as CXCL4 or platelet factor 4, a chemokine not implicated as a product of DCs before.

Conclusion: These data confirm and expand the central role of chemokines and lipid mediators as effector molecules of DC-mediated immune responses in systemic inflammation after severe trauma. Furthermore, the

central role of the 5-LO/LTB4 pathway in DC for the pathogenesis of asthma would be consistent with a role in trauma-associated acute lung injury, a leading cause of morbidity and mortality in multiple trauma.

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PHAGOCYTOSIS, INTRACELLULAR DEGRADATION AND PHENOTYPIC CHANGES OF CORD BLOOD MACROPHAGES (MF) AND PERIPHERAL BLOOD MF AFTER BACTERIAL CHALLENGE

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Objective: Neonates are more susceptible to systemic bacterial infections than adults. Early events of pathogen-host interaction are phagocytosis, intracellular degradation and consecutive receptor-modulation of the phagocyte. Our own results showed, that phagocytic activity and intracellular killing of gfp-labelled Escherichia coli (E. coli-gfp) of cord blood MF (CBMF) were not reduced compared to MF of adults (PBMF). We asked now, weather upregulation of HLA-DR and costimulatory molecules by CBMF would be impared.

Methods: PBMF and CBMF of healthy term neonates were isolated. Escherichia coli DH5α, expressing a prokaryotic variant of green fluorescent protein (E. coli-gfp) were added in a ratio of 50:1 (bacteria: cells) for 60 minutes. After removal of free bacteria, cells were seeded in new medium supplemented with gentamycin (10μg/ml). Phagocytic activity was checked immediately by flow cytometry by measuring Phagocytosis Index (CD14+gfp+: CD14+ cells) and Phagocytosis Capacity (mean gfp-fluorescense intensity, MFI of CD14+). Expression of CD14, HLA-DR and CD80 were analyzed 4 hours and 24 hours after removal of bacteria. Uninfected samples served as controls. Intracellular degradation of E. coli-gfp was assesed by loss of gfp-fluorescence.

Data: Phagocytosis Index of PBMF vs. CBMF after 60 minutes (58±15% vs. 57±18%) and Phagocytic Capacity (127±27 MFI vs. 128±14 MFI) was not different confirming earlier results. 4 hours after bacterial challenge less than 5% of PBMF an CBMF were positiv for gfp. CD14 expression of PBMF 4 hours after exposition to E. coli-gfp vs. control was slightly downregulated (57±41 MFI vs. 89±34 MFI) wereas there was a significant downregulation on CBMF (19±17 MFI vs. 58±30 MFI; $p<0.05$). After 24 hours CD14 expression was diminished on both, exposed PBMF vs. respective control samples (14±16 MFI vs. 87±68 MFI; $p<0.05$) and exposed CBMF (26±14 vs. 75±46; $p<0.05$). HLA-DR expression after 4 hours was significantly higher on exposed PBMF compared to control (39±8 MFI vs. 27±8 MFI) while after 24 hours upregulation of HLA-DR was the same in both groups (108±34 vs. 106±38). There was no difference in HLA-DR expression on exposed CBMF vs. control after 4 hours (18±11 MFI vs. 21±9 MFI). After 24 hours HLA-DR expression of exposed CBMF vs. control was again not different (47±19 MFI vs. 80±41 MFI) but compared to PBMF, HLA-DR upregulation was diminished. CD80 was not detectable after 4 hours of culture. After 24 hours CD80 expression on exposed PBMF vs. control was not different (47±7 MFI vs. 49±23 MFI), while on exposed CBMF, CD80 expression was diminished vs. control (23±8 MFI vs. 49±26 MFI; $p<0.05$).

Conclusion: CD14 downmolulation after challenge with E. coli-gfp was accelerated on CBMF compared to PBMF. HLA-DR and CD80 upregulation on CBMF was diminished. These differences between CBMF an PBMF could not be explained by different phagocytic activity. The functional consequence of diminished HLA-DR and CD80 upregulation on CBMF after bacterial challenge has to be determined.

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INFLAMMATORY AND ANTI-INFLAMMATORY REACTIONS IN THE NEONATAL IMMUNE SYSTEM

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Background: Sepsis and its sequelae remains the main challenge in neonatology worldwide. Monocyte-derived macrophages (MΦ), equipped with costimulatory and cytotoxic receptors and cytokines, are central mediators in sepsis and capable to initiate and modulate immune reactions. Although defects in neonatal Th1 production are known, the capability of neonatal MΦ to orchestrate immune reactions remains controversial. Hypothesis: Compared to MΦ of adults, the capability of neonatal MΦ to display both inflammatory and anti-inflammatory reactions is reduced.

Methods: Cord blood mononuclear cells and MΦ from term and preterm neonates (CBMΦ) and from healthy adults (PBMΦ) were isolated, stimulated and phenotyped by flow cytometry. Cytokine production was analyzed by ELISA; cDNA-copy numbers were measured by quantitative real-time PCR (LightCycler) using external standards. Cellular apoptosis was detected by Annexin V-stain and T cell proliferation by CSFE.

Results: Basal and IFN-γ-induced costimulatory MΦ phenotypes, inflammatory cytokine production (IL-8, IL-12, IL-18) and costimulatory MΦ functions were reduced on CBMΦ compared to PBMΦ. These effects were more pronounced in preterm neonates ($p < 0.05$ vs. term neonates). Consecutively, neonatal MΦ dependent T cell activation, as analyzed by stimulation with a polyclonal T cell mitogen, was diminished but could be almost restored when CBMΦ were replaced by PBMΦ. In parallel, basal and IL-10-induced inhibitory CBMΦ phenotypes, IL-10 cytokine production, and ADCC

functions using CD4 T cell targeting antibodies were impaired in CBMΦ as well (all $p < 0.05$ vs. PBMΦ).

Conclusion: With respect to neonatal immune reactions, mediated by MΦ, our data point towards both an impaired capacity to mediate activating as well as inhibitory signals. Although the newborn is prone to bacterial infection, these mechanisms may serve as a protection against hyperinflammatory reactions in utero.

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A 30

TLR4 SIGNALING MODULATES ARGINASE ACTIVITY IN MYELOID SUPPRESSOR CELLS AFTER TRAUMA

Petar Popovic, Benjamin Matta, Jose Prince, Juan Ochoa

Introduction: T-lymphocyte dysfunction after trauma increases susceptibility to severe infections leading to increased morbidity, mortality and cost. Trauma induces myeloid suppressor cells (TIMSC), which express arginase, an enzyme that destroys arginine, an essential amino acid for T cells. Th2 cytokines induce arginase in macrophage cell lines. Toll-like receptors (TLRs), such as TLR4, are required for activation of proinflammatory cellular signaling pathways in response to microbial products, but can also recognize endogenous molecules released from damaged tissues. Here, we hypothesized that danger signal recognized through TLR4 might modulate arginase activity in TIMSC induced by Th2 cytokines.

Methods: TIMSC were harvested from the spleens 16h after trauma and cultured in the presence of IL-4, IL-13 with or without LPS for additional 48h. TLR4-mutant (C[3H]/HeJ) mice and wild-type (WT) controls were used to address role of TLR4. Arginase activity was measured through a colorimetric assay.

Results: A large accumulation of TIMSC was observed in spleens after trauma and exhibited significant arginase activity (423 ± 68 vs. 32 ± 12, $p<0.01$). Arginase expression was increased further in TIMSC and modestly in control cells when cultured in the presence of IL-4 (2439 ± 199 vs. 531 ± 62, $p<0.01$) or IL-13 (1522 ± 167 vs. 419 ± 52, $p<0.01$) cytokines. Addition of LPS in the culture drastically decreases arginase activity in TIMSC by more than 80% (2593 ± 245 vs 449 ± 73 for IL-4 and 1150 ± 193 vs 223 ± 37 for IL-13) while effect on control CD11b cells was in the range of 35-55%. Interestingly, spontaneous arginase activity in CD11b cells after trauma was more that two times higher in TLR4 mutant mice compared to WT (560 ± 86 vs. 231 ± 79 , $p<0.01$). Additionally, IL-13 in vitro induced two times higher arginase activity in the TLR4 mutant CD11b cells comparing to WT (2179 ± 221 vs. 1049 ± 225, $p<0.01$). Finally, presence of LPS in the culture diminish IL-13 induced arginase activity by more then 80% in the CD11b cells from WT (830 ± 91 vs. 119 ± 24, $p<0.01$)) but only by 30% in the cells from TLR4 mutant mice (1220 ± 185 vs. 831 ± 127, $p<0.01$).

Conclusions: Th2 cytokines, which are increased after trauma, stimulate arginase expression in TIMSC, playing an important role in creating trauma induced immune deficiency. Simultaneously those cells show higher sensitivity to danger signal (LPS), which might oppose Th2 mediated signal. Our results suggest that TLR4 signaling might have important role in the regulation of immune functions after trauma, by modulating immunosuppressive function of TIMSCs.

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PANCREATIC PROTEASES ARE NECESSARY FOR GUT BARRIER FAILURE AND THE PRODUCTION OF BIOLOGICALLY ACTIVE LYMPH

Francis Caputo, Bobby Rupani, Anthony Watkins, Dimitrios Barlos, DaZhong Xu, Edwin Deitch

Objective: The loss of gut barrier function during trauma and hemorrhagic shock (T/HS) has been implicated as a major contributor to the development of distant organ injury and subsequent multiple organ failure (MODS). This process of gut barrier failure-induced MODS appears to occur when inflammatory mediators and 'toxic' factors generated in the ischemic/reperfused gut and contained in mesenteric lymph reach the systemic circulation and distant organs. Based on work indicating that the unstirred mucus layer is an important component of the gut barrier, we investigated the hypothesis that pancreatic proteases contribute to gut injury and the production of biologically-active T/HS mesenteric lymph, at least in part, by disrupting the mucus layer of the gut thereby promoting the translocation of gut luminal contents, which in turn exacerbate gut injury and inflammation.

Material and Methods: To test our hypothesis, we measured the effect of pancreatic duct ligation (PDL) prior to trauma and hemorrhagic shock on the mucus layer of the gut, gut permeability as well as the biological activity of lymph. Male Sprague-Dawley rats were subjected to T/HS (MAP 30-35 mmHg x 90 min) with and without pancreatic duct ligation. In addition, all the animals also underwent cannulation of the main mesenteric lymph duct for mesenteric lymph collection prior to the induction of T/HS. Gut permeability was measured using both in vivo as well as ex vivo methodologies. Subsequently, the animals were sacrificed and ileal samples were obtained for morphometric mucus analysis, gut histology and ex-vivo gut permeability (everted gut sac method). The MTT viability assay was used to measure the cytotoxic effects of mesenteric lymph on human umbilical vein endothelial cells (HUVECs) and flow-cytometry was used to test its ability to activate neutrophils.

Table

Gender Hazard Ratio(F vs. M, 95% CI)	All Patients	PRE	POST	Pre vs. Post Hazard Ratio Comparison
Mortality	0.81 (0.6-1.1)	0.77 (0.5-1.2)	0.87 (0.6-1.4)	
MOF	0.61 (0.5-0.7)	0.57 (0.4-0.8)	0.65 (0.4-0.9)	p=0.52
NI	0.74 (0.6-0.9)	0.75 (0.6-0.9)	0.65 (0.4-0.9)	p=0.89

Data: PDL reduced the magnitude of T/HS-induced villous injury by about 50% ($p<0.01$). Also, PDL prior to T/HS essentially totally abrogated T/HS-induced increases in gut permeability whether measured by an in vivo (6±4 vs 18.9±5.5 μg/ml; $p<0.05$) or ex-vivo assay (18.2±0.1 vs 27.3±4.6 ng/min/cm2; $p<0.05$. Thus, PDL was more effective in preventing T/HS-induced changes in gut permeability as well as limiting villous injury. As hypothesized, the beneficial effects of pancreatic duct ligation on T/HS-induced gut injury and dysfunction were associated with preservation of the intestinal mucus layer. Specifically, PDL totally prevented T/HS-induced mucus loss whether measured as mucus thickness (31.3±5.2μm vs 20.6±2.4μm; $p<0.05$) or the percentage of the intestinal lumen covered with mucus (77±4% vs. 50±6%; $p<0.05$). Mesenteric lymph collected from rats subjected to T/HS lymph manifested significant endothelial cell cytotoxic activity with only 11.2% viable HUVECs ($p<0.01$). In contrast, mesenteric lymph collected from the T/HS rats subjected to PDL was not cytotoxic for endothelial cells and was comparable to the non-toxic T/SS (92±13 vs 95±3.5 % viability; $p>0.05$). Likewise, PDL prevented the ability of T/HS-lymph to prime neutrophils as measured by respiratory burst (361±120 vs 598±153 mean fluorescent intensity; $p<0.05$).

Conclusion: In summary, the results of the current study support the hypothesis that both the mucus layer and pancreatic proteases are involved in the pathogenesis of T/HS-induced gut injury and that pancreatic proteases are involved in the generation of biologically active lymph.

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GENDER DIMORPHISM FOLLOWING INJURY: ARE HORMONAL DIFFERENCES RESPONSIBLE?

Jason Sperry, Avery Nathens, Heidi Frankel, Ernest Moore, Ronald Maier, Joseph Minie

Objective: Experimental studies have shown that sex hormones are responsible for gender based differences in outcome following traumatic injury and hemorrhage. The hormonal milieu of the pro-estrous female has been shown to be protective following injury, however clinical studies have not reproduced these experimental findings. We sought to characterize the gender dimorphism after injury relative to the reproductive age of the female (PRE, $\leq$ 50 years old vs. POST, $>$ 50 years old) in a cohort of severely injured trauma patients where significant variation in post-injury care is minimized. We hypothesized the protective effects afforded by female gender would be evident only in women of reproductive age, with no protection afforded to post-menopausal females, when compared to similarly aged males.

Methods: Data were obtained from a multi-center prospective cohort study evaluating clinical outcomes in blunt injured adults with hemorrhagic shock. Patients with isolated brain injury were excluded from the analysis. Standard operating procedures (SOPs) were employed to minimize variation in clinical management across centers, including: early goal directed resuscitation, strict glycemic control, venous thromboembolism prophylaxis, low tidal volume strategy, ventilator associated pneumonia management, and restrictive transfusion protocols. Separate Cox proportional hazard regression (PHR) models were formulated based on all patients to evaluate the effects of gender on mortality, multiple organ failure (MOF, maximum organ dysfunction score $>$ 5) and nosocomial infection (NI), after controlling for all important covariates and adjusting for the effects of early death on later MOF and NI rates. These models were then used to characterize the effect of gender in PRE and POST age groups.

Results: Overall mortality, MOF, and NI rates for the entire cohort (n=923) were 20%, 40% and 45%, respectively. Mean ISS was 32±14 (mean±SD). Males (n=601) and females (n=322) were clinically similar except that males required higher crystalloid volumes, more often had a history of alcoholism and liver disease, and had greater ventilatory and ICU requirements. PHR revealed that gender is not a significant independent risk factor for mortality, yet a trend does exist in favor of female gender overall and in PRE (n=634) and POST (n=288) groups.(Table) Female gender is independently associated with a 40% and 25% lower risk of MOF and NI, respectively. Gender remained an independent risk factor in PRE and POST subgroup analysis, with the protection afforded by female gender remaining unchanged.

Conclusions: In this select cohort of severely injured patients, the independent effect of gender on MOF and NI rates remains unaltered in pre and post-menopausal women when compared to similarly aged males, after controlling for all important covariates. This is contrary to prior experimental studies, and the known physiologic sex hormone changes which occur after menopause in

		IFNγ (pg/ml)	IL-12 (pg/ml)	MHC II (MFI)
Group 1	S Tc + S APC	1383,4±19,1	180,5±2,8	6,6±0,26
Group 2	S Tc + Th APC	1272,7±20.4	170,7±3,1	5,3±0,32
Group 3	Th Tc + S APC	787,9*#±13,5	135,9*#±3,4	4,7*±0,23
Group 4	Th Tc + Th APC	645,0*#±15,7	112,7*#±2,8	4,1*±0,4

N=6-7 group, Mean ± SEM, ANOVA, *p<0.05 vs Group 1 # p<0.05 vs. Group 2

women. Further investigation is required to decipher the mechanism by which this dimorphism exists. However, these results suggest that factors other than sex hormones may be responsible for gender based differences following injury.

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DEPRESSED T-CELL FUNCTION IS RESPONSIBLE FOR PROLONGED IMMUNOSUPPRESSION FOLLOWING TRAUMA-HEMORRHAGE

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Trauma-hemorrhage (Th) leads to suppressed immune response resulting in increased susceptibility to sepsis. Nevertheless, the contribution of antigen presenting cells (APC) versus T-cells (Tc) to this immunosuppression remains unknown.

To study this, male mice underwent a midline laparotomy (trauma) and hemorrhage (arterial blood pressure of 35±5 mmHg for 90 min and resuscitation) or sham (S) operation. 24 hours thereafter, spleens were harvested and T-cells (via Microbeads) and APC (via adherence) were isolated. Co-cultures of combined T-cells and APC were stimulated with ConA and LPS. IFN-γ (Tc) and IL-12 (APC) were measured in the supernatants (with Multiplex-assay). In addition, the expression of co-stimulatory surface molecules (CD28, CD80, and CD86) and MHC class II on CD11b (+) or CD11c (+) APC was determined by flowcytometry.

IFN-g was suppressed by Th T-cells - irrespective whether S or Th APC were added. Th APC did not affect IFN-g release by S T-cells. In contrast, Th T-cells depressed the release of IL-12 by APC. The release of IL-12 by Th APC was not altered when S T-cells were co-cultured. Interestingly, co-stimulatory surface molecules were not altered following Th. MHC II expression on CD 11c (+) APC, respectively Denditic cells, was suppressed following Th.

Thus, following Trauma-hemorrhage the interaction of innate and adoptive immunity appears to be predominantly suppressed by T-cells. Although Th Dendritic cells displayed suppressed MHC II expression they did not affect T-cells function. (Supported by DFG AN 357/1-1)

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A 34

THE OUTCOME OF HEMATOPOIETIC PROGENITOR CELLS IN THE AREA OF INJURY DURING SUSTAINED BONE MARROW SUPPRESSION FOLLOWING TRAUMA/HEMORRHAGIC SHOCK

Chirag Badami, Ziad Sifri, Francis Caputo, Alicia Mohr, Edwin Deicth, David Livingston

Background: We have previously shown that injured tissue accumulates hematopoietic progenitor cells (HPCs). As shock is introduced and the bone marrow (BM) is suppressed there is an amplification of HPCs to the area of injury. The goal of this study was to investigate the long term effects of hemorrhagic shock and lung contusion on BM function and the presence of progenitor cells at the site of injury.

Methods: Sprague-Dawley rats (250-400g) sustained a unilateral lung contusion (LC) using the blast wave of a percussive nail gun (Craftsman 968514 Stapler) applied to a small metal plate onto the right chest. The rats were then subjected to either hemorrhagic shock (HS) (MAP 40-45 mmHg for 45 min) or sham shock (SS) (n= 4/group). BM mononuclear cells from each individual lung and femurs were isolated and plated (2×10^6) in duplicate for BM colonies.

Results: BM colonies were significantly less in HS when compared to SS, at both 3 and 72 hours. (Data not shown) The contused right lung had a greater number of BM colonies 3 hours after the injury when compared to 72 hours.

Conclusion: Early BM failure following HS is partially attributable to the mobilization of HPCs to the site of injury. Three days following injury the rat BM remains suppressed, however the initial increase in HPCs now shows a considerable reduction in cells. The function of these cells in the site of injury needs further investigation.

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Table

Contused (Right) Lung of HS		
Colony	3 Hours	72 Hours
CFU-GM	30.8 ± 7.6	9.8 ± 3.2*
CFU-E	31.0 ± 6.3	8.5 ± 2.1*
BFU-E	19.5 ± 2.1	4.8 ± 2.5*

* $p < 0.05$ vs. 3 hours

A 35

LOW MONOCYTE HLA-DR PREDICTS MORTALITY AND SECONDARY SEPTIC SHOCK IN SEVERE BURN PATIENTS

Fabienne Venet, Sylvie Tissot, Anne-Lise Debard, Caroline Faudot, Alexandre Pachot, Guillaume Monneret

Objective: Although prevention campaigns have decreased its incidence, severe burn still represents a frequent trauma and a major health care problem in industrialized countries. Despite recent medical advances, sepsis and subsequent multiple organ failure remain a major cause of burn morbidity and mortality. In patients with severe burns, 75% of all deaths are currently related to sepsis. Severe thermal injury causes immune dysfunctions involving pro and anti-inflammatory mechanisms. It subsequently leads to a state of immune deficiency that shares some similarities with sepsis-induced immunosuppression. These dysfunctions may participate in severe burn patients' susceptibility toward infections. A hallmark of sepsis-induced immunosuppression is established by decreased monocyte Human Leukocyte Antigen-DR (mHLA-DR) measurements. The main objective of the current study was to characterize the appearance and the duration of low mHLA-DR expression after severe burn as well as to determine its correlation with mortality and septic complications.

Patients and Methods: Severe burn patients (n = 14, Total Burn Surface Area >30%) were prospectively included and followed during 15 days after burn, 29 healthy volunteers served as controls. We quantified mHLA-DR expression with a standardized flow cytometry protocol (Quantibrite™ system). Quantitative RT-PCR was used to determine mRNA expression of HLA-DRA chain and transcription factor class II transactivator. IL-6, TNF-a and IL-10 were measured in plasma using commercial ELISA kits.

Results: Every patient presented with severe decreased mHLA-DR expression as little as day 2 after burn. This decrease was confirmed at the molecular level. After 6 days, mHLA-DR expression increased in patients who will survive whereas it remained diminished in non-survivors. As early as days 7-10 after burn, patients who will present with septic shock complications exhibited significantly lower monocyte HLA-DR expression in comparison with non-septic patients. We did not observe any relationship between cytokines concentrations and the development of septic complications or pejorative issue.

Conclusion: Severe burn induced a marked mHLA-DR expression decrease. As observed after septic shock, its magnitude and duration were associated with pejorative outcome and the development of septic complications. In the present study, using either 30 % (HLA-DR positive monocyte) or 5,000 AB/C (antibodies bound per cell) for cut-off values, non-surviving patients and patients developing secondary septic shock could be rapidly identified. In the other patients, mHLA-DR expression never declined under these thresholds. Using a standardized flow cytometry protocol, decreased mHLA-DR expression could thus be proposed to monitor burn patients. Multicenter studies are now needed to confirm these preliminary results.

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EFFECT OF 17B-ESTRADIOL ON SIGNAL TRANSDUCTION PATHWAYS AND SECONDARY DAMAGE IN EXPERIMENTAL SPINAL CORD TRAUMA

Salvatore Cuzzocrea, Tiziana Genovese, Emanuela Mazzon, Emanuela Esposito, Rosanna Di Paola, Carmelo Muia

Since studies have shown that 17b-estradiol produces anti-inflammatory effects following various adverse circulatory conditions, we examined whether administration of 17b-estradiol prior to spinal cord injury (SCI) has any salutary effects in reducing SCI. In order to gain a better insight into the mechanism of action of the anti-inflammatory effects of 17b-estradiol, the following endpoints of the inflammatory process were evaluated: (1) spinal cord inflammation and tissue injury (histological score); (2) neutrophil infiltration (myeloperoxidase activity); (3) expression of iNOS, nitrotyrosine and COX-2; (4) apoptosis (TUNEL staining and Bax and Bcl-2 expression); (5) tissue TNF-a, IL-6, IL-1b and MCP-1 levels. In another set of experiments, 17b-estradiol was found to significantly ameliorate the recovery of limb function (evaluated by motor recovery score).

In order to elucidate whether the protective effects of 17b-estradiol were mediated via the estrogen receptors, we investigated the effect of an estrogen receptor antagonist, ICI 182,780, on the protective effects of 17b-estradiol. ICI 182,780 (500 mg/kg administered subcuta-

neously 1 h prior to treatment with 17b-estradiol) significantly antagonized the effect of the 17b-estradiol and abolished the protective effect against SCI. Taken together, our results clearly demonstrate that administration of 17b-estradiol prior to spinal cord injury reduces the development of inflammation and tissue injury associated with spinal cord trauma.

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A 37

ANALYSIS OF SYSTEMIC INTERLEUKIN-18 IN MULTIPLE INJURED PATIENTS

Bernd Roetman, Christian Schinkel, Thomas Frangen, Gert Muhr, Manfred Koeller

Interleukin-18 (IL-18) belongs to the IL-1-family of cytokines and was originally described as interferon (IFN)-gamma-inducing factor. IL-18 is mainly produced by antigen-presenting cells (e.g. macrophages, monocytes, dendritic cells) but is also produced by other cell types. Beside its role in acute inflammation during host defense IL-18 plays an important role in chronic inflammation and autoimmune related diseases.

Thus, it was the purpose of this study to analyze systemic IL-18 in severely injured patients.

During a period of 5 years blood samples were daily collected (between 7 a.m. and 8 a.m.) from 229 multiple injured patients (54 female, 175 male; ISS> 16) admitted to the Surgical ICU of the BG Kliniken Bergmannsheil, University Hospital, Ruhr-University Bochum. All but one patient were admitted from the site of the accident or secondary within the first week after trauma. Quantitation of plasma IL-18 and plasma IL-18-binding protein (IL-18BP) was performed by ELISA (R&D Systems, Wiesbaden, Germany).

Compared to the median value 23 pg IL-18/ml measured in the plasma of normal healthy donors (n=110, 42 male, 68 female) systemic IL-18 was significantly ($p<0.001$) elevated (91 pg /ml) in the overall group of severely injured patients. Additionally, a significant ($p<0.001$) correlation between plasma IL-18 and plasma IL-18-binding protein was calculated (r=0.344). A higher IL-18 median value (205 pg/ml) was detected in patients with a higher Injury Severity Score (ISS $\geq$25, n=166) compared to patients with an ISS <25 (n=63, 57 pg IL-18/ml). Survivors presented a significantly ($p<0.05$) higher IL-18 plasma median value (n=193, median 98 pg/ml) compared to non-survivors (n=36, median 63 pg/ml) which was due to the subgroup the non-survivors among the elderly patient group. Patients older than 60 years had significantly lower plasma levels of IL-18 (median 32 pg/ml) compared to patients who were younger (90 pg/ml). No gender differences in IL-18 plasma values were observed between male or female patients.

These data may improve our knowledge on the regulation of traumatic inflammation and the etiology of the systemic inflammatory response syndrome.

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A 38

PRO-INFLAMMATORY TH1-RESPONSE IN SIRS IS ATTENUATED BY CANNABIS (Δ9-THC) SUPPLEMENTED OPIOID THERAPY INDEPENDENTLY OF THE MU-OPIOID RECEPTOR (ÁOPR) POLYMORPHISM A118G

Ute Bauer, Wolf Dieter Seeling, Weidong Du, Hsin Yun Hsu, Uwe Brueckner, Marion Schneider

Recent findings suggest a crosstalk between the Cannabis and μ-opioid receptors (Rios et al., 2006). The inflammatory response after surgery may be dependent on medication in perioperative pain therapy. We studied changes induced by partial substitution of the μ-opioid induced analgesia by δ^9-THC. While doing this, we considered the μOPR single nucleotide polymorphism (SNP) at position A118G to influence individual patient's opioid response.

Ninety-two patients with prostate carcinoma underwent radical prostatectomy and received the opioid Piritramide. Before and till day 2 post surgery 48 patients received a partial substitution by the cannabinoid δ^9-THC (40mg in total) as placebo-controlled trial. Patients' plasma samples were tested for cytokines and metalloproteinases (MMP) by Luminex Multiplex Assays before (day0) and on the first and second postoperative day (day+1, day+2). DNA was analysed for the μOPR SNP A118G genotype by allele-specific pyrosequencing using a PSQ^{TM} 96 MA (Biotage).

We defined six groups regarding δ^9-THC test (positive/negative) and genotype for μOPR SNP A118G (wildtype/heterozygous/ homozygous mutated genotype). On day+1 post surgery plasma concentrations were found to be significantly higher in patients w/o δ^9-THC medication ($p < 0.05$, Table 1) independently of their μOPR genotype.

By contrast, the levels of anti-inflammatory IL-10 and IL-13 did not differ in δ^9-THC positive and negative patients. No difference in cytokine and MMP distribution could be observed in patients with different genotype for the SNP A118G.

We conclude that perioperative administration of δ^9-THC combined with μ-opioid analgesics attenuated the inflammatory Th1 response, independently of the μOPR SNP A118G genotype. The use of Cannabis may therefore prevent immune dysfunction induced by trauma.

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Table 1

Median (range)	TNF-α (pg/ml)	IL-1β (pg/ml)	Eotaxin (pg/ml)	GM-CSF (pg/ml)	MIP-1α (pg/ml)	MMP1 (ng/ml)	MMP10 (ng/ml)	MMP13 (ng/ml)
δ^9-THC positive, wildtype (n = 37)	5.8 (1.4/33)	3.9 (1.1/27)	490 (178/1768)	15 (3.2/98)	386 (155/1021)	7.2 (1.1/24)	3.3 (0.4/10)	1.3 (0.2/5.5)
δ^9-THC negative, wildtype (n = 37)	9.1 (1.7/53)	6.0 (0.6/38)	628 (128/2417)	20 (3.1/136)	530 (316/1448)	10.6 (1.7/39)	4.4 (1.1/17)	2.2 (0.3/9.2)
δ^9-THC positive, heterozygous (n = 10)	4.8 (2.5/24)	4.1 (0.7/17)	400 (128/1560)	19 (5.5/72)	439 (209/967)	7.2 (1.6/29)	3.4 (1.2/13)	1.0 (0.4/6.3)

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MECHANISMS OF ANTI-INFLAMMATORY ADAPTATION TO HYPOXIA

Sean Colgan, Joseph Khoury, Juan Ibla, Andrew Neish

A major adaptive pathway for hypoxia is hypoxic preconditioning (HPC), a unique phenomenon of endogenous protection that renders cells tolerant to more severe challenges of hypoxia. In recent work, we sought to contribute to what is currently known about mechanisms of HPC. In particular, our work has implicated anti-inflammatory properties of HPC. For these purposes we are currently using protocols of HPC in mice and cultured epithelial cells. As guided by cDNA microarray analysis of lung tissue from mice subjected to hypoxia or HPC, we identified a cluster NF-κB-regulated genes transcriptionally repressed by HPC. Using a NF-κB luciferase reporter assay subsequent studies confirmed a significant suppression of NF-?B activation during HPC ($p<0.01$). HPC-elicited activity was conferrable as a soluble supernatant from HPC-treated cells ($p<0.05$), and on biophysical principles of size, stability and UV spectroscopy, the active fraction was purified and identified as adenosine (Ado). Guided by recent studies demonstrating bacterial inhibition of NF-κB through Cullin-1 (Cul-1) deneddylation, we found a dose dependent dennedylation of Cul-1 by Ado receptor stimulation both in vitro and in vivo. Further, siRNA-mediated repression of CSN5, a subunit of the COP9 signalosome responsible for the deneddylation of Cul-1, partially reversed HPC-mediated inhibition of NF-κB. Cul-1 deneddylation was evident in a murine model of HPC, and was lost in animals lacking the ability to make extracellular Ado (cd73-/-). Taken together these results demonstrate that HPC induces extracellular accumulation of Ado and suppresses NF-κB activity through deneddylation of Cul-1. These results define a new molecular regulatory pathway by which Ado provides potent anti-inflammatory properties.

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A 40

THERMAL INJURY INDUCED LYMPHOCYTE APOPTOSIS - A ROLE FOR A2A RECEPTORS?

Charles C. Caldwell

Objective: Trauma leads to a number of systemic physiological changes associated with alterations in the immune system. These systemic effects include tissue damage, inflammation, lymphocyte apoptosis, and subsequently, immunosuppression. Of the leukocytes that are involved in the immunological response to trauma, the macrophage and the lymphocyte play important roles. It is known that the adenosine A2a receptor (A2aR) is expressed on these cells. The A2aR mediate leukocyte functions by increasing the intracellular concentration of cAMP. It has been reported that increased intracellular cAMP can cause the loss of cell viability. This is considered significant in that it has been recently demonstrated that the adoptive transfer of apoptotic lymphocytes worsens survival during sepsis due to increased immunosuppression. Here, our objective was to determine the molecular mechanisms by which the A2aR mediates lymphoycte apoptosis following thermal injury.

Materials and methods: Adenosine A2a receptor (A2aR) and wild type (WT) mice on a C57BL/6J background were given an 18% total body surface area dorsal scald burn. This method yields a full thickness burn with a mortality of less than 10%. Data: Here, we show that our scald burn results in a 90% depletion of the total number of splenic naive T and follicular B lymphocytes within one day of the trauma. Analysis of TUNEL staining of the spleen 12 hours following the injury show a significant increase in DNA strands with exposed 3′-hydroxyl ends, a hallmark of late stage apoptosis. In this model, there was a significant reduction of lymphocyte depletion in mice either genetically or pharmacologically devoid of the A2aR. This is significant in that we and others have shown that lymphocytes are protective against a subsequent infection following injury. To determine the A2aR-mediated mechanism, we isolated CD4 expressing lymphocytes 8 hours following the scald burn. Analysis of these cells show that there is increased CREB phosphorylation in the wild type as compared to the A2aR deficient CD4 T cells. As activated CREB has been shown to increase p53 mediated cell apoptosis, we injected the p53 inhibitor, Pifithrin-?, following thermal injury and found that CD4 apoptosis was decreased similarly as to mice deficient in the A2aR.

Conclusion: These data reveal a novel mechanism through which interference with CREB or p53 increases cell survival following thermal injury.

Support: SHC Projects 8560 (CCC) and 8904 (CCC)
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A 41

ADENOSINE A2A RECEPTOR INACTIVATION INCREASES SURVIVAL IN POLYMICROBIAL SEPSIS

Gyoergy Haskó

The physiological processes that are responsible for the impaired resistance against bacterial invasion and immune function in sepsis are unclear. Adenosine concentrations are increased during tissue hypoxia and injury that accompany sepsis. A2A adenosine receptors are expressed on immune cells and their stimulation leads to decreased immune functions. Here we addressed the hypothesis that A2A receptor stimulation by increased endogenous adenosine contributes to the dysregulation of immune function in sepsis. A2A receptor KO mice were resistant to the lethal effect of sepsis that was induced by cecal ligation and puncture (CLP). A2A KO mice were more effective in clearing bacteria when compared to WT animals. cDNA microarray analysis and flow cytometry revealed increased MHC II expression in A2A receptor KO mice, indicating improved antigen presentation as a mechanism of protection. Apoptosis was suppressed in spleens of A2A KO mice indicating preserved lymphocyte function. Concentrations of the immunosuppressive cytokines IL-10 and IL-6 were markedly lower following A2A receptor blockade. Similar to observations with A2A receptor KO mice, an A2A receptor antagonist increased survival even when administered in a delayed fashion. These studies demonstrate that A2A receptor blockade may be useful in the therapeutic management of infection and sepsis.

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HSP70 SECRETION BY MACROPHAGES IN RESPONSE TO TRAUMA AND UPTAKE OF BACTERIA

Stuart Calderwood, Salamatu Mambula

Heat shock protein 70 is both an intracellular molecular chaperone and an extracellular signaling molecule. Even though Hsp70 does not possess a leader sequence for secretion, it is nonetheless secreted from a range of cells. Our studies show that Hsp70 is actively secreted by macrophages through a non-canonical pathway involving lysosomal endosomes, which is markedly similar to a pathway previously characterized for IL-1ß secretion. Secretion can be stimulated by a number of agents, most notably febrile heat and exposure to live E coli. When macrophages are expose to E coli, Hsp70 is secreted at high concentrations along with IL-1ß and other cytokines. However, these pathways appeared to be independent, as while secretion of IL-1ß is largely in response to lipopolysaccharides (LPS) in the bacterial cell wall, Hsp70 secretion was not markedly stimulated by LPS. In terms of kinetics, Hsp70 secretion preceded IL-1ß release after infection and although not affected by LPS, Hsp70 secretion was markedly stimulated by the E. coli intracellular protein GroEL. Our experiments therefore indicate that Hsp70 secretion by macrophages is an independent response to infection. As Hsp 70 has marked effects on both inflammation and immunity, the finding of Hsp70 release during infection suggests it may play a significant role in response to invading pathogens.

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HSP70 IS PRESENT IN EXTRACELLULAR VESICLES RICH IN GM1 AND CHOLESTEROL

Antonio De Maio, Monica Rodriguez, Diego Nino, J.C. Diaz, Gabrielle Multhoff, Nelson Arispe

Heat shock proteins (hsp), which are intracellular proteins, play a major role in a variety of cellular processes, including the restoration of homeostasis following stress. Recently, Hsp70, one of the major stress-induced hsp, has been found in the extracellular medium. Moreover, Hsp70 has been observed to activate immune system cells. Thus, circulating Hsp70 has been thought to act as a danger signal that triggers the response to injury. We have previously reported that Hsp70 is capable of interacting with lipid membranes, with high selectivity for phosphatidylserine (PS). The incorporation of Hsp70 within PS-containing membranes was enhanced by the presence of spingolipids (GM1) and cholesterol, which are major components of lipid rafts. Hsp70 was detected in the lipid raft fraction isolated from Triton X-100 solubilized HepG2 cells after heat shock (HS). The presence of Hsp70 in this fraction was reduced by depletion of cellular cholesterol by treatment with β-cyclodextrin. Moreover, Hsp70 was visualized within the plasma membrane of HepG2 cells after HS. Hsp70 was also detected in extracellular vesicles secreted by HepG2 cells after HS. These vesicles were highly enriched in GM1 and cholesterol, but were depleted of other cellular components, such as actin. Hsp70 within these vesicles was associated with the membrane rather than being present in the lumen. We propose that part of the extracellular Hsp70, which may be involved in the activation of immune cells, is present in the membrane of secretory vesicles.

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HSF1 AND THE CONTROL OF APOPTOSIS IN INFLAMMATION AND CANCER

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Mammalian cells have evolved networks of different responses for the detection and control of diverse forms of stress. Among these, the heat shock response (HSR) represents a fundamental protective mechanism utilized by living cells to preserve cellular function and homeostasis under stress conditions, and contributes to establish a cytoprotective state in several human diseases, including inflammation. In cancer cells activation of the HSR has been associated with both anti- and pro-apoptotic responses. HSR induction requires the rapid activation of one or more heat shock factors (HSF) which control the expression of a specific set of genes encoding cytoprotective heat shock proteins. We have shown that cyclopentenone prostanoids and several other bioactive molecules, which activate HSF1 and have potent anti-inflammatory activity, inhibit the induction and function of nuclear factor-kB (NF-kB), indicating a link between the regulatory pathways of these stress-regulated transcription factors. NF-kB, a critical regulator of the inflammatory response, was found to be constitutively activated in several types of malignancies and to suppress cell death pathways by switching on genes that dampen pro-apoptotic signals. We have recently designed and developed a new class of prostanoid mimetics with anticancer activity which are potent HSF1 inducers. The pro-apoptotic activity of these molecules is dependent on the coordinated activation of HSF1 and inhibition of the transcription factor NF-kB. In addition, we have found that different types of HSF1 inducers, including hyperthermic treatment itself, have potent pro-apoptotic activity in different types of chemoresistant B cell malignancies presenting aberrant NF-kB regulation. The results suggest that the block of anti-apoptotic signaling pathways utilizing the IkB kinase IKK is an important factor in modulating HSR pro-apoptotic effects in chemoresistant cancers. The understanding of the molecular mechanisms underlying the interactions between HSF and NF-kB may lead to innovative approaches to therapeutic intervention in inflammation and cancer.

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REGULATION OF PHAGOCYTOSIS BY HEAT SHOCK PROTEINS

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Phagocytosis is a fundamental process necessary for maintaining systemic homeostasis. This process is responsible for the clearance of invading pathogens, necrotic tissue as well as the uptake of apoptotic cells that appear as part of tissue remodeling or stress. The rapid clearance of foreign particles and damaged cells reduces the activation of a secondary inflammatory response, thereby contributing to improved survival. Recently, heat shock proteins (hsp), which are important components of the cellular stress-response, have been found to modulate the immune system. In the present study, we investigated whether hsp could modulate phagocytosis. The heat shock response was induced in macrophages (Mf) by exposure to elevated temperatures (42°C) or by incubation with geldanamycin (GA). Phagocytic capacity was monitored using either bacteria particles or latex beads. Both treatments resulted in an identical elevation of phagocytosis, which required new gene expression. This increase in phagocytosis was not due to an increase in the level of cell surface receptors involved in the recognition of foreign particles. In contrast, these treatments resulted in accelerated internalization of the phagocytic ligand. This data suggests that hsp may participate in phagosome formation and/or maturation (trafficking). We speculate that this increase in phagocytosis is important in the rapid clearance of cell debris and apoptotic cells after injury. This faster phagocytic response decreases cell damage propagation and controls the inflammatory response. It also reduces post-injury infections, which is a common cause of secondary complications and mortality in trauma patients.

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ROLE OF HMGB1 IN APOPTOSIS-MEDIATED SEPSIS LETHALITY

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It has been suggested that apoptosis contributes to the pathogenesis of severe sepsis, because administration of caspase inhibitors to mice prevents apoptosis and improves survival from experimental sepsis induced by cecal ligation and puncture (CLP, Hotchkiss et al, Nature Immunol, 2000). However, the relationship between apoptosis and HMGB1, a critical mediator in lethal sepsis, is unknown (Wang et al, Science, 1999, Yang et al, PNAS, 2004). Accordingly, here we examined the effects of caspase inhibitors on HMGB1 release. In murine macrophage-like RAW 264.7 cells, Z-VAD-FMK, a broad-spectrum caspase inhibitor, dose-dependently attenuated LPS-induced HMGB1 release by decreasing NF-κB-dependent translocation of HMGB1 from the nucleus to cytoplasm (Xin et al, JEM, 2006). In animal studies, BALB/c mice underwent CLP and received treatment with either Z-VAD-FMK or control peptide (0.5 mg/mouse) injected intraperitoneally at 90 minutes after CLP surgery, and they were euthanized at 24 hours after CLP. Besides reducing sepsis-induced apoptosis in the spleen and thymus in CLP mice (data not shown), treatment with Z-VAD-FMK significantly reduced sepsis-induced serum HMGB1 levels compared to control peptide treated animals (control CLP group $=160 \pm 20$ vs. Z-

VAD-FMK group = 38 ± 7 ng/ml. N=7-11 mice per group, P<0.05). Monoclonal antibodies against HMGB1 conferred significant protection against organ damage, but did not prevent accumulation of apoptotic cells in spleen. Thus, our data indicate that caspase inhibitor Z-VAD-FMK may exert protection against lethal sepsis by inhibiting cell apoptosis and by reducing HMGB1 release. Although it had been generally accepted that clearance of apoptotic cells is via a non-inflammatory pathway, it now appears that accumulation of apoptotic cells may provoke other cells to release HMGB1, which in turn mediates severe organ damage. (Supported in part by grants from the North Shore-Long Island Jewish Health System, GCRC, M01RR018535, and from NIH, NIGMS; to KJT).

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ROLE OF HMGB1 IN AUTOIMMUNE AND INFLAMMATORY DISEASES

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High Mobility Group Box (HMGB) proteins are highly abundant chromatin binding proteins located primarily in the cell nucleus where they bind to the minor groove of Their principal function is to bend or distort the double helix and regulate a number of transcriptional events. More recently HMGB1 has been shown to be released from cells via two distinct mechanisms, being either liberated from cells undergoing necrosis, but not apoptosis or actively secreted from cells following inflammatory cytokine stimulation. The function of HMGB1 in autoimmune and inflammatory disorders however remains to be fully eludicated. However, in addition to binding to nuclear DNA, HMGB1 also binds to A class CpG, but not B class -containing ODNs and HMGB1 functionally interact and this complex activates pDCs and augments IFN-a production through a MyD88/TLR9 and RAGE dependent mechanism raising the possibility that this protein maybe involved in autoimmune diseases such as lupus that are driven by type I IFNs. Moreover, HMGB1 is present in DNA-containing immune complexes and is a key factor in immune complex-triggered activation of autoreactive B cells and the induction of type I interferon genes after stimulation with DNA immune complexes. The ability of HMGB1 to induce IFNs is dependent on TLR9, MyD88 as well as requires the immunoglobulin superfamily member RAGE. While the precise mechanisms underlying this effect remain to be determined, HMGB1/DNA complexes result in RAGE internalization and localization of RAGE in early endosomes. The importance of this mechanism in autoimmune disease is suggested by the demonstration that moncloncal antibodies to HMGB1 can delay the onset of proteinuria in experimental models of autoimmune predisposed animals.

HMGB1 also plays an important role in the pathogenesis of rheumatoid arthritis. Monoclonal antibodies to HMGB1 inhibit disease inflammation and joint erosion and are as effective as mAbs to TNF-a in models of collagen induced arthritis and adjuvant induced arthritis.

These data suggest that under circumstances where cells are undergoing necrosis the liberation of HMGB1 may play an important role in systemic autoimmune disease such as lupus as well as organ specific inflammatin such as that which occurs such rheumatoid arthritis.

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CAN CYTOKINES PREDICT MOF IN CRITICALLY ILL TRAUMA PATIENTS?

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Both trauma and infection elicit the systemic inflammatory response. Once triggered, both pro-inflammatory and anti-inflammatory mediators are released in an attempt to eliminate the infectious process or to initiate repair and healing. If successful, inflammation subsides and normal bodily functions return. However, if inflammation is allowed to proceed unabated, progressive and sequential organ dysfunction ensues, culminating in multiple organ failure (MOF) and ultimately death in many. In fact, MOF remains the leading cause of death in the ICU for critically injured trauma patients or patients in septic shock. Consequently, it is crucial to identify the various elements or mediators that might predict the development of MOF such that novel or alternative treatment strategies can be devised. Historically, injury severity scores, base deficit in the emergency room, units of blood received in the first 6 hours, and admission coagulopathy have been used to predict MOF, although the complexity and diversity of shock renders prediction difficult. Recently, mathematical modeling has been used in animals to address the inherent biologic complexity from endotoxic and hemorrhagic shock. Interestingly, changes in cytokine levels were found to exhibit significant variability at points of maximal elevation, but the timing of the peaks was quite predictable. Whether similar changes in serum cytokines occur in critically injured trauma patients over time remains to fully elucidated. This work represents a prospective observational study at an urban level 1 trauma center. Serum cytokine levels were determined at multiple time points in patients sustaining major torso trauma (excluding severe head injuries) who met criteria for standardized shock resuscitation (defined as patients with systolic blood pressure <90 mm Hg and/or and base deficit >6 mEq/ml in whom blood transfusion was required). Controls were similarly aged, healthy volunteers. Outcome measures studied included MOF and mortality. Fifty-one trauma patients were studied with a median age of 33 years. Seventy-five percent were male and 82 % sustained blunt trauma. The median injury severity score was 25. Sixteen percent of the patients developed MOF, 18% died, and 24% developed MOF and/or died. Serum cytokines, eotaxin, interferon gamma, interleukin 1

receptor antagonist, IL6, IL8, IL10, G-CSF, and GM-CSF, displayed dramatic differences in patients who developed MOF when compared to those who did not. While this study lacked the statically power to correlate changes in serum cytokines to the development of MOF, the data suggests that serum cytokines may be used as predictors to assist in the identification of patients at risk for the development MOF.

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OPPOSITE ROLES OF HYPOXIA-ADENOSINERGIC IMMUNE REGULATION IN ACUTE VS CHRONIC INFLAMMATION AND SIRS VS CARS

Michail Sitkovsky

The genetic evidence of the critical role of the local tissue hypoxia-associated accumulation of extracellular adenosine and stabilization of HIF-1a in down-regulation of immune response and in tissue protection also offered new insights into mechanisms of trauma, shock and sepsis. It has also attracted attention to A2 adenosine receptor (A2R)-mediated and Hypoxia Inducible Factors (HIF) - mediated pathways as possible drug targets in engineering inflammation depending on the stage of the inflammatory disease. We will discuss both beneficial and detrimental roles of collaborating A2R and HIF-1 in sepsis (SIRS vs CARS), in acute vs chronic inflammation and in misguided protection of cancerous tissues from anti-tumor T cells.

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ROLE OF GENETIC UP- AND DOWN-REGULATION

Steve Calvano

Systemic inflammation is a hallmark of major injury and contributes importantly to organ dysfunction and sepsis. A useful model of systemic inflammation that avoids the confounding variables of physical injury and other co-morbidities found in hospitalized patients is that of endotoxin challenge to otherwise healthy human volunteers. Using such a model, four endotoxin-challenged and four placebo challenged volunteers were studied. Peripheral blood leukocytes were obtained at six time points after endotoxin or saline administration and were evaluated for RNA abundance using Affymetrix gene chips. 5,093 probe sets were identified as significantly modulated by endotoxin administration (FDR $< 0.1\%$). Using a structured network knowledge-base approach, the genome-wide transcriptional response revealed that the human blood leukocyte response to an acute systemic inflammation includes the transient dysregulation of leukocyte bioenergetics and modulation of its translational machinery. In a subsequent study, six additional volunteers were studied, and purified neutrophils, monocytes and T-lymphocytes were evaluated for genome-wide transcriptional changes at 2 and 6 hours after endotoxin administration. As was seen in the previous study of blood leukocytes, transcripts for mitochondrial respiratory transport chain components were decreased in monocytes and neutrophils, but not T-lymphocytes. The findings provide new insight into the regulation of global leukocyte and leukocyte subset activities and will be discussed as they might relate to organ dysfunction.

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LYMPHOCYTE-DIRECTED AUGMENTATION OF CANONICAL NF-KB ACTIVATION REDUCES THYMOCYTE APOPTOSIS AND IMPROVES T CELL NUMBERS AND OUTCOME IN MURINE SEPSIS

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Objective: Sepsis induces extensive lymphocyte apoptosis that contributes to immunosuppression and mortality. Interestingly, prevention of lymphocyte apoptosis improves survival of septic mice. Moreover, activation of the canonical NF-kB pathway, however, prevents TNFa-induced lymphocyte apoptosis. Thus, due to cytokine overproduction TNFα-induced anti-apoptotic NF-κB signalling may play a decisive role in the context of lymphocyte apoptosis during sepsis. In this study the function of canonical NF-kB activation in T cells was studied in the context of murine sepsis.

Material and Methods: Sepsis was induced in female C57BL/6J-mice by cecal ligation and puncture [20G] (CLP). Control mice underwent laparatomy and manipulation of the cecum (Sham). At different time points up to 48hrs NF-kB activity (Electrophoretic Mobility Shift Assay) was determined in protein extracts of thymus and spleen. Thymocyte apoptosis was evaluated via TUNEL-Assay and flow cytometric detection of permeabilized PI-hypochromic cells. Cells of lymphoid organs were analyzed by flow cytometry of surface antigens. Mortality rates were determined up to 6 days. Ikbα - deficient cells demonstrate increased activation of NF-kB upon stimulation. To further analyze the effects of enhanced NF-kB activation in lymphocytes, we adoptively transferred Ikbα-/- - or wild-type (wt) fetal liver stem cells into sublethally irradiated lymphopenic Rag1-/– host mice (radiation chimeras, RCs) and did the same investigations subsequent to lymphopoetic reconstitution.

Data: Thymocyte apoptosis was more pronounced following CLP surgery compared to sham mice. In parallel, NF-κB DNA binding activity was decreased at

late time points only in septic thymocytes. Upon sepsis induction, IκBα-deficient RCs demonstrate increased NF-κB activation in thymocytes throughout the observation period compared to wt-reconstituted host thymi. Also, apoptotic rates of IκBα-/- - thymocytes were clearly lower compared to wt thymocytes. Interestingly, peripheral T cell numbers and numbers of CD69-positive, activated T cells are increased in septic IκBα-/–host mice compared to wt controls. Moreover, survival was improved in septic IκBα-deficient RCs (40%) compared to wt RCs (0%).

Conclusion: The most intriguing result of this study is the thymocyte NF-κB downregulation in the course of sepsis. This stands in clear contrast to its well known proinflammatory key role. However, the anti-apoptotic function is another hallmark of NF-kB signaling. Thus, our data clearly show that enhanced NF-kB activation in lymphocytes improves lymphopoesis and is likely to improve immunosuppression during sepsis. These data provide evidence for a new approach in sepsis therapy.

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SEPSIS REPROGRAMS BONE MARROW HEMATOPOESIS TOWARD THE MYELOID LINAGE IN A MYD88 AND TRIF DEPENDENT MANNER

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Objective: Polymicrobial sepsis is thought to induce bone marrow suppression evidenced by anemia, leukopenia, and thrombocytopenia. However, little is known about the cellular fluctuations that occur in the bone marrow during sepsis with the current knowledge being inferred from peripheral blood smears and leukocyte counts. In this study we determined the effects of sepsis on bone marrow cells at various time points before, during, and after sepsis.

Material and Methods: Female, 6-12 wk old $TRIF^{-/-}$, $MyD88^{-/-}$ and B6.129 mice underwent cecal ligation and puncture (CLP) (LD10) or sham procedure (n = 5 each group). At various times after treatment, total bone marrow cells were isolated and stained for stem cell (Lin, c-kit, sca-1,), myeloid (GR-1 and CD11b), erythroid (TER 119), and lymphoid (B220) markers and analyzed by flow cytometry.

Data: The hematopoetic stem cell population ($Lineage^{-}c\text{-}Kit^{+}Sca\text{-}1^{+}$) was increased over 6-fold on day 1 and remained increased 2-fold on day 7 in the wild type CLP group as compared to the sham group ($p<0.05$), suggesting that the bone marrow is not suppressed in sepsis. The percentage of total myeloid ($GR\text{-}1^{+}CD11b^{+}$) and erythroid cells as well as $B220^{low}AA4.1^{+}CD19^{+}$ pre-B cells were all decreased 24 hours after CLP ($p<0.05$) with the remaining bone marrow cells in the sepsis group staining for $B220^{high}AA4.1^{-}$ more mature B cells or $GR\text{-}1^{+}CD11b^{+}F480^{-}$ immature myeloid cells ($p<0.05$). While the percentages of erythroid and lymphoid cells decreased and remained low 7 days after sepsis, the $GR\text{-}1^{+}CD11b^{+}$ cells increased to almost 90% of the total bone marrow population beginning at 3 days after CLP compared to sham treatment ($p<0.05$) with a concomitant decrease in all cells including $B220^{high}AA4.1^{-}$ B cells. However, when the bone marrow cells from $TRIF^{-/-}$ and $MyD88^{-/-}$ mice were analyzed, the sepsis induced decreased in $B220^{low}AA4.1^{+}$ B cell precursors observed in the wild type animals was attenuated on day 1 and day 7 indicating that both the TRIF and MyD88 signaling pathways are instrumental in bone marrow B cell precursor production after sepsis.

Conclusion: The results of this study indicate that, rather than suppressing the bone marrow, sepsis reprograms the bone marrow towards the production of cells of the myeloid linage at the expense of the erythroid and lymphoid lineages. This bone marrow reprogramming is at least partially dependent on the TRIF and MyD88 cell signaling pathways.

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CELLULAR REPROGRAMMING BY BACTERIAL LIPOPROTEIN IS INDEPENDANT OF ST2

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Members of the Interleukin-1 Receptor superfamily signal inflammation via activation of NF-κB, leading to increased transcription of pro-inflammatory genes. One member of the IL1-R superfamily, ST2, does not activate NF-κB, and has been implicated in the negative regulation of TLR signaling, and in the development of endotoxin tolerance.

Our study sought to elucidate the role of ST2 in cellular reprogramming by bacterial lipoprotein. Peritoneal macrophages from ST2 deficient (Il1rl1-/-) mice produce significantly greater TNFα and IL-6 compared to wild type macrophages in a dose dependant fashion following stimulation with a variety of TLR ligands. Ex-vivo induction of BLP tolerance results in significant attenuation of cytokine production from both WT and ST2 deficient mice ($p=0.002$). Age and weight matched wild type and ST2 deficient mice were given 10mg/kg BLP or equivalent dose of PBS by intraperitoneal injection 24 hrs before challenge with BLP 35mg/kg. BLP tolerance conferred a significant survival advantage on both WT ($p=0.0001$) and ST2 deficient mice ($p=0.0014$) rechallenged with BLP. Serum cytokine analysis revealed significant attenuation in TNFα ($p=0.012$) and IL-6 (0.036) production in tolerised compared to naïve WT and ST2 deficient mice. In a caecal ligation and puncture model of polymicrobial sepsis, bacterial clearance from blood and solid organs was significantly inpaired in ST2 deficient mice ($p=0.021$). BLP tolerance rescued these animals from lethality, with increased survival, decreased

serum cytokine production and enhanced bacterial clearance ($p<0.05$).

We have demonstrated that cellular reprogramming by BLP can be induced in ST2 deficient mice, thus concluding that cellular reprogramming by BLP is independent of ST2.

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ACTIVATION OF LXR IN KUPFFER CELLS INHIBITS P38 MAPK PHOSPHORYLATION AND TNFA PRODUCTION AT THE POST-TRANSCRIPTONAL LEVEL

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Objective: The transcription factor Liver X receptor (LXR) of the nuclear receptor family exists in two isoforms, LXRα and LXRβ, which are both expressed in liver Kupffer cells. Treatment of cultured Kupffer cells with LXR agonist (GW3965) leads to attenuation of lipopolysaccharide-mediated TNFα production by 50% at peak levels (6 hours), and agonist treatment of endotoxemic rats leads to a similar 50% reduction of plasma TNFα and protection from liver injury (Wang YY, Shock, 2006). The specific role of LXRα was established when Kupffer cells isolated from mice deficient in LXRα was found to produce elevated levels of TNFα when treated with LPS, compared to wild type and LXRβ-deficient Kupffer cells (unpublished results). In this study, we aimed to elucidate the mechanisms behind the LXR-mediated attenuation of TNFα.

Methods: Primary cultures of Kupffer cells isolated from male Sprague Dawley rats were treated by LXR agonist GW3965 (1 μM) for 30 min, followed by LPS stimulation (10 μg/ml). Cells were harvested for p38 MAPK activation analyses (15 min), TNFα mRNA analyses (2 hours) or TNFα protein analyses (6 hours). In some experiments cell nuclei were isolated for preparation of nuclear and cytosolic RNA and protein. Levels of TNFα mRNA were analysed by real-time RT-PCR, and TNFα protein levels were assessed by ELISA. Levels of p38 MAPK phosphorylation were assessed by immunofluorescence and western blotting.

Results: A 50 % reduction of both secreted and intracellular TNFα was observed in Kupffer cells pretreated with GW3965 before LPS stimulation. However, no reduction in peak mRNA levels were observed, either in cytosolic or nuclear cell fractions. Surprisingly, we observed that pretreatment of cells with GW3965 reduced p38 MAPK phosphorylation, reflecting p38 MAPK activation level.

Conclusion: TNFα appear to be regulated by LXR activation at the post-transcriptional level in Kupffer cells. In addition, we found that LXR activation inhibits p38 MAPK activation. A role of p38 MAPK in the regulation of TNFα translational initiation was recently demonstrated, and may serve as a possible explanation for our observations.

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ARNT2 IS A REGULATOR OF A TNF-ALPHA-DEPENDENT, ANTI-INFLAMMATORY FEED-BACK MECHANISM IN THE RESPONSE OF MACROPHAGES TO LPS

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Objective: Recognition of lipopolysaccharide (LPS), a major constituent of Gram-negative bacteria, by monocytes and macrophages initiates a rapid release of inflammatory mediators resulting in vascular activation and recruitment of immune cells. In contrast to immediate reactions a reliable analysis of the late gene expression program in monocytes elicited by LPS is not yet done.

Material and Methods: We used oligonucleotide microarrays covering more than 13,000 genes (Affymetrix) to define the delayed response of macrophages to LPS. Signalling cascades were inhibitied by specific pharmacologic inhibitors. Microarray data were confirmed by independent assays (RT-PCR, FACS, ELISA, siRNA, ChIP).

Data: Our analysis of the delayed LPS-triggered response in monocytes after 16 hours demonstrated a TNF-dependent up-regulation of anti-inflammatory rather than pro-inflammatory molecules at this time point, which was functionally confirmed by medium transfer experiments and by specific blockade of TNF. Systems biology approaches of involved regulatory pathways revealed a major importance of p38 MAP kinase and contribution of bHLH-PAS transcription factor ARNT2 (aryl-hydrocarbon receptor nuclear translocator 2) which was confirmed by chromatin immuno-precipitation and transfection of ARNT2-specific siRNA.

Conclusions: Our data demonstrate for the first time an autocrine delayed induction of anti-inflammatory molecules in monocytes via TNF. Downstream activation of transcription factor ARNT2 is a fundamental aspect in the LPS-induced late expression pattern. Our results point to negative feedback mechanisms of inflammatory responses protecting against excessive tissue damage. These regulatory mechanisms may at least partly explain the lack of efficiency of anti-TNF treatment strategies in sepsis and opens door for development of novel therapeutic interventions in inflammatory diseases.

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HEAT SHOCK PROTEIN 70 IS AN ENDOGENOUS MODULATOR OF THE INNATE IMMUNE RESPONSE

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Mammalian responses to bacterial products can lead to an uncontrolled inflammatory response that can be deadly for the host. It has been shown that the innate immune system employs at least three cell surface receptors, TLR4, CD14 and MD2, in order to recognise bacterial products. We have previously shown that heat shock proteins (Hsps) are also involved in the innate immune recognition. Hsps are a family of highly conserved proteins that act as molecular chaperones and assist in proper folding, assembly and intracellular trafficking of proteins. How hsps reach the cell surface and how they are involved in the innate immune response still remain unclear. In the present study we investigated their association with the TLR4/CD14/MD2 complex in response to bacterial products and provide evidence that the hsp70 and hsp90 associate with TLR4 in response to stimulation by bacterial products. These associations seem to take place within lipid rafts. The addition of exogenous recombinant hsp70 to cells in vitro results in a dose-responsive inhibition of the inflammatory signal cascade, including NF-kB activation, phosphorylation of mitogen-activated protein kinase (MAPK) proteins, and cytokine production in response to different microbial stimuli. We are currently beginning to explore their administration in vivo in order to limit the clinical signs and symptoms of inflammatory conditions. Our studies reveal that hsps may play an important role as endogenous regulators of the innate immune response.

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EXPRESSION OF ADENYLYL CYCLASE MRNA IN CLP SEPSIS

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Objective: During an infection, pathogen-associated molecules may trigger the expression of proinflammatory cytokines in macrophages. When dysregulated, the proinflammatory response may lead to systemic inflammation and sepsis that carries high mortality. Elevation of intracellular cyclic AMP, produced by ten isoforms of adenylyl cyclases (AC1-10), reduces the expression of proinflammatory cytokines in Kupffer cells from liver and other macrophages, and may thereby control inflammation. It has been demonstrated that elevation of cAMP by inhibiting phosphodiesterases protects against liver injury in endotoxemia and limits the systemic inflammatory response. Low expression of AC may lead to decreased cAMP production and loss of protection. We have recently shown that the mRNA expression of AC5, AC6, AC7 and AC9 were significantly attenuated in endotoxemia in the liver, kidney and spleen, and in cultured macrophages treated with lipopolysaccharide. Since endotoxemia is only part of the septic response, we explored AC regulation in a rat model of experimental sepsis.

Methods: A model of cecal ligation and puncture (CLP) was used. Anaesthetized male Wistar rats were subjected to a mid-line laparatomy, followed by cecal ligation and double puncture. Sham operated animals received laparatomy, but no cecal interference. Rats develop a severe peritonitis during the following 24 hours. In separate sets of experiments, rats were sacrificed at 10, 18, and 24 hours following operation. RNA was isolated from liver, kidney and spleen, and gene expression of AC5, AC6 and AC7 were examined by real-time RT-PCR.

Results: At 18/24 hours after CLP, AC5 mRNA was significantly suppressed in liver and kidney, compared with levels in sham animals. No difference was observed in the spleen, however. Levels of AC6 were significantly reduced at all time points in the spleen, and also appeared to be reduced at all time points in liver, but not significantly. In contrast to the data obtained in the rat endotoxemia model, the expression of AC7 mRNA was not attenuated. Elevation of AC7 mRNA was observed at 10 h after CLP in liver and kidney, but not at later time-points.

Conclusion: Our results demonstrate that gene regulation of AC5 and AC6 is altered during the course of experimental sepsis, in line with the observations from the endotoxin model. This may suggest a role for these adenylyl cyclases in the course of the septic response.

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INHIBITION OF C-JUN N-TERMINAL KINASE AFTER HEMORRHAGE BUT BEFORE RESUSCITATION REDUCES LIVER DAMAGE, HEPATIC AND SYSTEMIC INFLAMMATION IN RATS

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Objective: Hemorrhagic shock and resuscitation (H/R) induces an inflammatory response that is associated with hepatocellular damage. Inhibition of the mitogen activated protein kinase (MAPK) c-JUN NH2-terminal kinase (JNK) using a protease resistant peptide (D-JNKI-1) before hemorrhage blunted liver damage and proinflammatory responses. Here, we studied the effects of JNK inhibition after hemorrhage but before resuscitation. Material and Methods: Male Sprague Dawley rats were hemorrhaged to a blood pressure of 32-37±5 mmHg for 1 h. Before resuscitation (shed blood and twice the shed blood volume as lactated Ringers solution), rats received either D-JNKI-1 (11mg/kg i.p.) or vehicle. 2 h

after resuscitation, serum alanine aminotransferase (ALT), hepatic necrosis and hepatic polymorphnuclear leucocytes (PMN) infiltration (chloroacetat-esterase stain) were evaluated. In addition, IL-6 levels were measured in serum and liver tissue (ELISA). A $p<0.05$ was considered significant (ANOVA).

Data: After H/R, ALT levels rose to 1766±202 IU/L (mean±S.E.M.) in the vehicle treated group as opposed to 657±113 IU/L after D-JNKI-1 treatment. Liver necrosis was also largely diminished after JNK inhibition as compared to vehicle treatment. Hepatic IL-6 level increased to 2212±635 pg/ml in the vehicle treated group and was blunted by 80% after JNK inhibition. Hepatic PMN infiltration strongly increased after H/R to 12.5±1.9 (positive cells/high power field) in the vehicle treated group but was largely diminished after D-JNKI-1 treatment (2.2±1.5, $p<0.05$). JNK inhibition reduced systemic Il-6 levels compared to vehicle treatment (2721±426 vs. 1338±579 pg/ml, $p<0.05$).

Conclusion: JNK inhibition after accomplished hemorrhage but before resuscitation blunts hepatic damage as well as systemic and hepatic inflammation. Therefore, JNK contributes to resuscitation induced hepatic damage and proinflammatory changes. This accentuates JNK as promising therapeutic target even after hemorrhage but before resuscitation. Supported in part by DFG MA 1119/3-3

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HEME OXYGENASE-2 IS NECESSARY FOR HYPOXIC SIGNALING IN HEPATOCYTES

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Objective: The injury from hemorrhagic shock is multifactorial and includes injury from tissue hypoxia. Hypoxia results in acute signaling changes and the upregulation of a subset of genes regulated by the hypoxia inducible factor. We have previously shown that hepatocyte hypoxia or experimental hemorrhagic shock results in the phosphorylation of c-Jun N-terminal Kinase (JNK). Several genes have been implicated in the sensing of hypoxia including heme oxygenase-2 (HO-2). The purpose of these investigations was to test the hypothesis that HO-2 acts as a hepatic sensor for hypoxia leading to adaptive signaling via JNK and HIF1-a stabilization.

Methods: Primary mouse hepatocytes were harvested and cultured from male C57/BL6 mice. Hypoxia was performed by exposing hepatocytes to 1% oxygen, 94% nitrogen, and 5% CO_2. Heme oxygenase activity was inhibited by tin-protoporphyrin (SnPP) and HO-2 was 'knocked down' by siRNA transfection. JNK phosphorylation and HIF1-a protein levels were determined by Western blotting.

Results: Hypoxia results in hepatocyte JNK phosphorylation. Hypoxia-induced JNK phosphorylation was inhibited by SnPP, which functions as a non-specific inhibitor of HO activity. siRNA transfection efficiently decreased protein levels of HO-2 and inhibited hypoxia-induced JNK phosphorylation. Furthermore, hypoxia increased protein levels of HIF1-a and SnPP or HO-2 siRNA abrogated this effect. Additionally, HO-2 siRNA reversed the hypoxia-induced upregulation of the HIF-regulated protein VEGF.

Conclusions: HO-2 is involved in the hypoxia-induced phosphorylation of JNK and stabilization of HIF1-a. HO-2 appears to be an important protein involved in hypoxic signaling and further investigation is needed to determine further mechanism(s) of signaling. These investigations may lead to the development of therapeutics to protect against injury from hemorrhage and hypoxia.

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LIPIDS RAFTS AN INITIATION OF INNATE IMMUNE CELL SIGNALING

Joseph Cuschieri

Lipid rafts are sphingolipid rich membrane microdomains that serve as a platform for TLR4 receptor complex formation following LPS exposure within the macrophage. The mechanism responsible for this receptor formation remains complex and poorly investigated. Recently, we have demonstrated that this complex is composed of several different receptor proteins including CD11/CD18, CD55, HSP70 and HSP90 in addition to TLR4, CD14 and MD2. Based on our recent studies, it appears that initial formation of this complex on lipid rafts requires initial binding of LPS to CD14 on lipid rafts. Once bound, activation of a membrane bound phospholipid flippase occurs resulting in the internalization of the external phospholipid phosphatidylcholine, and the externalization of the internal phospholipid phosphatidylserine. Internalized phosphatidylcholine, in turn, serves as a substrate for phosphatidylcholine-phospholipase C resulting in the generation of diacylglycerol (DAG). The generation of DAG results in subsequent downstream activation of acid sphingomyelinase. Acid sphingomyelinase activation results in the degradation of lipid raft sphingolipids to ceramide which in turn fuses within the raft resulting in a gel phase fluidity allowing protein migration. This protein migration into and out of the raft is responsible for TLR4 complex formation within mononuclear cells. As a result, LPS on CD14 is presented to TLR4 allowing its conformational change, and subsequent downstream signaling and activation. Although this process is rapid, alterations in a number of different components have been demonstrated by us to regulate subsequent activation. Additionally, it appears that these alterations may be, in part, responsible for conditions such as tolerance and excessive inflammation characteristic of dysregulated immunity. Although further work is

obviously needed, these novel insights provide further insight into the receptor regulation of LPS-mediated activation within mononuclear cells and provide targets for future therapies in the regulation of sepsis and organ dysfunction.

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A 61

A ROLE FOR TYPE I INTERFERONS IN ENDOTOXEMIA AND SEPSIS?

Claude Libert

Endotoxemia is mediated by a complex host response which is triggered by activation of the TLR4 pathway by LPS. Numerous genes are induced by means of activated transcription factors, which are the result of several signaling cascades. We have described that an inbred strain of mice, SPRET/Ei, which is derived from Mus spretus, exhibits an extreme and dominant resistance to LPS, when compared to other mouse strains such as C57BL6. Although normal MyD88-dependent activation of NFkB was observed, a greatly reduced induction of IFNb was observed in SPRET/Ei-derived macrophages. This leads to reduced IFN-dependent phenomena, such as STAT-1 activation and nuclear inport, as well as IRF-7 induction. Also in vivo, less IFN was induced in SPRET/Ei, whether they were challenged with LPS or with virus. Furthermore, SPRET/Ei mice exhibited typical phenotypes associated with reduced IFNb, such as resistance to Listeria monocytogenes and sensitivity to Leishmania infection. The important role of type I IFNs in endotoxemia was strengthened by the finding that IFNAR1-deficient mice indeed resisted, to some extend, endotoxemia. The mechanism of reduced IFNb induction is unclear, but the data indicate that type I IFNs may be considered as important drug targets in endoxemia and perhaps in sepsis too. Further research is needed to evaluate inhibition of type I IFNs as a new therapeutic intervention in endotoxemia ans/or sepsis.

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GI PROTEIN AND ß-ARRESTIN CONTROL OF INFLAMMATORY CELL SIGNALING

James Cook, Hongkuan Fan, David Williams, Basilia Zingarelli

Heterotrimeric guanine nucleotide binding regulatory proteins of the G inhibitory class (G_i) are involved in signaling to microbial stimuli. The availability of Gαi deficient mice has provided *invivo* and *invitro* approaches to examine the role of G_i proteins as modulators of Toll-like Receptor (TLR) signaling. $G\alpha_{i2}$ (-/-) mice challenged *invivo* with LPS exhibited significantly augmented inflammatory responses as measured by increased plasma cytokines and lung and liver leukosequestration relative to wild type (WT) mice. The latter findings suggest a predominant pro-inflammatory phenotype in the $G\alpha_{i2}$ knockout mice to LPS challenge. This pro-inflammatory phenotype paralleled the *in vitro* response of splenocytes from $G\alpha_{i2}$ (-/-) mice. LPS or *Staphylococcus aureus* (SA) induced production of TNFα, IL-6, IFNγ, IL-12, IL-17, GM-CSF, MIP-1α, MCP-1, MIG and IP-10 were significantly increased (1.2 to 33 fold, $p<0.05$) in splenocytes harvested from $G\alpha_{i2}$(-/-) mice compared to WT mice. Analysis of splenic macrophage ,T cells ,B cells, and dentritic cells demonstrate that differences in splenic cellular composition do not account for the disparate cytokine and chemokine responses .Collectively the data demonsrate that $G\alpha_{i2}$ plays an inhibitory role in TLR signaling.

G_i protein coupled receptor signaling is regulated by β-arrestins. Therefore in subsequent studies we investigated if β-arrestins modulate TLR signaling. β-arrestin 1 and 2 are ubiquitously expressed proteins that alter signaling by G protein coupled receptors by sterically inhibiting coupling of the receptor G protein interaction. β-arrestins also function as adaptor proteins that target G protein coupled receptors for endocytosis , and as signaling scaffolds connecting G protein coupled receptors to an ever-growing list of signaling pathways. Our recent data demonstrate that β-arrestin 1 and 2 depletion with siRNA techniques significantly alters LPS activation of MAP kinase and NFkB, and pro-inflammatory gene expression. Additionally we demonstrated that mouse embryonic fibroblast deficient in β-arrestin 2 exhibited impaired LPS induced cytokine production and reconstitution of β-arrestin 2 gene expression restored LPS activation . These composite studies demonstrate an important role of G_i proteins and β-arrestins as novel modulators of inflammatory cell signaling. Supported by NIH GM 27673.

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MAPK AND THE REGULATION OF IMMUNE RESPONSE TO INJURY

Mashkoor Choudhry, Xiaoling Li, Martin Schwacha, Irshad Chaudry

Mitogen activated protein kinases (MAPK) is a network of signaling cascade that influences cell growth, differentiation and apoptosis. While most of the proteins phosphorylate either on tyrosine, threonine or serine; MAPK have been reported to phosphorylate on two residues tyrosine and threonine and thus are referred as dual phosphorylated proteins. They are also known to play the central role in the regulation of inflammatory responses associated with injury conditions. There are 3 major MAPK-dependent pathways, extracellular-regulated protein kinase (ERK), c-Jun NH2-terminal kinase (JNK), and the p-38. ERK is 42 and 44 kD and is referred to as ERK-1 and -2 respectively. Similarly, JNK is a complex of 46 and 54 kD proteins. Studies have shown that the activation of these pathways is altered in cells of

the immune system following burn, trauma and sepsis. While such alterations in MAPK may cause inappropriate immune cell effector responses under those conditions; the mechanism responsible for altered MAPK activation following burn, trauma and sepsis remains unknown. We investigated the role of MAPK in altered intestinal T cell function following a combined insult of alcohol and burn injury. The reason for this combined insult is that alcohol exposure at the time of injury is found to be a significant factor in post burn complications. To perform the study, rats were gavaged with alcohol to achieve a blood alcohol level in the range of ~100 mg/dL at the time burn injury. Animals were sacrificed and mesenteric lymph node T cells were harvested, stimulated with anti-CD3 antibodies and lysed. Western blot analyses of T cell lysates prepared on day 1 and 2 after injury showed a significant decrease in T cell p-38 and ERK-1/2 phosphorylation following a combined insult of alcohol and burn injury compared to T cells from rats receiving either alcohol intoxication or burn injury alone. Since the decrease in p-38 and ERK-1/2 could result from an inappropriate activation of protein phosphatases (PP), we further investigated the role of serine/threonine specific PP1, PP2A and MAPK phosphatase-1 (MKP1) in altered p-38 and ERK-1/2 activation. The results from this study suggest that treatment of T cells with inhibitors of PP1/PP2A [calyculin A (CA) and okadaic acid (OA)] prevented the suppression in p-38 and ERK-1/2 activation. In addition, alcohol plus burn-mediated decrease in T cell effector responses as determined by IL-2 and IFN-γ production was also prevented in T cells cultured in presence of CA and OA. MKP-1 inhibitor triptolide did not prevent the suppression in T cells p-38/ERK-1/2 and cytokine production following a combined insult of alcohol and burn injury. Furthermore, the finding of an increase in PP1 activity in T cells following a combined insult of alcohol intoxication and burn injury suggests that activation of PP1 is likely to play a predominant role in T cell p-38 and ERK-1/2 suppression following a combined insult of alcohol intoxication and burn injury. These findings together with reported by others suggest that alterations in MAPK may cause inappropriate immune cell effector responses following injury; however more studies are needed to understand the mechanism by which injury causes those alterations in MAPK activity. (Support: R21AA015979-01A1).

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NEUTROPHIL DEPLETED MICE ARE ABLE TO CLEAR ENTEROCOCCUS FAECIUM PERITONITIS

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Objective: The prevalence of colonization and infection with multiresistant Enterococcus faecium in hospital-settings is still increasing worldwide. In particular patients with hematologic malignancies that are accompanied by neutropenia, suffer from enterococcal bacteremia frequently. Because these bacteria are resistant to most commonly used antibiotics, such infections are difficult to treat and cause significant morbidity and mortality. To gain better understanding of the immune response during E. faecium infections we studied the role of neutrophils during E. faecium peritonitis.

Material and Methods: C57BL/6 mice were depleted of neutrophils by intraperitoneal injection of monoclonal rat anti-mouse antibodies directed against Ly-6G, an antigen on the surface of murine neutrophils. Control mice were injected with rat IgG. Antibodies were injected 1 day before and 2 and 4 days after intraperitoneal injection with $9x10^7$ CFU E. faecium.

Mice were sacrificed at different timepoints up to 5 days after infection to determine the immune response by measuring cyto- and chemokines, counting and differentiating blood and peritoneal cells and to determine bacterial loads in different organs including the circulation, peritoneal lavage fluid, liver and lung.

Data: All anti-Ly6G injected mice showed < 50 neutrophils/ µl blood during the entire experiment (i.e they were neutropenic). As a consequence, hardly any neutrophils were attracted to the primary site of infection, the peritoneal cavity. Surprisingly, all mice were able to clear the enterococci from the tested organs within 5 days. Control mice eliminated the bacteria in 2-3 days. In both groups cytokine responses were very low, yet the neutropenic mice showed modestly higher values of TNFα and IL-6. One day after infection KC, a neutrophil attractant, was significantly higher in the neutropenic mice. During the experiment none of the mice showed any signs of illness.

Conclusion: These data show that mice are able to clear a high inoculum of E. faecium, even though they are neutropenic. Although E. faecium could be cultured from different organs at least for 3 days, the infection was not accompanied by noticeable signs of illness.

Apparently, other cells of the immune system or other actors in the immune response can compensate for the deficiency of neutrophils.

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IMMUNE MODULATION BY THE PSEUDOMONAS TYPE III SECRETION SYSTEM

Rudi Beyaert, Marlies Galle, Mira Haegman, Peter Schott

Immune modulation by the Pseudomonas Type III secretion system Rudi Beyaert, Marlies Galle, Mira Haegman and Peter Schotte Unit of Molecular Signal Transduction in Inflammation, Department for Molecular Biomedical Research, Ghent University – VIB, Gent-Zwijnaarde, Belgium Pseudomonas aeruginosa infection

is the leading cause of mortality and morbidity in patients with cystic fibrosis. Due to the ubiquitous nature of P. aeruginosa and its ability to develop resistance to antibiotics, it continues to be problematic from a treatment perspective. The pivotal host defence mechanisms responsible for determining outcome of this infection remain incompletely defined. Improved understanding of critical molecular mechanisms of host-pathogen interactions could lead to the development of immunomodulatory treatments to improve patient outcomes. Several bacterial species such as P. aeruginosa can take control of the host cell by injecting so-called Type III effector proteins into the cytosol of the cells they infect. Using P. aeruginosa strains that are deficient in one or more effector proteins, we could show in cultured macrophages and in a mouse model of acute pneumonia that the effector protein exoenzyme S (ExoS) inhibits the caspase-1 mediated maturation of the cytokine pro-interleukin-1b. ExoS is a bifunctional toxin with an N-terminal Rho GTPase activating protein (GAP) activity and a C-terminally encoded ADP-ribosyl transferase (ADPRT) activity. Infection of macrophages with P. aeruginosa expressing ExoS mutants in the GAP or ADPRT domain showed that the caspase-1 inhibitory effect of ExoS involves its ADPRT activity but not its GAP activity. Furthermore, we provide evidence that instillation of the type III secretion system into the membrane of the infected macrophage induces an inflammatory type of cell death called pyroptosis, which is switched by ExoS in a non-inflammatory type of cell death called apoptosis. These results highlight a previously unknown function of ExoS in the regulation of innate immunity and give new insight on the role of ExoS in immune-escape mechanisms of Pseudomonas aeruginosa.

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EOS-FP, A RECOMBINANT FLUORESCENT DYE TO MONITOR PHAGOCYTOSIS AND DIGESTION OF PATHOGENS IN PATIENTS AT RISK OF SEPSIS AND SEPTIC SHOCK

Marion Schneider, Leonard Schreiner, Manfred Weiss, Joerg Wiedenmann, Franz Oswald

Background: The function of phagocytic cells is of crucial importance to i) ingest and degrade the infectious agent, and ii) to sensitize the host against secondary infections. Measuring phagocytosis and oxygen radical formation has long been accepted to monitor granulocyte function. Thus chemiluminescence measures were applied to study host defense against pathogens and to evaluate damage of endothelial and epithelial lining cells in various diseases including sepsis. We aimed at a new method to study phagocytosis and concomitant digestion using whole blood.

Methods: Based on the pH-sensitive properties of a green-fluorescent protein- (GFP) like dye (Eos-FP), cloned from a coral Lobophyllia hemprichii that switches its fluorescence emission from green (516 nm) to red (581 nm) upon irradiation with approximately 400-nm light (1), we developed a whole blood assay using fixed Eos-FP transfected E. coli. Following incubation of 106 bacteria in 1ml of 1+4 diluted whole blood, we followed the by conventional dual fluorescence emission spectrum in granulocytes, monocytes two colour flow cytometry.

Results: Patients with local infections, and sepsis were compared with patients suffering from septic shock. Remarkably, the uptake of bacterial numbers per cell as well as the digestive capacity of the PMN fraction varied in patients but was fairly constant in healthy individuals. The fluorescent light emission ratio of green (fluorescence 1) and red (fluorescence 2) after 1h of incubation was a suitable measure to evaluate the digestive capacity of the PMN fraction of an individual patient. In healthy individuals, whole blood incubation with Eos-FP E. coli resulted in a mean channel ratio of 2.5 to 3.5 after 60min. During septic shock, some individuals had severely impaired digestive capacity resulting in barely detectable loss of red fluorescent light and a mean fluorescence ratio of only 1.9-1.6 whereas phagocytosis remained at a normal level. Degradation of Eos-FP transfected bacteria was stimulated to ratios of 4.5 to 6.5 by 24h of G-CSF infusion even in patients who did not respond with elevation of leukocyte counts. The relative amount of patients with impaired Eos-FP digestive function was found in septic shock.

Conclusions: The Eos-FP E. coli whole blood phago/digestion assay is useful and very easy to handle. By flow cytometry, quantitative and qualitative analysis can be simultaneously performed at different time points using less than 0.5ml of whole blood per assay. Dual function analysis is relevant in patients with major trauma and a high risk to manifest sepsis. (1) Nienhaus et al. Proc Natl Acad Sci U S A 2005; 102:9156-9159

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A NOVEL ROLE OF CASPASE-12 IN MUCOSAL IMMUNITY TO ENTERIC PATHOGENS

Amal Nadiri, Maya Saleh, Samantha Gruenheid

Caspase-12 deficient mice are more resistant to sepsis, and are able to clear bacterial pathogens more efficiently than wild-type mice by dampening the production of pro-inflammatory cytokines. We have previously shown that caspase-12 exerts its negative effects on inflammation through inhibition of caspase-1. Here we report a novel role for caspase-12 as a key factor during the first steps of innate immunity at the mucosal interface. We have used an enteropathogenic Escherichia coli (EPEC) mouse infection model to study the role of caspase-12 in mucosal immunity. Citrobacter rodentium, a mouse enteric pathogen closely related to EPEC, was used in our studies. C. rodentium colonizes the cecum and colon of its host and induces a colitis phenotype at the peak of

infection, which in C57BL/6 mice, is at 12 to 14 days following bacterial oral gavage. During the first 7 days of infection, there was no sign of inflammation in the infected colon of both wild-type and caspase-12 defficient mice. Nonetheless, caspase-12 deficient mice showed a significantly lower counts of Citrobacter rodentium than wild-type mice, as early as 24 hours after infection. At this time point, infiltrating immune cells were not yet detected in colonic sections, and the levels of IL-1β (a caspase-1 substrate) were not different between the two mouse genotypes. These results suggested that the effects of caspase-12 on bacterial growth in the colon were caspase-1 independent. We have mapped the site of action of caspase-12 to the epithelium, and showed that within this tissue, caspase-12 specifically blocked the production of different antimicrobial peptides including that of β defensins and the cathelicidin CRAMP. Colon epithelial cell-extracts from infected caspase-12-/- mice had significantly greater antimicrobial activity against C. rodentium than those of caspase12+/+ mice, in a zone inhibition assay. These results establish a novel role of caspase-12 as an important component of innate antimicrobial defense in the epithelial compartment. Thus, caspase-12 controls not only bacterial clearance by phagocytic cells but also acts in non-hematopoeitic tissues earlier during innate immunity to control bacterial growth via the actions of antimicrobial peptides. This function of caspase-12 provides an additional line of defense against bacterial infections as well as in sepsis.

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DIFFERENTIAL EXPRESSION PATTERNS OF TOLL-LIKE RECEPTORS IN NEONATAL SEPSIS

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Objectives: In the innate immune system the main cellular components express specific pattern recognition receptors (PRRs) which recognize distinct conserved microbial structures. Thus, PRRs are supposed to represent the starting point in the activation cascade of innate immune cells induced by microbial pathogens. However, studies regarding the expression of PRRs on protein level during clinically relevant infectious disease in man are still very limited. The role of PRRs in human infectious diseases therefore still remains to be elucidated.

Material and Methods: We obtained blood samples from 53 healthy neonates and 20 healthy adults as well as 32 newborns with early and late onset sepsis. We used flow cytometry to determine the expression of the Toll-like receptor (TLR) 2 and TLR4 on granulocytes and monocytes. In newborn patients the analysis was started when clinical sepsis was first suspected and before antibiotic treatment, and continued at four defined time points during follow up. At the same time serum levels of CRP were routinely acquired whereas the concentrations of IL-8 and IL-6 were determined flow-cytometrically using the human inflammation cytometric bead array.

Data: The comparison with adult samples revealed a slightly lower basal expression of TLR2 in neonatal phagocytes whereas no differences could be detected for TLR4. Analysing neonates with sepsis we found an impressive up-regulation of TLR2 on blood phagocytes already at initial presentation of symptoms. Comparison with CRP, IL-8 and IL-6 suggested that TLR2 expression on monocytes is comparably valuable as an early sepsis marker. In the follow up TLR2 was differentially regulated on neonatal phagocytes during sepsis showing a constant up-regulation on monocytes but only a transient increase on granulocytes. Surprisingly, TLR4 showed no remarkable changes during neonatal sepsis.

Conclusions: Our results revealed a mild deficiency of TLR2 expression in newborns possibly contributing to the special susceptibility of neonates to infections with gram-positive bacteria. The differential expression of TLR2 but not TLR4 in the course of neonatal sepsis could reflect the specific inflammatory responses to gram-positive pathogens. Moreover the prolonged increased expression of TLR2 on monocytes suggests a predominant role for monocytes in the later phase of sepsis and implies specific roles of PRRs during different stages of human infectious diseases. Being able to recognize characteristic response patterns in systemic inflammatory response syndromes might be useful to define more specific therapeutic strategies in sepsis.

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EOSINOPHILS INDUCE DENDRITIC CELL MATURATION: IMPLICATIONS FOR REGULATION OF IMMUNITY

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Objective: Even though several clinical disorders including asthma and helminth infections as well as cancer are associated with blood and lung-tissue eosinophilia, the role of eosinophils in their pathogenesis is still controversial. Eosinophil depletion using anti-IL-5 does not influence asthma symptoms in patients for example. We hypothesized that eosinophil-matured DCs, rather than eosinophils themselves, may be responsible for regulating immunity during infection and in particular chronic inflammation. For the first time we show that in presence of pathogen-associated molecular pattern molecules (PAMPs-CpG DNA) or pathogens (adenovirus, influenza virus) eosinophils are capable of inducing DC maturation in vitro.

Material and Methods: Granulocytes were isolated from normal human whole blood by density gradient centrifugation followed by ACK lysis of remaining red cells. Eosinophils were negatively separated using magnetic beads. Immature dendritic cells were generated from CD-14 positively separated monocytes which have been then treated 6 days with GM-CSF and IL-4. CpG ODN 2395

(CpG-C) as a PAMP-surrogate, adenovirus or influenza-virus were used to induce eosinophil based DC maturation. Transwells were used in order to assess cell-cell interaction between eosinophils and DCs. Eosinophil survival was assessed by flow cytometry, cells which did not stain with Sytox-orange were considered as viable.

Data: In the presence of CpG-C, adenovirus, and influenza-virus eosinophils induced DC maturation. Similar results were obtained when eosinophils were pretreated with CpG or pathogens for 4h and cocultured afterwards with DCs. Eosinophil-induced maturation of DCs directly correlated with the eosinophil:DC-ratio. Transwell studies showed that the direct cell-cell interaction between eosinophils and DCs enhances the maturation-inducing effect but was not obligatory. CpGs did not have any negative effect on eosinophil survival, thus we could exclude the possibility that DC maturation was caused by sensing eosinophil cell death.

Conclusions: CpG activated eosinophils mature conventional DCs. The role of viral or bacterial products or potentially host derived DNA as eosinophil activators with consequent DC maturation should be considered in more detail in the inflammatory settings in which eosinophils have been observed.

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L. PNEUMOPHILA INDUCED NF-KAPPAB- AND MAPK-DEPENDENT CYTOKINE RELEASE BY LUNG EPITHELIAL CELLS

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Legionella pneumophila causes community-acquired pneumonia with high mortality, but little is known about its interaction with alveolar epithelium. We tested whether L. pneumophila-infection of lung epithelial cells (A549) resulted in proinflammatory activation.L. pneumophila-infection induced liberation of IL-2, IL-4, IL-6, IL-8, IL-17, MCP-1, TNFalpha, IL-1beta, IFNgamma, G-CSF, but not of IL-5, IL-7, IL-10, IL-12 (p70), IL-13 or GM-CSF. We focused on IL-8 and found induction by L. pneumophila strains 130b, Philadelphia 1, Corby, and to a lower extent, JR32. Knock out of dotA, a central gene involved in type IVB secretion, did not alter IL-8 induction, whereas lack of flagellin significantly reduced IL-8 release by Legionella. Moreover, p38 MAP kinase was activated and kinase inhibition reduced secretion of of induced cytokines with exception of IL-2 and G-CSF. In contrast, inhibition of MEK1/ERK pathway only reduced expression of few cytokines. L. pneumophila also induced binding of NF-kappaB subunit RelA/p65 and the RNA polymerase II to the il8 promoter and a specific inhibitor of the IkappaBalpha- complex dose-dependently lowered IL-8 expression.Taken together, L. pneumophila activated p38 MAP kinase- and NF-kappaB/RelA pathway-dependent expression of a complex pattern of cytokines by human alveolar epithelial cells, presumably contributing to immune response in Legionellosis.

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DIFFERENTIAL EFFECT OF PRETREATEMENT WITH BACTERIAL PEPTIDOGLYCAN AND LIPOPOLYSACCHARIDE (LPS) ON LPS-STIMULATED MACROPHAGE CYTOKINE RELEASE

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Objective: In vitro pretreatment (PreRx) of macrophages (MØ) with LPS induces a stare of LPS-tolerance, with altered response to subsequent stimulation. LPS-tolerance is associated with impaired TNF secretion and MAPK activation. We hypothesized that PreRx with a TLR2-dependent stimulus, peptidoglycan (PDG), would result in a qualitatively different "tolerant" or "reprogrammed" response than that seen with LPS PreRx, a predominantly TLR4-dependent stimulus.

Material and Methods: RAW 264.7 MØ-like cells were PreRx 4 hrs in medium (None), PDG (10 ug/mL), or E. coli LPS (100 ng/mL), then washed and incubated in medium alone for 18 hrs. Medium was discarded and the cells were rechallenged with 0, 1, 10, or 100 ng/ml LPS. Supernatant TNF and IL-6 were measured at 4 hours using ELISA. Phospho-ERK was examined by Western blot 30 minutes after rechallenge. Statistics by ANOVA.

Data: The table shows that PreRx with either LPS or PDG inhibited LPS-stimulated TNF production. In contast, PreRx with LPS had no effect on MØ IL-6 release, whereas PreRx with PDG dramatically augemented LPS-stimulated IL-6 release. PreRx with LPS and PDG both increased p-ERK and prevented further LPS-stimulated p-ERK activation (data not shown).

Conclusion: We observed qualitatively different effects of PreRx with TLR2- or TLR4-dependent stimuli on subsequent rechallenge LPS-stimulation. In particular, we noted that PDG, but not LPS, dramatically augmented subsequent IL-6 release. If the in vitro findings were confirmed in vivo it would suggest that high levels of IL-6 might be more likely to be associated with Gram-positive, than Gram-negative infections.

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Table

LPS PreRx:	TNF (pg/mL)			IL-6 (pg/mL)		
	None	LPS	PDG	None	LPS	PDG
0	190	185	200	60	62	65
1 ng/mL	540	240 *	200 *	58	60	225*
10 ng/mL	600	250 *	218 *	71	48	360*
100 ng/mL	1148	443 *	319 *	67	60	670*

* $p < 0.05$ versus No PreRx

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IMPORTANT ROLE OF COMPLEMENT-REGULATORY PROTEINS (CREG) EARLY AFTER TRAUMA AND DURING SEPSIS IN HUMANS

Umme Amara, Uwe Bruckner, Manfred Weiss, Daniel Rittirsch, Florian Gebhard, Markus Huber-Lang

Purpose: The complemente system as part of innate immunity plays an important role after severe trauma and during systemic inflammatory response against various danger-associated molecular patterns. There is increasing evidence that complement activation products (such as C3a and C5a) may trigger and progress the systemic inflammation leading to sepsis and sepsis-related fatal complications. However, little is known about the regulatory system of complement activation. Especially the role of membrane bound complement-regulatory proteins (CReg) on neutrophils (PMN) early after trauma and resulting sepsis in humans is still unknown.

Materials and Methods: After permission of the local ethics committee, whole blood from patient with severe trauma (ISS>18) was drawn at various time points after trauma (at the emergency room, 4,12,24,48,120, and 240 h post trauma) and compared with blood obtained from patients in septic shock (n=60) or healthy volunteers (n=20). The expression of the complement-regulatory proteins CD35 (complement receptor 1, CR1), CD46 (membrane co-factor protein, MCP), CD55 (decay accelerating factor, DAF), CD59 (MAC inhibitor) and expression of CD88 (C5aR) and C3aR was determined on the surface of neutrophils (PMN).

Results: There was a specific post trauma pattern of Creg expression on PMN with a significant reduction of CD46 early after injury (nadir at 24 h) and a significant increase in CD35, CD55 (peak at 48 h) and CD59 early after trauma. In patients who survived severe trauma, the altered Creg-expression pattern returned to normal levels of sex/age-matched healthy volunteers 240 h after injury. This was in striking contrast to neutrophils obtained from patients in septic shock, which exhibited a further increase in CD55 and CD59 expression, whereas CD46 on PMN was even more suppressed. After trauma, CD88 expression was found to be continuously decreased as time elapsed, and almost abolished during septic shock, especially in those patients who did not survive the fatal consequences of sepsis for 28 d.

Summary: The data suggest for the first time an important role of complement regulation proteins (CReg) early after injury and during development of septic shock. The determination of CRegs and complement receptors might be used as markers to define stage and prognosis of trauma patients with sepsis. In addition, Cregs might be a new target for immunomodulatory therapy after injury and during sepsis to prevent the harmful effects of uncontrolled complement activation during sepsis.

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CROSS TALK OF THE COAGULATION AND COMPLEMENT SYSTEM

Markus Huber-Lang, Umme Amara, John Lambris, Peter Ward, Florian Gebhard, Uwe Bruckner

Purpose: Hemorrhagic shock and subsequent systemic inflammation remain a leading cause of death in trauma victims. During severe hemorrhage, both the coagulation and the complement system are extensively activated and seem to trigger a generalized inflammatory response. Both systems belon belong to the phylogentetically ancient "first line of defense" against danger-associated molecular patterns. Interactions between both cascades have often been proposed, but the precise molecular pathways of this cross-talk have remained elusive.

Material and Methods: In vitro cleavage experiments with incubation of native C5/C3 ± coagulation factors (FXIa, FXa, FVIII, thrombin) or plasmin were performed at 37°C dose- and time-dependently and analyzed by standard ELISA kits (C3a Quidel REF A015, C5a DRG Diagnostic, REF EIA3327) and western blot analysis and functionality of proteins determined by chemotaxis assays.

Results: The coagulation factors FXIa, FXa, and thrombin (FII) were found to time- and dose-dependently generate C3a or C5a in vitro in the presence of C3 or C5, respectively, as determined by immunoblotting and C3a or C5a ELISAs. The C5-cleavage products were biological active in a dose-dependent manner as determined by chemotaxis assays for neutrophils. FVIII failed to either cleave C3 or C5 nor to develop biological activity. Plasmin as a serine protease of the fibrinolytic system

also generated significant amounts of functionally active C3- and C5-cleavage products.

Summary: For the first time, molecular mechanisms of a cross-talk between the coagulation and complement system are found. These insight to "novel pathways" of complement and coagulation activation may identify therapeutic targets for modulation of hemorrhagic shock associated with systemic inflammatory response.

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MOLECULAR BASIS OF VASCULAR DISEASE AFTER TRANSPLANTATION

Jeffrey Platt

Residing at the interface between the blood of a recipient and the cells of a graft, blood vessels, and particularly endothelial lining cells of wholes, are ideally situated to regulate the fate of organ transplants. Consistent with their key anatomic positioning, endothelial cells exist in various physiologic states. Resting endothelial cells inhibit coagulation, inflammation, and ischemic injury and provide a barrier to egress of plasma proteins and blood cells from blood vessels. Injured endothelium promotes coagulation and inflammation and allows blood cells and plasma proteins to exit blood vessels. Endothelial cells stimulated by complement can, depending on blood flow, promote inflammation and coagulation and allow the selective egress of leukocytes, or it can resist these changes. Injured endothelium can also acquire resistance to agonists and noxious substances that would otherwise induce damage. We have elucidated some of the molecular pathways that explain the various changes in endothelial cell physiology, and thus some of the pathologic outcomes of organ transplants and tissues in disease. These molecular pathways reflect in part interaction of components of innate immunity with blood vessels.

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INNATE IMMUNITY IN RENAL TRANSPLANTATION

Didier Ducloux, Cécile Courivaud, Philippe Saas

Innate immunity, a major component of host defense against pathogens, is activated very quickly after infection and precedes the development of acquired immunity. Recently, considerable progress has been observed in the knowledge of the innate immune system and its implication in organ transplantation. Both experimental data and human clinical studies support the role of different components of the donor 's and the recipient 's innate immunity in the outcome of organ transplantation. As in the general population, innate immunity is crucial in defense against infection after transplantation and probably implied in the progression of atherosclerosis. More interestingly, different effectors of the innate immune system, such as toll-like receptors or complement, have been implied in allograft acute and chronic rejection. By instance, the common polymorphisms in TLR-4, which are associated with hyporesponsiveness to inhaled-endotoxin or LPS, increase the risk of infections and decrease atherosclerosis progression in renal transplant recipients. However, both donor's and recipient's TLR-4 polymorphisms may mitigate the risk of acute rejection. These new data suggest that strategies to suppress innate immunity such as the inhibition of complement activation, and blockade of innate effector functions are future potential therapeutic in organ transplantation. Both pharmacological drug therapy and gene therapy have been tested in experimental models and will probably lead to the design of appropriate clinical trials in humans.

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INNATE IMMUNITY IN HEART TRANSPLANTATION

Heiko Methe

Chronic rejection with the development of cardiac allograft vasculopathy remains the major impediment to long-term graft survival after cardiac transplantation. Non-immune and immune risk factors contribute to its development. The role for adaptive immune reactivity has been clearly defined over the last decade. Recent research highlighted the involvement of innate immune mechanisms and especially the Toll-like receptor (TLR) system for rejection in allograft transplantation.

Various animal models have demonstrated release of innate immune ligands during organ harvest and implantation as a result of an initial ischemia-reperfusion injury. This injury is mediated via TLRs recognizing endogenous immune ligands, e.g. heat shock proteins.

We could identify that development of endothelial dysfunction as an early clinical indicator of transplant vasculopathy early after cardiac transplantation is associated with an expansion of TLR4-expressing CD14+-monocytes. In patients with a coronary flow reserve (CFVR) <2 mRNA transcript levels for TLR4 ($P<0.05$) and surface expression of TLR4 ($P<0.005$) were significantly higher than in patients without endothelial dysfunction. This was associated with a significant higher expression of the costimulatory molecule B7–1 ($P<0.05$) on circulating monocytes as well as increased secretion of interleukin-12 ($P<0.02$) and tumor necrosis factor-α ($P<0.05$). Interestingly, expression levels of TLR4 ($r=-0.827$; $P<0.0009$) and B7-1 ($r=-0.796$; $P<0.002$) on peripheral circulating monocytes correlated negatively with CFVR values. Immunohistochemistry revealed increased infiltration with TLR4-expressing immune

cells and strong TLR4 expression on endothelial cells in biopsies of hearts from patients with endothelial dysfunction.

These results were compared with mRNA levels in a mice model of acute and chronic rejection. Compared with native grafts, acutely and chronically rejected grafts exhibited significantly elevated transcript levels for mTLR4 ($P<0.05$). Relative gene transcript levels for the costimulatory molecule mB7–1 in graft-rejecting mice showed the same expression pattern as was seen with mTLR4 ($P<0.05$). Furthermore, acute and chronic rejection of grafts was associated with significant up-regulation of mMac-1 ($P<0.0001$).

The current data on innate immunity and heart transplantation elucidate a significant association between innate immune signaling processes in circulating monocytes and endothelial dysfunction in patients early after heart transplantation. Signaling via TLR4 might be part of the cascade activating acquired immune responses known to be essential for the development of alloimmune responses in the transplanted organ with subsequent advancement towards cardiac allograft vasculopathy. Further studies are necessary to delineate fully the importance of innate immune mechanisms in development of acute and chronic rejection of heart transplants before TLRs might be considered potential therapeutic targets.

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DETECTION OF MYELOPEROXIDASE-CAERULOPLASMIN COMPLEX IN VITRO: IMPACT ON REDOX IMBALANCE

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Introduction & Objective: The systemic inflammatory response syndrome (SIRS) is seen in association with a wide variety of non-infective insults, including surgery necessitating cardiopulmonary bypass (CPB). Data from our institution suggest that 90% of over 1200 patients sequentially admitted through the cardiothoracic adult ICU fulfil the criteria for SIRS. SIRS is associated with organ dysfunction, resulting in significant morbidity and mortality amongst the critically ill. Pro-oxidant mediators are released as a consequence of CPB, including myeloperoxidase (MPO)-generated hypochlorous acid (HOCl). HOCl, a strong oxidant, is the major end product of the neutrophil respiratory burst. At high concentrations it not only mediates molecular damage, but also affects cellular signalling mechanisms. Caeruloplasmin (CP) is an acute phase protein with anti-oxidant capacity, whose properties include an affinity for MPO, resulting in inhibition of its enzymatic function and hence HOCl production. In previous studies we have demonstrated a significant increase, in the immediate post-operative period in CPB-patients in whom SIRS persists, of both MPO protein levels and MPO activity. In addition we have demonstrated a decrease in CP levels, due to CPB-associated haemodilution and a time lag in CP synthesis. An imbalance between MPO and CP may result in an increase in MPO activity, the latter of which is implicated in the pathogenesis of SIRS. The dynamics of binding and complex formation of MPO and CP have not been explored in detail, such that the MPO/CP protein interaction in patients with SIRS has not been demonstrated. Here we investigate the interaction of MPO and CP.

Hypothesis: CP and MPO bind to form complexes in vitro that are detectable by immunoprecipitation (IP) and gel chromatography (GC).

Methods: (1) IP, using standard protocols, was performed using anti-CP antibody and Western blotting (WB) for MPO, followed by anti-MPO antibody and WB for CP, in control, MPO, MPO/CP and CP equimolar solutions. (2) GC was performed on equivalent solutions using standard protocols (25cm-sephadex-200 gel, at 0.2ml/min). Eluted samples were analysed for protein (Bradford assay), MPO (ELISA) and CP (immunodiffusion).

Data: WB of MPO/CP immunoprecipitate was positive for MPO when using anti-CP antibody and positive for CP when using anti-MPO antibody (n=3). Gel chromatography demonstrated three distinguishable curves, with distinct absorbance peaks for MPO and CP, and two peaks for MPO/CP (2.8 and 4.4ml elution respectively) with a corresponding change in absorbance in MPO and CP peaks. The absorbance peak seen at 2.8ml in the MPO/CP curve tested positively for MPO and CP proteins. At this same elution volume the CP curve tested negatively for both MPO and CP, and the MPO curve was negative for CP with only small amounts MPO detected (n=4).

Results: IP demonstrated MPO-CP binding. GC confirmed the MPO-CP complex of a molecular weight greater than the individual proteins.

Conclusion: MPO-CP binding and complex formation can be demonstrated in vitro. This methodology will facilitate the investigation of MPO-CP as a plasma marker, or even CP as a therapeutically relevant intervention, in patients with SIRS.

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DECREASE OF ADAMTS13 ACTIVITY IS A COMMON FEATURE OF THE INFLAMMATORY RESPONSE

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Background and Aims: ADAMTS13 is a protease that specifically acts on the multimeric von Willebrand factor (VWF). Congenital or acquired deficiency of

ADAMTS13 can result in the appearance of ultralarge VWF multimers with subsequent activation of circulating blood platelets, thrombus formation in the microcirculation and (multi) organ failure. There is some evidence that systemic inflammation also results in restriction of microcirculation and that the development of multiple organ dysfunction syndrome (MODS) is associated with decreased ADAMTS13 activity and increased VWF levels.

Methods: In a prospective study on patients with various degrees of systemic inflammation we aim to provide evidence for a crucial role of an imbalance between ADAMTS13 activity and VWF level in the development of inflammation induced MODS.

Data: Four groups of patients were studied: healthy volunteers with moderate SIRS after strenuous physical exercise, heart surgery patients with low risk for systemic inflammation and MOF, heart surgery patients with SIRS and moderate MODS, and patients with severe sepsis or septic shock. Consecutive plasma samples were analysed for ADAMTS13 activity and VWF and compared with various markers of systemic inflammation and activated coagulation as well as with parameters of describing the extent of MODS.

After physical stress, we found a marked decrease of ADAMTS13. With the exception of pre-operative values, in all patient groups ADAMTS13 was found to be significantly decreased compared to values measured in age- and sex-matched controls. The decrease in ADAMTS13 activity was related to the extend of systemic inflammation and was most pronounced in patients with MODS, specifically in patients who died in septic shock. Exemplarily we demonstrate an association of low ADAMTS13 activity/ MOF with the appearance of ULVWF multimers. Furthermore, some of the patients with low ADAMTS13 activity and high VWF levels revealed evidence of DIC and drastic changes in platelet count.

Conclusion: Our data provide further evidence that systemic inflammation may cause an imbalance between ADAMTS13 activity and VWF level, and that this imbalance may contribute to inflammation-mediated MODS.

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THE USE OF PROTEIN S-100B AS SCREENING FACTOR IN PEDIATRIC HEAD TRAUMA PATIENTS

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Background: Blunt head trauma is a frequent injury pattern in children. The consecutive traumatic brain injury remains a leading cause of mortality and morbidity. Management strategies for major head trauma with neurological symptoms are well established. In minor head trauma, however, the amount of necessary primary diagnostic procedures is not clearly defined. In most investigations it is remarked that a real brain injury is difficult to catch by clinical symptoms only. Most patients get a CCT scan or are hospitalized for one to three days for observation. An additional problem is the necessity of anaesthesia in children under the age of six years to perform a CCT scan. S-100b is a well known marker of neuronal damage after traumatic brain injury in adults, but it has rarely been evaluated in paediatric patients and its use as a screening factor has not been investigated.

Methods: We present a prospective study in which S-100b is measured in serum of patients within the first six hours after head trauma. According to our protocol patients with clinical symptoms of a mild brain injury such as recurrent vomiting, nausea, headache or skull fracture in the x-ray are admitted to the ward. A CCT scan is done if the symptoms are getting worse, or initially for patients with GCS under 14. If available, a correlation between positive CCT scans and S-100b is evaluated.

Results: Between January and April 2006, in 269 patients the serum concentration of S-100b was measured. In 249 patients the S 100b was taken within 6 h. The prevalence was higher in boys (160) compared to girls (89). 71 patients (28,5 %) underwent CCT-scans due to ongoing neurological symptoms. In 34 of these patients (48%), scans revealed intracranial pathologies (haemorrhage, fractures, epi-, subdural haematoma). In 37 patients (n=52%) the CCT scan was negative. All of these had S-100b serum levels above the adult cut-off value. 19 patients showed elevated S-100b levels despite negative CCT-scans, while in the remaining 18 cases the S-100b was also negative. The positive predictive value was only 64%, but the negative predictive value was 100%.

Conclusions: The preliminary results show the diagnostic value of S-100b as a marker of neuronal damage after traumatic brain injury in children in correlation with CCT scans. In our opinion the negative predictive value of 100% makes S-100b a helpful screening factor. Children who have a negative value need not to be hospitalized and do not need a CCT scan. All patients with a positive S-100b level should be admitted for observation and if necessary a CCT scan has to be done.

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ADMISSION SERUM PROCALCITONIN AND IL-6 LEVELS, BUT NOT SERUM LACTATE OR TIME OF LACTATE CLEARANCE CORRELATE WITH MAJOR INFECTION IN SEVERELY TRAUMATIZED PATIENTS

Matthias Turina, Adrian Billeter, Ladislav Mica, Thomas Lustenberger, Otmar Trentz, Marius Keel

Introduction: Identification of clinical and laboratory parameters to recognize trauma patients at risk of developing major infection and/or increased mortality remains a key goal of trauma research. Lactate and IL-6 levels were shown to correlate with mortality and multiple organ dysfunction following trauma, but their predictive value for the development of major infection remains poorly understood.

Patients and Methods: 1601 consecutive adult trauma patients with an injury severity score (ISS) > 16 admitted over a 10-year period were analyzed with respect to physiological and laboratory parameters over a 21-day period. Patients not surviving the first 72 hours (24.5%) or those admitted after more than 24 hours following trauma (8.0%) were excluded from further analysis. Data are stated as mean ± SEM.

Results: 1079 trauma patients (75.8% male) with an average age of 41 ± 0.5 years and a mean ISS of 30.5 ± 1.6 (mean APACHE II score 14.5 ± 2.5) have been evaluated. The unadjusted late (> 3 day) mortality rate was 11.7%. Admission lactate correlated with overall mortality, but not with infectious complications in patients surviving > 3 days. Admission and early serum procalcitonin and IL-6 levels strongly correlated (all $p<0.01$) with the subsequent development of pneumonia (incidence 25.2%), sepsis (incidence 20.7%), and surgical site infections (incidence 9.4%).

Conclusions: Although serum lactate and time to lactate clearance are predictors of mortality in trauma patients, only early procalcitonin and IL-6 levels correlate with the subsequent development of infectious complications in severely traumatized patients. Definition of specific cut-off values and early monitoring may help identify patients at risk and direct early surgical and antibiotic therapy.

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PLASMA NT-PROCNP LEVELS DIFFER IN MULTIPLY TRAUMATIZED PATIENTS WITH OR WITHOUT SEPTIC COMPLICATIONS

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BACKGROUND & AIM: C-type natriuretic peptide (CNP) is a member of the natriuretic peptide family which is produced in vascular endothelial cells and may play an important role in sodium regulation and blood pressure. Few studies have compared the relative prognostic value of different natriuretic peptides in polytraumatized patients. This study describes the long term profiles of NT-proCNP, the N-terminal fragment of the CNP precursor, in multiply traumatized patients with and without traumatic brain injury (TBI) with relationship to septic complications and outcome. NT-proCNP was chosen because it circulates in higher amounts and is more stable than the active hormone CNP.

METHODS AND RESULTS: We assessed profiles of NT-proCNP in 53 multiply traumatized patients (MTP) with or without TBI verified by computer tomography . We found distinct NT-proCNP profiles in patients with or without TBI. While NT-proCNP levels were significantly higher in MTP developing septic complication without TBI, NT-proCNP levels were lower in MTP with TBI developing septic complications. In non-survivors NT-proCNP levels increased dramatically before death. During follow-up (14 days), 30 patients developed septic complications and 12 died. NT-proCNP levels at admission did not have predictive value for outcome.

CONCLUSION: NT-proCNP plasma profiles differ distinctly between MTP with and without TBI, developing septic complications. The prognostic value of NT-proCNP for septic complications in MTP needs to be verified further in larger patient collective.

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NGAL AS A MARKER FOR RENAL INJURY IN SEPSIS

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Circulating levels of neutrophil gelatinase-associated lipocalin (NGAL) are reported to be raised in both inflammation, especially that due to bacterial infection, and in renal injury leading to acute renal failure (ARF). The aim of the present study was to see whether urine or plasma NGAL levels, determined by a recently developed ELISA, can be used for the early diagnosis of renal injury leading to ARF in patients with sepsis, who are all expected to show increased NGAL levels.

Plasma and urine NGAL was monitored (daily to alternate days) in 53 consecutive patients with sepsis admitted to intensive care. ARF was defined in biochemical terms as a 50% or greater increase in plasma creatinine over basal values. Six patients were excluded because of incomplete data, e.g. due to early transfer. NGAL was determined with a commercially available ELISA kit (AntibodyShop A/S, Denmark). Data are reported as median (with 5th and 95th centiles in brackets). NGAL levels of 5 (1-12) ng/mL in urine and 63 (45-108) ng/mL in plasma were found in healthy

volunteers. Maximal NGAL levels in patients with and without ARF groups were analyzed nonparametrically by the Mann-Whitney U-test.

All patients had increased levels of NGAL in both urine (962 (47-18,390) ng/mL) and plasma (577 (163-2658) ng/mL). ARF occurred in 31 patients; these had a slightly higher mean APACHE II score (26 (13-35)) than those in whom ARF did not occur (21 (12-30); p 0.036) and a markedly increased mortality (46% vs. 0%; p 0.004, chi-squared test). The maximal urinary NGAL level was significantly ($p < 0.0001$) higher in patients with ARF (2672 (216-19,997) ng/mL) than in those without ARF (147 (28-1046) ng/mL). The maximal plasma NGAL was also significantly ($p<0.0001$) higher in the patients with ARF (1144 (258-3085) ng/mL) than in those without (285 (110-587) ng/mL). ROC analysis was used to determine the best cutoff values for NGAL to separate patients with ARF from those without, and closely similar values were found for urine and plasma, giving negative predictive values of 87.5% and 84.8%, respectively, and positive predictive values of 80% and 73.3%, respectively.

It is concluded that NGAL levels rise to much higher values in patients with sepsis who develop ARF than in those who do not, despite the general elevation of NGAL levels in sepsis. NGAL determination may therefore be useful for the early diagnosis of renal injury leading to ARF during the course of sepsis.

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INCREASED POLYCYTHEMIA RUBRA VERA-1 MRNA EXPRESSION IS ASSOCIATED WITH SEPSIS AND NOT SEVERITY OF ILLNESS OR LEUKOCYTOSIS

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Polycythemia rubra vera-1 gene (PRV-1) codes for a variably expressed unique neutrophil-specific cell surface glycoprotein associated with the myeloproliferative diseases polycythemia vera and essential thrombocythemia . We have previously demonstrated that gene expression for PRV-1 is markedly increased in patients who become septic compared to patients with sterile SIRS. These changes occur prior to the onset of clinical sepsis1. Objectives: The purpose of this study was to determine if PRV-1 is associated with severity of critical illness (APACHE II), WBC, or neutrophil count in systemic inflammation due to infected and sterile etiologies. Further we sought to determine if plasma protein changes in PRV-1 occurred in sepsis and whether these changes were related to acuity of illness. Methods: Critically ill, uninfected SIRS patients were prospectively evaluated for development of sepsis. Patients admitted to an intensive care unit were prospectively divided into two groups: 1) Pre-septic SIRS(n=45): SIRS patients who developed clinical sepsis, and 2) Uninfected SIRS(n=45): SIRS patients remaining uninfected. Uninfected SIRS patients were time-matched to pre-septic SIRS patients. Isolated whole blood RNA from 1 day, 2 days, and 3 days (T-12, 36, and 60 hours) prior to clinical sepsis, and day of study entry was analyzed with RT-PCR. Protein profiling was performed on pooled plasma samples using LC3 MS2 and/or electrospray ionization (ESI) LTQ-FTMS mass spectrometry. Daily APACHE II scores for each time point were calculated and WBC count were collected and correlated to PRV-1 mRNA gene expression. Data: At study entry, patients in both groups were matched for APACHE II, age, and gender. PRV-1 mRNA expression was similar between groups at study entry and did not correlate with APACHE II (r2=0.044). At T-12, pre-septic patients had a 10.75 median fold change increase compared to uninfected patients (previously reported1). Despite the difference in PRV-1 mRNA expression between groups, there was no correlation to APACHE II for all patients (r2=0.23), pre-septic patients (r2=0.01), or uninfected patients (r2=0.04). Similarly changes in APACHE II score over time in both groups did not correlate with PRV-1 mRNA expression changes (all r2=0.019, pre-septic r2=0.018, uninfected r2=0.003). PRV-1 mRNA did not correlate with either WBC (r2 = 0.0912) or neutrophil count (r2 = 0.0745). Mass spectrometry failed to detect circulating PRV-1 protein in either group at any time point. Conclusions: This study demonstrates that neither severity of illness, WBC or neutrophil count are associated with PRV-1 mRNA expression. Further PRV-1 plasma protein expression is not present suggesting a post-transcriptional alteration or lack of PRV-1 cellular shedding. These findings suggest that similar to molecular diagnostic specificity in myeloproliferative diseases, PRV-1 gene expression differentiates sepsis from sterile etiologies of inflammation, and is not associated with severity of illness plasma levels, or WBC count. 1 Lissauer ME, Johnson SB, Scalea TM, et al: Chest. 2006; 130:136S.

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EVALUATION OF AN ASSAY TO DETERMINE NF-KB ACTIVATION CAPACITY DURING CRITICAL DISEASES

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Objective: Signalling via NF-kB is essential for the development of inflammation. In critically ill patients NF-kB activaty in mononuclear blood cells is known to positively correlate with the incidence of complications, the Apache-II-score and lethality. Currently, NF-kB inhibitors are developed to treat hyperinflammation e.g. during sepsis. Thus, monitoring NF-kB activation would help to establish new anti-inflammatory therapies. However, NF-kB assays such as p65-Elisas or flow cytometry-based assays are not specific enough to analyse material of patients. Moreover, NF-kB activation is a very dynamic process. Simply measuring NF-kB activity in peripheral blood cells may not reflect its role during

critical diseases. The gold standard to determine NF-kB activity, the electro-phoretic-mobility-shift-assay (EMSA), is rather semi-quantitative. In order to precisely elucidate the importance of NF-kB during critical diseases a standardized quantitative assay is necessary.
Material and Methods: Blood mononuclear cells (MNCs) were isolated by standardized Ficoll preparation from 26 healthy volunteers. T cells were purified by means of magnetic bead separation and negative selection of T cells. Purity was checked by flow cytometric analysis of CD4 and CD8 expressing cells and was usually >95%. Preliminary tests were performed in order to establish optimal conditions for full NF-kB activation in MNCs and T cells. 4x106 MNCs and T cells, respectively, were stimulated with 10ng TNFa for 30 minutes and 10Ág of nuclear protein was used for EMSA analysis. Nuclear factor-1 (NF-1) DNA binding activity of the same samples was used as loading control and for normalization. For supershifts nuclear extracts were incubated with anti-p50 and anti-p65 antibodies. Upon densitometric analysis of autoradiographic band shifts relative NF-kB induction was calculated: (NF-kBstimulated/NF-1stim)/(NF-kBctrl/NF-1ctrl). MNC isolation and analysis of NF-kB activation capacity in 5 septic patients was performed as described above.
Data: Supershift analysis demonstrates that canonical p50p65 heterodimers are only partially involved upon TNFa activation of MNCs and T cells. NF-kB activation capacity is similar in both cell populations. The MNC composition of septic patients is heterogenic with respect to the lymphocyte fraction which is reflected by NF-kB activation capacity. Data of surgical patients will be presented.
Conclusion: Differential blood (MNC) counts are necessary to interpret NF-kB activation capacity. However, this assay provides an applicable means to quantify the capacity of NF-kB activation upon ex vivo stimulation of peripheral human immune cells. Its application in the context of clinical studies will show whether NF-kB signalling essentially contributes to e.g. hyperinflammation in the course of human sepsis.

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ELEVATED SERUM-PROCALCITONIN MEASURED BY THE KRYPTOR-ASSAY INDICATES SEVERE ABDOMINAL INJURY IN MULTIPLE TRAUMATIZED PATIENTS

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Objective: Changes of serum concentrations in a number of different inflammatory markers have been described after severe trauma. However, the correlation between injury pattern and a specific inflammatory mediator has not been fully elucidated. Our goal was to determine if the serum concentration of the inflammatory mediator procalcitonin (PCT) is directly associated with abdominal trauma in multiple traumatized patients.

Material and Methods: In 96 patients with multiple trauma (ISS $\geq$ 20) PCT, IL-6, and CRP were measured on admission (D0), and day one and two (D1, D2) after trauma. 39 of these 96 patients suffered from severe abdominal trauma ($AIS_{abdomen} \geq 3$). The patients were further subdivided into groups of ISS greater or lesser than 25 to determine if overall injury severity can influence the prognostic value of PCT regarding abdominal trauma. We used the KRYPTOR®-Assay for PCT, ELISA-technique for IL-6 and standard laboratory procedure in our clinic for CRP measurement. Data are given as mean, significances between the groups were calculated using one way ANOVA with alpha correction for multiple testing. A p-value of less then 0.05 indicates statistical significance.

Data: While IL-6 and CRP were similar in patients with and without severe abdominal trauma PCT was significantly increased in patients with severe abdominal trauma (D1 2.79 ng/ml vs 0.56 ng/ml ($p < 0.05$), D2 2.90 ng/ml vs. 0.49 ng/ml; ($p < 0.0.5$)). Further testing revealed that differences remained significant between the ISS-subgroups as well. On day 1 mean serum concentrations were 3.48 ng/ml in patients with abdominal trauma vs. 0.70 ng/ml in patients without abdominal trauma for ISS $\geq$25. For ISS < 25 mean serum concentrations were 1.27 ng/ml and 0.25 ng/ml respectively. On day 2 similar results were measured. Additionally, PCT values tended to be higher in patients with spleen or liver injury.

Conclusion: In our setting CRP and IL-6 were not helpful in determining the trauma localization. In contrast, PCT can help to identify patients suffering from severe abdominal trauma during the immediate period after multiple trauma regardless of overall injury severity.

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SECRETION OF N TERMINAL-PRO BRAIN NATRIURETIC PEPTIDE IN PATIENTS WITH SEVERE TRAUMATIC BRAIN INJURY

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Objective: The pathophysiological consequence of TBI is influenced by primary and secondary brain damage; in this context secondary damage might be worsened by dysregulation of cerebral water - sodium homeostasis in terms of cerebral salt wasting (CSW). Therefore, recent studies gave evidence to the important role of BNP in CSW in patients suffering from aneurysmatic subarachnoidal haemorrhage (SAH). Moreover it has been shown, that in patients with SAH serum BNP levels correlate with intracranial pressure (ICP) and clinical outcome. Therefore, the aim of this study was to assess the influence of the systemic and intrathecal excretion of NT-proBNP on the dysfunction of the blood brain barrier

(BBB) and the correlation of NT-proBNP levels to increased ICP in patients with severe TBI.

Patients and Methods: 14 patients suffering from TBI (GCS <8) were enrolled in this study. Immediately after the placement of an extraventricular drainage (EVD), as well as 12, 24, 48 and 72 hours post trauma, 3ml cerebrospinal fluid (CSF) samples as well as 5ml peripheral blood samples were drawn. Pro-BNP levels were determined in CSF and blood, using ®NT-proBNP-test systems (Roche Diagnostics). ICP was monitored on a permanent basis via the EVD (TraumaCath®, Integra-Neurosciences, Plainsboro, USA) and recorded at each drawing time point. Patients were divided into two subcollectives according to ICP; group I (n=8) exhibited an ICP<15 over the complete observation period, whereas group II (n=6) was at least once above that level. Due to its reliability as a parameter for the assessment of BBB function, the ratio of CSF/serum albumin (Q_a) was daily calculated in all patients (normal value<0.007). 90 days post trauma the clinical outcome was evaluated using GOS. Statistical analysis was done using t-test (group I vs. group II) and spearman rank correlation for assessing relationships between NT-proBNP and ICP levels ($p<0.05$).

Results: Patients in group I had a mean ICP of 11.1±3.5mmHg (mean±SD,), group II a mean of 27.6±9.2. GOS of patients in group II accounted for 2±2 vs. 4±1 in group I. Serum (800±150 pg/ml) as well as CSF levels (120±15pg/ml) of NT-proBNP rose in group II in correlation to ICP-values and were therefore significantly elevated in comparison to the measured levels of group I (serum: 40±8.8 pg/ml), respectively in CSG 110±13pg/ml as well as to admission and control group values. However, there was no significant disruption of BBB as the Q_a accounted for 0.006±0.0008.

Conclusions: In this study we showed significant increases of serum and intrathecal NT-proBNP levels, directly correlating to the increase of the ICP in patients with severe TBI. We could demonstrate that this increase seems not to be influenced by integrity of the blood brain barrier. A possible explanation could be the intrathecal synthesis of proBNP. However, the physiologic role of NT-proBNP in TBI needs to be elucidated in further studies.

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ELIMINATION OF HEAD TRAUMA SERUM MARKER S-100: A MULTICENTER TRIAL ON 830 PATIENTS

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Clinical decision rules for the indication of cranial computed tomography (CCT) in patients suffering from minor head injury (MHI) still result in a high frequency (90%) of normal findings (negative CCT). Recently, serum measurement of astroglial S-100 was introduced as a rule out parameter for intracranial complications in patients suffering from MHI on a highly significant level (Shock 2006, 25, 446-53). However, falling short below the cut-off level due to physiological elimination might have a substantial impact on this clinical rule out decision. In this context, the elimination kinetic remains incompletely clarified, so far. Hence, the aim of this study was to clarify the elimination kinetic of S-100 by repetitive serum analysis within a multi-centric study approach.

Into this study, 830 patients were enrolled after suffering from MHI and blood samples were drawn on admission and 2h later. In between, a CCT scan was performed to identify patients suffering from intracranial complications. Out of the obtained results, a linear regression analysis was calculated to obtain an elimination equation and establish from this the half life time of S-100.

In 90% of the patients the second blood sample was drawn within an interval of 2 up to 2.5h after first sample. The linear regression analysis revealed the following equation: [µg/l/h]=-0.378 x S-100 + 0.044 meaning that a given serum concentration of 1.0µg/l will fall down about -0.334 µg/l per hour. The r^2 for this linear regression analysis was 0.92 and thereby highly significant.

This data is the first calculation of S-100 elimination kinetics within a multicentric study on 830 patients. Concluding from the obtained results, the elimination half life time of S-100 in serum of patients suffering from MHI in this multicentric collective was approximately 1.5h. This important information has be taken into account for interpretation of serum concentrations as rule out parameters in MHI patients.

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INFLAMMATORY INDICATORS IN THE EVOLUTION OF ACUTE MYOCARDIAL INFACTION

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Objectives: The aim of this work is to study the role of molecules involved in the inflammatory process the onset of myocardial infarction and during the recovery period.

Methods: Seventeen patients with confirmed acute myocardial infarction (AMI) in the first 24 hours of evolution were compared with 16 patients with chest pain complains but normal coronary arteries, as proved by angiography and with a reference group of 15 healthy non-smoker volunteers. A longitudinal follow-up of AMI patients was carried out at 3 different occasions: in first 24 hours of the acute myocardial infarction before any

medication or treatment; two days after; and 40 days after the infarction. The determination in serum of soluble adhesion molecules P-selectin and ICAM-1, as well as CD40 ligand and TNF-alpha was assessed in all groups using commercial kits.

Results: Immediately after infarction the sP-selectin levels were increase, while the soluble concentrations of CD40L and TNF-alpha were decreased. At day 2, a decrease of sP-selectin and CD40L was observed reflecting medication. Two months after the infarction the levels of TNF-alpha and P-selectin significantly increased and CD40L show the same trend. No significant variations were observed for ICAM-1.

Conclusion: The variations observed suggest a mediation of the inflammatory markers measured in the AMI evolution. The increased concentration of sP-selectin at the infarct onset evidenced thrombosis and platelet activation. Remarkably the levels of soluble CD40L, which can also be expressed in activated platelets, were depressed at the infarct onset proposing a delayed action for this molecule and/or a slow rate of release of the CD40L soluble form. Therefore, the soluble levels of CD40L may not constitute a true indicator of the expression of this molecule. After 40 days of recovery the increasing levels of TNF-alfa, P-selectin and sCD40L in circulation may indicate an apparent worsening in the inflammation status that can also derive from compensation mechanisms or the healing of the damaged myocardium and epithelium area.

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NOSOCOMIAL INFECTIONS AFTER SEPTIC SHOCK: RELATIONSHIP WITH DECREASED MONOCYTE HLA-DR EXPRESSION

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Monocytes from patients with severe infections undergo significant reprogramming, also known as immunoparalysis. Diminished monocyte HLA-DR (mHLA-DR) expression is an accepted marker of this immune suppression. It is an independent predictor of mortality in septic shock (SS). Reduced expression of mHLA-DR has also been shown to be a marker of an increased risk for infectious complications after various injuries (trauma, surgery, pancreatitis). The purpose of this study was to examine whether there is a relationship between mHLA-DR decreased expression after septic shock and the occurrence of nosocomial infections (NI).

Methods: A cohort of 209 patients presenting with a septic shock episode was studied during their ICU stay. mHLA-DR expression was serially measured by flow cytometry during the first 21 days and expressed as percentages of HLA-DR-positive monocytes out of total monocyte population. Control values are > 90%. NI were recorded, according to the definitions of the European Community (HELICS program), as a part of a NI surveillance program.

Results: Among the 209 patients, 43 had at least one device-related nosocomial infection (20.6 %): 24 Pneumonia, 16 Urinary Tract Infections, 9 Bacteraemia, 10 Catheter-Related Infections. Overall mortality was 43.1 % during the ICU stay (58.1 % [N = 25] among the infected (IN+) and 39.2 % [N = 65] among the non infected (IN-), including 23 patients deceased during the first 48 hrs). SAPS II did not differ between SS patients with (IN+) or without (IN-) secondary infection: (56.3 vs 50.8, p = 0.065).

The mean value of HLA-DR at day 7-8 after the onset of SS was 50.1 (median: 51.8 [5.8-92.6]) in the patient surviving at this time point. There is a significant difference ($p<0.001$) between HLA-DR at the same date in the IN+ group: 38.6 (median: 32.7 [10.9-92.6]) and in the IN- group: 54.1 (median : 57.4 [5.8-89.6]). The ROC curve of predicting value for nosocomial infection indicates that the best discriminating value of mHLA-DR expression is 40 %, with a sensitivity of 75 % and a specificity of 65 %. AUC is 0.709 (p = 0.001).

Conclusion: A decreased monocytes HLA-DR expression a week after a septic shock indicates a persisting immunoparalysis and is associated with an important risk of secondary nosocomial infection.

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THE NEURAMINIDASE ACTIVITY INCREASES RAPIDLY IN SEPTIC PATIENTS

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Introduction: Sialic acid (SA), a N-acetylated derivative of neuraminic acid, is positioned in the terminal positions of glycoprotein and glycolipid oligosaccharide side-chains, the sialylation patterns of these being highly variable. SA contributes to cell-to-cell interactions and serves as a chemical messenger in tissue and body fluids (1). SA plays also a determinant role in the circulating half-life of proteins and red blood cells (RBCs). We previously showed an inverse correlation between decreased membrane SA content and changes in RBC shape in critically ill patients (2). Free SA concentrations were also elevated in the sera of septic patients, suggesting a leakage of SA by its degrading enzyme, the neuraminidase (3). We hypothesize that neuraminidase activity is increased in septic patients.

Materials and Methods: We collected the sera of 45 (17 septic and 28 non-septic) patients on ICU admission. Exclusion criteria were: RBC transfusion in the previous 72 hours, acute bleeding, hematologic disorders, diabetes

mellitus, malignancy and cirrhosis. Neuraminidase activity was measured by the Amplex® Red Neuraminidase Assay Kit (Molecular Probes) and expressed as mU/mL. We also collected the following parameters: age, hemogram, C-reactive protein (CRP), lactate, PaO2/FiO2 ratios, bilirubin, creatinine concentrations. We also calculated APACHE II and SOFA scores, the control group consisted of 20 healthy volunteers.

Statistical Analysis: Results are expressed as median values (25-75%). Variables were compared by Wilcoxon Signed Rank Test. Correlation was evaluated by the Spearman's test.

Results: Neuraminidase activity was higher in septic than in non-septic patients and in volunteers (5.42 [4.85-6.00] vs 4.53 [4.23-5.23] and 1.26 [0.83-1.83] mU/mL respectively, all $p < 0.05$ compared to volunteers). A significant correlation was found between neuraminidase activity and CRP concentrations ($r = 0.44$; $p < 0.0001$).

Conclusions: Neuraminidase activity is rapidly increased in critically ill patients, especially in septic patients. This increased enzyme activity may contribute to desialylation observed in RBC membrane, and result in rheologic alterations. Inhibition of this enzyme could be a new therapeutic option for improve the RBC rheology in critically ill patients.

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WEIGHING EXOGENOUS VERSUS ENDOGENOUS TRIGGERS OF SIRS

Jeffrey Platt, Gregory Brunn, Geoffrey Johnson

The systemic inflammatory response syndrome (SIRS) poses a significant challenge in many conditions. SIRS resembles the condition of sepsis that is clearly triggered by the products infectious organisms acting on inflammatory cells and blood vessels, but SIRS arises in the absence of known triggers for systemic inflammation. SIRS, as such, has been shown to be induced by the stimulation of toll like receptors, particularly toll like receptor 4 (TLR4). What agonist or agonists might trigger TLR4 in this setting has been uncertain. Having shown that heparan sulfate and other saccharides can stimulate TLR4 in cell culture systems and in vivo, we tested whether heparan sulfate might induce SIRS in experimental animals. Injection of heparan sulfate into mice reliably induces SIRS, demonstrated by the presence of TNFα in blood, followed by death. Signs of SIRS were heightened by treatment of experimental animals with elastase, which we had shown to prime TLR4 for responsiveness. Inhibitors of lipopolysaccharide did not prevent SIRS and death, indicating that it was heparan sulfate and not a contaminant that accounted for this observation. Identifying the specific agonist in various types of SIRS may allow the devising of specific therapies to prevent or reverse that process.

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REGULATION OF INFLAMMATION BY HYALURONAN

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Hyaluronan (HA) is a major component of the extracellular-matrix of many tissues. Apart from its function as immunologically inert hydration agent HA displays many, in part opposing, functions in inflammation depending on its molecular size. First and foremost HA synthesis is strongly enhanced in areas of inflammation and tissue injury. However, at the same time, degradation of HA occurs either caused by hyaluronidases or free-radical related mechanisms. Thereby generated HA fragments of different sizes have to been shown to act as important regulators of the inflammatory response.

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ENDOGENOUS CONTROLS OF TOLL-LIKE RECEPTOR 4 SIGNALING

Gregory Brunn, Jeffrey Platt

Toll-like receptor 4 triggers local and systemic inflammation. Toll-like receptor 4 is activated in vitro by subnanomolar concentrations of lipopolysaccharide, a component of the cell wall of Gram-negative bacteria, or by heparan sulfate, a complex carbohydrate associated with proteoglycans on cell surfaces and extracellular matrices. Given the ubiquitous nature of heparan sulfate, we reasoned that Toll-like receptor 4 activity may be constitutively suppressed to avoid inappropriate activation of inflammation. To address this question, we developed a model system in which human cells expressing components of the Toll-like receptor 4 complex and a reporter were grown in extracellular matrix representing the microenvironment in which vascular and extra-vascular cells are typically found. While the Toll-like receptor 4-reporter cells were activated by small concentrations of LPS or heparan sulfate the cells grown in matrix were inured to these agonists. The ability of the reporter cells to respond to LPS and heparan sulfate was fully restored by exposure to small amounts of elastase, indicating suppression had been relieved. Larger amounts of elastase mobilized heparan sulfate which activated the reporter cells without any other agonist. Stromal cell-derived factor 1 and its

receptor CXCR4 abolished activation of Toll-like receptor 4 by lipopolysaccharide and heparan sulfate. Since stromal cell-derived factor 1 is a widely-expressed chemokine that is bound by heparan sulfate and inactivated by elastase, it may be an endogenous suppressor of Toll-like receptor 4 signaling. These findings suggest that extracellular matrices may set a threshold for Toll-like receptor activation, perhaps by concentrating stromal cell-derived factor 1. The threshold prevents inappropriate activation of inflammation or contains inflammation to regions where degradation of matrix by protease(s) is ongoing.

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CELL DEATH AND ACUTE INFLAMMATION

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Cell death occurs in many pathological situations and as such may be the harbinger of an infection or other dangerous process. Dead cells also need to be removed and replaced to restore tissue integrity. Consequently, the immune system has the capacity to sense and rapidly respond to the presence of dying cells. In this process it very rapidly mobilizes leukocytes to the site of cell injury. Neutrophils are the first cells recruited and these are followed with somewhat delayed kinetics by monocytes. While this process can rapidly combat infection, clear dead cells and ultimately stimulate repair, the inflammatory response can also cause significant collateral damage and thereby contribute to the pathogenesis of many diseases. Remarkably little has been know about how dying cells stimulates inflammation. We will present data that identifies a key pathway required for the sterile inflammatory response to dying cells.

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MOLECULAR REGULATION OF NEUTROPHIL RESPONSIVENESS TO IL-10

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Objective: Neutrophils have been identified as cells able of producing a number of pro- and antiinflammatory cytokines, including TNFα, CXCL8 and IL-1ra in response to specific agonists, for instance LPS, opsonized pathogens or various proinflammatory mediators. In this regard, IL-10 is known to potently modulate neutrophil-derived cytokines, especially following LPS-treatment. However, the mechanisms whereby IL-10 mediates this and other biological effects on neutrophils are not well defined yet. We have recently shown that responsiveness of human neutrophils to IL-10 is dependent upon exposure of these cells to LPS for at least 3-4 hours, a time-frame necessary to determine an upregulation of IL-10R1 expression.
Material and Methods: Real-time RT-PCR and Primary Transcript (PT) real-time RT-PCR, Flow cytometry analysis, Immunoprecipitation and Western blotting.

Data: In this study, we have focused our analysis to the early and late transcriptional events that occur after the addition of IL-10 to neutrophils that have been in vitro cultured for 4 h in the presence of LPS.

Conclusion: Quantitative and kinetic analysis of the effects of IL-10 on TNFα, CXCL8 and IL-1ra gene expression performed in neutrophils pretreated with LPS revealed that IL-10 primarily targets the transcription of TNFα, CXCL8 and IL-1ra gene. Studies to establish whether these transcriptional effects of IL-10 require new protein synthesis, similarly to what prevously described in other cells, are undergoing and will be adequately discussed.

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INFLAMMATION: FROM CELSUS TO CYTOKINES, TOLLS, AND INFLAMMASOMES

Steve Calvano

Two thousand years ago the cardinal signs of inflammation, redness (rubor), heat (calor), swelling (tumor), pain (dolor), were proposed by Celsus. In 1858, a fifth sign, loss of function (functio laesa) was added by Virchow. About the same time, it was shown that white cells accumulate at sites of inflammation. In the 19th and 20th centuries, it was discovered that inflammation could be attenuated by salicylates, adrenocortical steroids and NSAIDs. However, it was not until recently that a comprehensive paradigm underlying inflammation has been revealed. In the early 1980s, Springer characterized three leukocyte surface heterodimers (LFA-1, Mac-1 & p150,95) that were involved in attachment of leukocytes to endothelial cells and/or with binding complement fragments. Shortly thereafter, Rothlein and Springer discovered ICAM-1, the endothelial cell ligand for LFA-1. Contemporaneously, Dinerello and colleagues isolated a potent endogenous pyrogen from activated leukocytes while Tracey, Beutler, Lowry and Cerami showed that a substance produced by activated macrophages could cause cachexia, and profound systemic inflammation and organ failure. Subsequently, it was shown that this substance was identical to tumor necrosis factor-alpha discovered previously by Old. These seminal findings in leukocyte adhesion and cytokine biology led to a quantum leap in understanding the inflammatory response as did the discovery of NFkB transcription factor by Baltimore and colleagues in the mid-1980s. In 1984, Blalock proposed that the immune system acted a sensory organ that could respond to microorganisms by differential production of peptide mediators. At this point in time, immunologists studying the adaptive immune system did

know that specificity resided in antibody and T-cell receptor diversity and clonal selection, but, with a few exceptions, the mechanisms for innate immune system specificity were not apparent. In the late 1980s, this began to change with the discovery of the LPS-binding proteins CD14 and LBP by Wright, Mathison, Tobias and Ulevitch. However, these proteins did not readily reveal a straightforward means by which LPS signaling could occur. It was not until nearly 10 years later that a mammalian homolog of the anti-fungal drosophila Toll was reported by Medzhitov, Preston-Hurlburt and Janeway, and it was subsequently shown by Beutler, Malo and colleagues that the long-utilized LPS hyporesponsive mouse strains had mutations or deletions in the Toll like receptor (TLR) for LPS, TLR4. Shortly thereafter, another large family of intracellularly located pathogen pattern recognition receptors, the NLRs comprised of the NODs and NALPs were recognized. Interestingly, the so-called NALP3 inflammasome can be activated not only by pathogen-associated molecular patterns (PAMPs) but also by damage-associated molecular patterns (DAMPs) thereby fitting in with the concept of an immune system that has evolve to recognize "danger signals", be they exogenous or endogenous, as proposed by Metzinger and colleagues.

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EARLY EVENTS IN INNATE IMMUNITY IN THE RECOGNITION OF MICROBIAL PATHOGENS

Jean-Marc Cavaillon

Humoral factors and cellular receptors are involved in the recognition of microbial pathogens. P. Ehrlich (1854-1915) coined the word "complement" to characterize a heat-labile activity present in plasma that contributes to destroy pathogens. "Alexin" was the name used by J. Bordet (1870-1961) who identified a striking parallel between humoral bacteriolysis and hemolysis. Natural IgM antibodies that interact with C1q to activate the classical pathway leading to the membrane attack complex can mediate the recognition of pathogens. The lectin pathway of complement is initiated by the "mannose binding lectin" that interacts with polysaccharides containing mannose, glucose or N-acetylglucosamine expressed on pathogen surfaces. Alternative pathway is initiated following the interaction of C3 with other sugars or the endotoxin of Gram-negative bacteria.
E. Metchnikoff (1845-1916) proposed the word "phagocytosis" to illustrate a process of cellular microbicidal activity occurring after the recognition of pathogens. The so-called "non-specific immunity" was renamed "innate immunity" when C. Janeway (1943-2003) identified in mammals that the recognition of microbial pathogens occurs thanks to specific receptors fixed in the genome. Evidences were accumulated that innate immunity uses the same building blocks in organisms as diverse as the worm C. elegans, plants, and insects. The Toll molecule, discovered in drosophila by C. Nuesslein-Volhard to be involved in the embryological development of the insect, was shown by J. Hoffmann's team to be a key element in the antifungal innate immunity in Drosophila adults. Toll appeared to be the prototype of a family of molecules (Toll-like receptors, TLR) sensing the signals of danger harbored by all types of pathogens. TLR are either expressed within cells or on their surface. TLR extracellular domains contain leucin rich repeats (LRR) that are involved in the recognition of the "pathogen associated molecular patterns" (PAMPs) [or rather "microbial associated molecular patterns" (MAMPs)]. The plant diseases resistance proteins are another great family of molecules that contain a LRR domain: more than 350 genes exist in Arabidopsis. Among those, around 150 include a nucleotide-binding oligomerization domain (NOD) similar to the domain present in the mammalian NOD-like receptors (NLR) of which certain serve as intracellular sensors for MAMPs. MAMPs recognition by leukocytes through these specific sensors leads to cell activation and the release of cytokines and defensins. In addition to the anti-microbial properties of defensins, peptidoglycan recognition proteins (PGRPs) (four in humans) that are secreted molecules recognizing peptidoglycan of the bacterial surface, also possess antibacterial activity. Cytokines orchestrate the anti-infectious process thanks to their capacity to increase the microbicidal activity of phagocytes, to favor the cellular recruitment within the infectious focus, to boost hematopoiesis, and to induce fever, a primitive anti-infectious mechanism. An excessive stimulation by MAMPs as seen in sepsis can lead to a "cytokine storm" that contribute to numerous deleterious events including organ dysfunction and eventually death. A better knowledge of MAMPs and their respective receptors should help to develop new therapeutic tools in fighting infectious diseases.

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THE COAGULATION-INFLAMMATION CONNECTION IN SEVERE INJURY

Marcel Levi

In the pathogenesis of vascular disease inflammation and coagulation play a pivotal role. Increasing evidence points to an extensive cross-talk between these two systems, whereby inflammation not only leads to activation of coagulation, but coagulation also considerably affects inflammatory activity. The intricate relationship between inflammation and coagulation may not only be relevant for vascular atherothrombotic disease but has also major consequences for the pathogenesis of microvascular failure and subsequent multiple organ failure, as a result of severe infection and the associated systemic inflammatory response.

Molecular pathways that contribute to inflammation-induced activation of coagulation have been precisely identified. Pro-inflammatory cytokines and other mediators are capable of activating the coagulation system and downregulating important physiological anticoagulant

pathways. Activation of the coagulation system and ensuing thrombin generation is dependent on an interleukin-6-induced expression of tissue factor on activated mononuclear cells and endothelial cells and is insufficiently counteracted by tissue factor pathway inhibitor. Simultaneously, endothelial-bound anticoagulant mechanisms, in particular the protein C system, is shut-off by pro-inflammatory cytokines. Tumor necrosis factor-a (TNF-a)-mediated downregulation of thrombomodulin on endothelial cells appears to be a key phenomenon in this respect. In addition, fibrin removal is severely inhibited, due to inactivation of the fibrinolytic system, caused by an upregulation of its main inhibitor, plasminogen activator inhibitor type 1 (PAI-1). Increased fibrin formation and impaired removal leads to (micro)vascular thrombosis, which may result in tissue ischemia and subsequent organ damage.

Modulation of inflammatory activity by activation of coagulation also occurs by various mechanisms. Activated coagulation proteases, such as the tissue factor-factor VIIa complex, factor Xa and thrombin can bind to protease-activated receptors on various cells and the ensuing intracellular signaling leads to increased production of pro-inflammatory cytokines and chemokines. Activated protein C can bind to the protein C receptor on endothelial cells and mononuclear cells, thereby affecting NFkB nuclear translocation and subsequently influencing inflammatory gene expression and inhibition of tissue factor expression on mononuclear cells.

Observations in experimental models of targeted disruption of the protein C gene and restoration of the downregulated protein C pathway by administration of recombinant activated protein C support this notion.

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IMMUNOLOGICAL MEMORY IN CHRONIC INFLAMMATION AND RHEUMATIC DISEASE

Andreas Radbruch

Current therapeutic strategies for the treatment of chronic inflammation are mostly based on immunosuppression, i.e. the suppression of ongoing immune responses. Although efficient, it remains controversial, why they do not provide a cure to many patients. One reason may be that state-of-the-art immunosuppression does not target pathogenetic immunological memory. Immunological memory is a hallmark of the adaptive immune response. It is dependent on signaling between specific T and B lymphocytes. T helper lymphocytes then provide critical signals for the differentiation of activated T and B cells into memory lymphocytes. Upon challenge with the memorized antigen, B and T memory lymphocytes will react faster and their reaction will be functionally imprinted, i.e. independent of the physiological (and therapeutic) regulation of primary immune responses. Already this qualifies them for resistance towards therapies targeting the regulation of primary immune responses. Moreover, apart from this reactive immunological memory, long-lived plasma cells and probably also effector memory T cells can secrete antibodies and cytokines, respectively, without proliferation or any continued instructive signals, making them resistant to any therapy targeting signalling or proliferation. However, the persistence of these effector memory cells depends on a special molecular competence, e.g. the expression of the gene twist1 in memory Th1 cells, and complex signals from their environment, forming distinct survival niches for competent memory cells. Targeting the survival of effector memory cells, in addition to the immunosuppressive targeting of reactive memory, may be critical to eliminate the entire pathogenetic memory of the immune system driving a chronic inflammation. Complete immunoablation and reconstitution of a new tolerant immune system from stem cells has shown that this approach can work in principle.

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NEURODEGENERATIVE DISEASES

Reinhard Hohlfeld

The nervous and immune systems maintain intensive communication. This involves not only anatomical integration by innervation, but also utilizes shared signalling pathways. The longstanding dogma that the central nervous system (CNS) is "immune-privileged" has been replaced by a more modern concept according to which the CNS is constantly patrolled by the immune system. The CNS has its own innate immune system, whose main components are glial cells. Invading immune cells (adaptive and innate) play a major role in initiating, maintaining, resolving, and repairing acute and chronic disorders of the CNS. The most important example of an inflammatory CNS disorder is multiple sclerosis, which is thought to be mediated by autoreactive T and B lymphocytes. Recent findings have shown that immune reactions are also involved in neurodegenerative diseases like like Alzheimer's and Parkinson's disease. In these disorders the immune system seems to have a role in clearing putatively pathogenic protein deposits. Novel therapies are aiming to strengthen the immune system's "waste deposal" function. Preventive and therapeutic strategies are currently being tested in Phase II and III randomized clinical trials.

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INNATE IMMUNITY AND INFLAMMATION IN HUMAN CHRONIC AND SEVERE VIRAL DISEASE

Jordan Orange

The innate immune response to viral infection represents a complex but coordinated series of immunological events. These have the ultimate goals of containing viral replication, limiting cellular proliferation, inducing adaptive immunity and eradicating virus. In the setting of chronic viral disease, an evasion mechanism most typically employed by the virus leads to ineffectual or incomplete virus elimination and can be associated with some degree of resulting persistent innate immune activation. In severe viral disease the immune response may be overly active or incomplete; leading to excessive inflammation, viral replication, or both. Particularly illustrative in understanding the fine balance that exists between the host and pathogen in this context are certain inherent defects of human immunity that either amplify or eliminate components of the antiviral response resulting in severe infection. Equally important are defects in immunity that create states of chronic viral infection and inflammation in settings where the virus would normally be contained or eliminated. Examples of these two paradigms in humans include a number of well-characterized deficits of adaptive immunity that can impair either T cell function or antibody production. Emerging examples giving rise to these paradigms, however, have also been identified as aberrations of innate immunity. These include deficiencies of NF-?B essential modulator (NEMO), signal transducer and activator of transcription (STAT) 1, uncoordinated-93 homolog B (UNC-93B), and specific individual variations in certain toll-like receptors. A common feature of these defects is the abnormal recognition of a viral signature that results in an inappropriate response leading to viral chronicity, excessive viral replication, or inflammation. As a group, the aforementioned defects likely represent a small facet of what will ultimately explain a substantial part of the variability inherent to human viral disease.

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MOLECULAR PATHOGENESIS OF ATHEROSCLEROSIS

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The autoimmune hypothesis of atherogenesis postulates that preexisting cellular and humoral immunity to either microbial heat shock protein 60 (HSP60) or bona fide autoimmunity to biochemically altered autologous HSP60 leads to an attack on stressed arterial endothelial cells (ECs). We have shown in various animal models with spontaneously occurring autoimmune diseases that two essential sets of genes have to be present for an autoimmune disease to develop, i.e. genes that code for autoimmune reactivity of the immune system and genes that are responsible for target organ susceptibility. In the case of atherosclerosis, the arterial ECs express HSP60 that is also transported to the cell surface after being subjected to classical atherosclerosis risk-factors. We have shown the HSP60-inducing effect of most of these risk-factors, including mechanical stress (hypertension), oxygen radicals, oxidized low-density lipoproteins (oxLDL), proinflammatory cytokines (TNFα), and cigarette smoke constituents. Exposure to these classical atherosclerosis risk-factors entail the simultaneous expression of HSP60 and various adhesion molecules (ICAM-1, VCAM-1, P-selectin). Due to their lifelong mechanical pre-stress by the arterial blood pressure, arterial ECs have a lower threshold for the HSP60 inducing effect of atherosclerosis risk-factors as compared to venous ECs. However, when veins are subjected to arterial blood flow conditions, e.g. after arterial-venous bypass operations, HSP60 expression and intimal infiltration with mononuclear cells with subsequent restenosis occurs similar to the pathogenetic events in classical atherosclerosis. During the past years part of our work has been devoted at elucidating the molecular mechanisms underlying the vascular damage induced by cigarette smoke as the most important atherosclerosis risk factor. Similar to the observations with other risk factors, cigarette smoke first acts as an endothelial stressor leading to the expression of HSP60 thus rendering the endothelium a target susceptible to the attack of preexisting innate and adaptive anti-HSP60 immune reactions. Supported by the Austrian Research Fund (P14741) and the EU-funded European Vascular Genomics Network (EVGN).

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THE ROLE OF TLRS IN THE IMMUNADRENAL CROSS TALK

Kai Zacharowski

Sepsis and septic shock remain major health concerns worldwide and rapid activation of adrenal steroid release is a key event in the organism's first line of defense during this form of severe illness. There is evidence that failure of the hypothalamic-pituitary-adrenal (HPA) axis is a cofactor for sepsis outcome leading to an uncontrolled immune response. The family of Toll-like receptors (TLRs) are critical in the early immune response upon bacterial infection, and particularly TLR2 and TLR4 polymorphisms are frequent in humans.

During sepsis, relative adrenal insufficiency may occur in a substantial number of patients and is responsible for increased mortality. Replacement therapy with low-dose hydrocortisone during septic shock or acute respiratory distress syndrome showed improved survival. At present, inadequate adrenal function as well as glucocorticoid treatment during sepsis is one of the most discussed topics in medicine.

In addition to hypothalamic hormones including corticotropin-releasing hormone and vasopressin, inflammatory cytokines such as interleukin (IL)-1, IL-6 and tumor necrosis factor α have been identified as important modulators of HPA axis function. During inflammation, these cytokines are capable of maintaining high glucocorticoid output, suggesting a shift from neuroendocrine to immune-endocrine regulation of the adrenal during septicaemia. In turn, enhanced adrenal glucocorticoid release is required to prevent an uncontrolled response of inflammatory cytokines, which could result in severe damage to the cardiovascular system. Therefore, a coordinated response of the adrenal and immune system is crucial for survival during severe inflammation.

Recent data from our lab demonstrate a novel link between the innate immune system and the endocrine stress response mediated by TLRs. TLR2, TLR4 and TLR9 are expressed in human and mice adrenals and TLR2 and TLR4 deficiency is associated with an impaired glucocorticoid response (1-4).

In conclusion, TLRs play a crucial role in the immune-adrenal crosstalk. This close functional relationship needs to be considered in the treatment of inflammatory diseases requiring an intact adrenal stress response.

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TLR9 IS ACTIVATED BY MULTIPLE CONFORMATIONAL CHANGES

Eicke Latz

Toll-like receptors (TLR) are a conserved family of type I transmembrane receptors that recognize diverse microbial products and can respond to endogenous molecules that signal the presence of tissue damage or infections. TLR9 recognizes CpG-rich microbial DNA and self-DNA present in immune complexes. Activation of TLR9 initiates innate immune responses and the production of inflammatory cytokines and type I interferons. Erroneous or excessive receptor activation can have detrimental effects with serious consequences such as is seen in autoimmune disorders. Thus, pharmacological modulation of TLR receptor activation has impact on the therapy of inflammatory diseases. We have analyzed the mechanism of TLR9 activation by a combination of biochemical and imaging methods and demonstrated that unliganded TLR9 homodimers exist in a conformation that prevents activation. Ligand binding induced conformational changes that resulted in close approximation of the cytoplasmic signaling domains, which likely enables adapter molecule recruitment. Our results demonstrate that dimerization is not sufficient for TLR9 activation and that TLR9 activity is regulated and tightly controlled by the nature and sequence of the DNA ligand. This molecular activation mechanism explains how different DNA sequences can quantitatively tune TLR9 responses and suggests the possibility to develop partial or complete TLR9 receptor antagonists.

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AUTOIMMUNE RECOGNITION OF DNA BY TOLL RECEPTORS

Terry Means

DNA is normally sequestered from the immune system inside the nuclear membrane. However, during an infection or due to a lack of clearance of DNA by host cells, nucleic acids can be released by microbes or dying cells. This nucleic acid material can activate immune cells and trigger the production of cytokines and chemokines. Activation of cells depends on the ability of the cell to internalize RNA or DNA and deliver it to intracellular vesicles containing TLR7/8 and TLR9, respectively. Systemic lupus erythematosus (SLE) is characterized by the presence of autoantibodies directed against DNA and RNA binding proteins. We and others have found that DNA- and RNA-autoantibody immune complexes purified from SLE patient sera stimulate dendritic cells. This activation was shown to require the expression of CD32 (Fc receptor gamma IIa), which binds and delivers RNA- and DNA-containing immune complexes to intracellular lysosomes containing TLR7/8 and TLR9, respectively. This TLR7,8,9/CD32 pathway used by dendritic cells is distinct from that used by B cells, in which the B cell receptor is required for delivery of RNA or DNA to intracellular vesicles containing TLR7/8/9. Ongoing studies are underway to examine the mechanism of delivery and the activation of other cell types by nucleic acid-containing immune complexes.

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TOLL-LIKE RECEPTORS IN SEPSIS: TLR2 IMPAIRS HOST DEFENSE IN GRAM-NEGATIVE SEPSIS CAUSED BY BURKHOLDERIA PSEUDOMALLEI

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Objective: Toll-like receptors (TLRs) are essential in host defense against pathogens by virtue of their capacity to detect microbes and initiate the inflammatory response. TLR2 is seen as the most important receptor for Gram-positive bacteria while TLR4 is regarded as the Gram-negative TLR. Melioidosis is a severe infection caused by the Gram-negative bacterium Burkholderia pseudomallei that is endemic in SE-Asia. We aimed to characterize the expression and function of TLRs in septic melioidosis.

Materials and Methods: Subjects: Patients with sepsis caused by B. pseudomallei and male wild-type (WT), TLR2 KO and TLR4 KO C57BL/6 mice intranasally infected with a lethal dose of B. pseudomallei.

Data, Patient studies: 34 patients with melioidosis demonstrated increased expression of CD14, TLR1, TLR2 and TLR4 on the cell surface of monocytes and granulocytes and increased CD14, TLR1, TLR2, TLR4, MD-2, TLR5 and TLR10 mRNA levels in purified monocytes and granulocytes when compared with healthy controls. In vitro experiments: Whole blood and alveolar macrophages obtained from TLR2 and TLR4 knockout (KO) mice were less responsive to B. pseudomallei in vitro, whereas in the reverse experiment transfection of HEK 293 cells with either TLR2 or TLR4 rendered these cells responsive to this bacterium. Mouse studies: Surprisingly, TLR4 KO mice were indistinguishable from WT mice with respect to bacterial outgrowth and survival in experimentally induced melioidosis. In contrast, TLR2 KO mice displayed a markedly improved host defense as reflected by a strong survival advantage together with decreased bacterial loads, reduced lung inflammation and less distant organ injury.

Conclusion: Severe sepsis caused by B. pseudomallei can probably be seen as the clinical manifestation of a TLR mediated dysregulation of the inflammatory response to this invasive pathogen. In addition, our data undermines the paradigm regarding TLR4 as "the Gram-negative receptor" and TLR2 as "the Gram-positive receptor". Patients with septic melioidosis display an upregulation of multiple TLRs in peripheral blood monocytes and granulocytes. Although both TLR2 and TLR4 contribute to cellular responsiveness to B. pseudomallei in vitro, only TLR2 impacts on the immune response of the intact host in vivo. Inhibition of TLR2 may be a useful adjunctive therapy for sepsis caused by B. pseudomallei.

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MRP8 AND MRP14 ARE NOVEL ENDOGENOUS LIGANDS OF TOLL-LIKE RECEPTOR 4 AND PROMOTE LETHAL ENDOTOXIN-INDUCED SHOCK

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Objective: Lipopolysaccharide (LPS) is an effective trigger of the inflammatory response during Gram-negative infections. An uncontrolled activation of LPS-induced mechanisms results in sepsis, septic shock, or systemic inflammatory response syndrome (SIRS). Myeloid related protein 8 (MRP8, S100A8) and MRP14 (S100A9) are the most abundant cytoplasmic proteins of phagocytes and are released during inflammatory activation of these cells. Analysing the physiological functions of these molecules by targeted gene deletion we identified these proteins as novel components promoting the inflammatory cascade during sepsis.

Material and Methods: MRP14 -/- mice were established by targeted gene deletion and the inflammatory response of these mice was investigated during LPS-induced lethal shock and E. coli-induced abdominal sepsis. Cytokine levels were determined by ELISA. Toll-like receptor 4 (TLR4) specific effects were confirmed by use of phagocytes obtained from TLR4 mutant mice and by transfection of TLR4 in HEK293 cells. Activation of signal transduction pathways was investigated by analyzing rearrangement of the adaptor protein MyD88, kinase activities as well as activation of transcription factors by Chromatin-Immuno-Precipitation (ChIP).

Data: Targeted deletion of the MRP14 gene results in a functional loss of both MRP8 and MRP14 proteins probably due to a higher turnover of isolated MRP8 in absence of its binding partner MRP14. We demonstrate that mice lacking MRP8/MRP14 are protected from LPS-induced lethal shock and E. coli-induced abdominal sepsis. Extracellular MRP8/MRP14 amplify LPS-triggered inflammatory responses of phagocytes. MRP8 is the active component of the complex. MRP8 activates a classical signalling cascade involving intracellular translocation of MyD88, activation of IRAK-1, activation of the mitogen activated protein kinase pathways ERK1/2 and p38 and enhances DNA-binding activity of NF-κB. Activation of this signalling pathway by MRP8 results in elevated expression of inflammatory molecules, e. g. TNFα. Using both, phagocytes obtained from mice expressing a non-functional Toll-like-receptor 4 (TLR4) or HEK293 cells transfected with TLR4/CD14/MD2 as well as TLR4-specific antibodies and antagonists we demonstrate that MRP8 specifically interacts with this receptor complex thus representing a novel endogenous ligand of TLR4.

Conclusion: We demonstrate for the first time that the major cytoplasmic proteins in granulocytes and monocytes, MRP8 and MRP14, are endogenous ligands of TLR4 and play an important role in the pathogenesis of sepsis upstream of TNFα-actions. The danger associated molecular pattern (DAMP) mechanism is known to play a

pivotal role in the pathogenesis of sepsis and other inflammatory reactions. Thus, MRP8/MRP14 complexes are inflammatory molecules representing new members in view of the DAMP mechanism.

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HIGH MOBILITY GROUP BOX 1 (HMGB1) INDUCES TOLL-LIKE RECEPTOR 4 (TLR4) CLUSTERING WITHIN THE LIPID RAFT IN MACROPHAGES

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Introduction: High Mobility Group Box 1 (HMGB1) is a 30 kD DNA-binding protein that may be released from cells during periods of stress or injury, allowing it interact with pattern recognition receptors (PRR's) on other cells and exert its pro-inflammatory cytokine-like effects. HMGB1 is known to interact with multiple PRR's including the receptor for advanced glycation endproducts (RAGE), toll-like receptor (TLR) 2 and TLR4. It has been previously demonstrated that optimal LPS signaling through TLR4 requires an accumulation of signaling molecules within the lipid raft. We hypothesize that HMGB1 stimulates migration of TLR4 and its co-receptors to the lipid raft. Further, we hypothesize that optimal HMGB1-dependent TLR4 activation requires an intact lipid raft.

Methods: RAW 264.7 cells were stimulated with recombinant HMGB1 1 μg/ml, LPS 10ng/ml, or PBS for 60 minutes. Cells were lysed and subjected to sucrose gradient fractionation. Fractions were run on SDS-page and raft fractions were identified by immunoblot for Flotillin-1. Immunoblots were preformed on the same fractions for TLR4, MD2 and CD14. RAW 264.7 cells then were pre-treated with Nystatin 60μg/ml, filipin 1μg/ml or PBS prior to stimulation as above. Nuclear protein was harvested for analysis of NF-κB DNA-binding via EMSA.

Results: After sucrose gradient fractionation, fractions containing lipid rafts were successfully identified by immunoblot for flotillin-1. Unstimulated cells demonstrated the presence of TLR4 and MD2 exclusively in non-raft fractions, while CD14 was found in both raft and non-raft fractions at baseline and after stimulation with HMGB1. After stimulation with HMGB1, significant translocation of TLR4 and MD2 to raft fractions was noted. NF-κB was activated in RAW cells stimulated with rHMGB1. This activation was significantly decreased in those cells pre-treated with Nystatin or filipin, two agents used to disrupt lipid rafts. Recombinant HMGB1 was made in yeast and has no detectable LPS contamination.

Conclusions: These data demonstrate that TLR4/MD2 clustering within the lipid raft occurs in macrophages after stimulation with HMGB1. Further, disruption of the lipid raft interferes with HMGB1-dependent NF-κB activation. Additional work is necessary to investigate what intracellular TLR4 adaptor molecules accumulate within the lipid raft after HMGB1 stimulation.

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OXLDL DIFFERENTIALLY INFLUENCES TOLL-LIKE RECEPTORS 2 AND 4

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The mechanisms by which the innate immune recognition of pathogens could lead to atherosclerosis remain unclear. Oxidized LDL is a key player in the pathogenesis of atherosclerosis. In this study we evaluated the interactions of Toll-like receptors with oxidized LDL. Using MM6 cells stimulated with TLR-agonists and incubated with oxidized LDL (oxLDL), we show that TLR-agonist dependent cell activation might be differentially modulated by oxLDL. Oxidized LDL was found to be able to inhibit TLR2-agonist-induced responses, whereas in the case of TLR4-ligands it seemed to suppress cytokine responses initially but subsequently augmented the response. In order to understand the molecular mechanisms involved, we investigated receptor associations in the presence and absence of oxLDL. In the case of TLR2, ox-LDL seemed to inhibit TLR2-TLR6 and CD36 associations in response to bacterial LTA. Similarly in the case of TLR4, oxLDL was found to initially inhibit the formation of the "LPS-sensing machinery", but in time the receptors were able to recover and form functional receptor complexes. The present data demonstrate an involvement of both TLRs and oxidized LDL on cell stimulation and subsequent cytokine secretion. Our results add to the body of literature that suggests an important role for the innate immune system for the initiation, development and, possibly even for later events such as plaque fissure and rupture with the consequence of myocardial infarction.

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TOLL-LIKE RECEPTOR-4 INITIATES THE INNATE IMMUNE RESPONSE OF THE KIDNEY TO RENAL ISCHEMIA-REPERFUSION INJURY

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OBJECTIVE: The mammalian Toll-like receptors (TLRs) are a family of conserved pattern recognition receptors that detect motifs of pathogens and endogenous (stress) molecules present during injury. It is already shown that there is constitutive renal TLR4 mRNA

expression, which is enhanced upon renal ischemia-reperfusion injury (IRI). However, the role of this organ-specific upregulation of TLR4 is unknown. In this study we investigated the specific role of TLR4 and its two downstream signaling cascades - MyD88-dependent and -independent (TRIF) - in renal ischemia-reperfusion injury in mice.

METHODS: We used TLR4-/-, MyD88-/-, TRIF mutant and Wt mice (n=8/group) that were subjected to bilateral clamping of the renal arteries for 45′ followed by reperfusion. After 1, 5 and 10 days, TLR4-/- and Wt mice were sacrificed for determination of cytokines (keratinocyte chemoattractant (KC), Tumor Necrosis Factor-α, Interleukin-1β by ELISA), renal neutrophil influx, and renal function (serum ureum/creatinine). MyD88-/- and TRIF mutant mice underwent the same procedure, and were sacrificed 1 day after surgery. Sham-operated animals served as controls. Values are expressed as mean±SEM. Statistics were done by student t-test.

RESULTS: One day after IRI induction, TLR4-/- mice had a more preserved renal function compared with Wt mice as reflected by significant lower serum ureum (42.87±5.09 vs. 25.71±3.43 mmol/l) and creatinine (87.25±18.32 vs. 38.00±3.04 μmol/l) levels ($p<0.05$). At 5 and 10 days after IRI, serum ureum/creatinine concentrations decreased to basal levels in both TLR4-/- and Wt mice and were not significantly different. We found that TLR4 plays a proinflammatory role after IRI, as reflected by significantly reduced amounts of interstitial neutrophils in kidneys of TLR4-/- mice compared to Wt mice (11.32±2.87 vs. 25.61±3.49 Ly6G+ cells/field after 1day, $p<0.05$). Moreover, after 1 day the level of KC was twofold reduced in TLR4-/- kidneys compared with Wt (53.94±7.76 vs. 24.89±3.38 pg/mg protein, $p<0.05$). The cytokines TNFα and IL-1β did not show a difference. Interestingly, 1 day after IRI MyD88-/- mice showed a tendency towards lower serum ureum (62.70±2.06 vs. 47.34±9.58 mmol/l) and creatinine (265.25±16.11 vs. 178.57±40.63 μmol/l) levels compared with their Wt, whereas in TRIF mutant mice the renal function was preserved as reflected by their ureum (47.86±4.84 vs. 50.64±0.70 mmol/l) and creatinine (161.33±24.36 vs. 155.20±8.33 μmol/l) values.

CONCLUSION: Taken together, these results suggest that TLR4 initiates an exaggerated pro-inflammatory response -most likely via the MyD88-dependent route - during renal ischemia-reperfusion injury in mice leading to renal dysfunction. This study strongly supports the idea that the function of TLR4 extends beyond host defense against invading pathogens. TLR4 appears to be capable of monitoring ischemic damaged kidney, and as a result is crucial for activating innate immune responses in this organ.

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ROLE OF TOLL-LIKE RECEPTOR 9 IN IMMUNE-ADRENAL RESPONSE TO CPG OLIGODEOXYNUCLEOTIDES

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Objective: Sepsis is a leading cause of death in intensive care units. There is evidence that failure of the hypothalamic-pituitary-adrenal (HPA) axis is a cofactor for sepsis outcome leading to an uncontrolled immune response. Within the immune response, Toll-like receptors (TLRs) play a crucial role by recognising pathogen-associated molecules such as bacterial DNA. TLR-9 can detect motifs of unmethylated CpG dinucleotides (CpG-DNA) being present in bacterial DNA. Here, we investigated whether TLR-9 plays a role in the immune-adrenal response in vivo.

Materials and Methods: Wild-type (WT; C57BL/6) and TLR-9 deficient (TLR-$9^{-/-}$) mice, both sensitized with D-galactosamine (D-GalN), were challenged with saline (1 mL/kg) or CpG-ODN (1 nmol/g) intraperitoneally and observed for 15min up to 6hrs. Then tissue and plasma samples were taken. TLR-9 expression, NF-κB activity, corticosterone and ACTH levels as well as various cytokines were determined.

Data

TLR-9 is expressed in murine adrenal glands (mRNA and protein). CpG-ODN challenge did not alter the expression of TLR-9 in adrenal glands. Baseline plasma levels of corticosterone and ACTH were similar in WT and TLR-$9^{-/-}$ mice. CpG-ODN challenge caused a three-fold increase in plasma levels of corticosterone in WT mice. This effect was abolished in TLR-$9^{-/-}$ mice. Furthermore, CpG-ODN challenge resulted in a strong release of several inflammatory cytokines, such as tumor necrosis factor-α and interleukin-1β, -6, -10 and -12. Again, this effect was abolished in TLR-$9^{-/-}$ mice.

Conclusion: TLR-9 is present in the murine adrenal gland and upon stimulation corticosterone and inflammatory cytokines are produced. TLR-9 could be an additional player in the regulation of the HPA-axis during conditions where bacterial DNA is present.

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PHOSPHOINOSITIDE 3-KINASE INHIBITION NEGATIVELY REGULATES BOTH TLR2- AND TLR4-MEDIATED PROINFLAMMATORY RESPONSES IN MURINE PERITONEAL MACROPHAGES

Edward McSwiney, Jiang Huai Wang, H.Paul Redmond

Introduction: Phosphoinositide 3-kinase (PI3K), a key enzyme in cell proliferation, survival and differentiation, has important functions in immune responses. TLR2 and TLR4 signalling both activate the PI3K/Akt pathway in monocytes/macrophages. However, the role of PI3K/Akt pathway in modulating TLR2 and TLR4 signalling is still controversial. This study was designed to examine whether PI3K/Akt pathway is involved in TLR2- and TLR4-mediated activation of proinflammatory responses.

Methods: Purified C57BL/6 murine peritoneal macrophages were pre-treated with the pharmacological PI3K-inhibitor LY294002 (10, 25, and 50 μM) for 1 hour and further stimulated with TLR2 agonist bacterial lipoprotein (BLP) (1,000 ng/ml) or TLR4 agonist lipopolysaccharide (LPS) (1,000 ng/ml) for 16 hours. Proinflammatory cytokines TNF-α and IL-6 in the culture supernatants were assessed by ELISA. Statistical analysis was performed using MINITAB (version 13.32).

Results: Both BLP and LPS stimulation resulted in marked increases in proinflammatory cytokines TNF-α and IL-6 release from murine peritoneal macrophages. Inhibition of PI3K/Akt pathway with LY294002 significantly reduced BLP-induced TNF-α (1667±872 Vs 408±200 pg/ml) and IL-6 (1821±254 Vs 476±303 pg/ml) (p=0.0005) production. A downregulated proinflammatory cytokine production following LPS stimulation in murine macrophages was also observed when PI3K/Akt pathway was blocked by LY294002.

Conclusion: These results indicate that the PI3K/Akt pathway is a positive regulator in both TLR2- and TLR4-mediated proinflammatory responses. Thus inhibition of PI3K/Akt pathway may represent a potential therapeutic target for preventing the development of fulminant septic shock.

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MORAXELLA CATARRHALIS IS INTERNALIZED IN RESPIRATORY EPITHELIAL CELLS BY A TRIGGER-LIKE MECHANISM AND INITIATES A TLR2- AND PARTLY NOD1-DEPENDENT INFLAMMATORY IMMUNE RESPONSE

Hortense Slevogt, Krishna Tiwari, Sebastian Bachmann, Norbert Suttorp, Bastian Opitz

Moraxella catarrhalis is an important pathogen in patients with chronic obstructive lung disease (COPD). While M. catarrhalis has been categorized as an extracellular bacterium so far, the potential to invade human respiratory epithelium has not yet been explored. Our results obtained by electron and confocal microscopy demonstrated a considerable potential of M. catarrhalis to invade bronchial epithelial (BEAS-2B) cells, type II pneumocytes (A549) and primary small airway epithelial cells (SAEC). Moraxella invasion was dependent on cellular microfilament as well as on bacterial viability, and characterized by macropinocytosis leading to the formation of lamellipodia and engulfment of the invading organism into macropinosomes, thus indicating a trigger-like uptake mechanism. In addition, the cells examined expressed TLR2 as well as NOD1, a recently found cytosolic protein implicated in the intracellular recognition of bacterial cell wall components. Importantly, inhibition of TLR2 or NOD1 expression by RNAi significantly reduced the M. catarrhalis-induced IL-8 secretion. The role of TLR2 and NOD1 was further confirmed by overexpression assays in HEK293 cells. Overall, M. catarrhalis may employ lung epithelial cell invasion to colonize and to infect the respiratory tract, nonetheless, the bacteria are recognized by cell surface TLR2 and the intracellular surveillance molecule NOD1.

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TLR2 POLYMORPHISM ARG753GLN IMPAIRS CYTOKINE RESPONSE AGAINST CANDIDA BUT NOT AGAINST STAPHYLOCOCCUS DURING SEPSIS

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Objective: Out of the known 12 mammalian Toll-like receptors (TLRs), TLR2 shows the widest range of ligands which is in part due to its ability to heterodimerize with TLR1 and TLR6. As a pattern recognition receptor for LTA derived from Staphylococcus and mannan from Candida, TLR2 induces the MyD88-dependent intracellular pathway, activates NF-kB and leads to the release of inflammatory cytokines. Two single nucleotide polymorphisms (SNPs) located to the Toll/Interleukin-1 receptor (TIR) domain of TLR2 have been reported to affect cellular activation: Arg677Gln and Arg753Gln. We asked

whether these SNPs play a role in the pathogen induced cytokine response in vivo, manifestation of sepsis, and outcome.

Patients and Methods: Thirty-eight ICU patients were studied for their clinical course, infectious complications, inflammatory cytokines and TLR2 genotype. Genomic DNA was extracted from whole blood, and TLR2 SNPs were determined by pyrosequencing. Blood samples were taken for blood culture analysis. Plasma was used to determine cytokines by multiplexing and Luminex technology.

Results: Cytokine patterns in TLR2 SNP753 heterozygous and wild type patients during Gram-positive and fungal sepsis were compared. SNP753 heterozygous patients were found to release the same cytokine patterns as wild type patients during Gram-positive and Gram-negative sepsis episodes. However, during systemic Candida infection, SNP753 heterozygosity was associated with a lack of IFNγ production and a remarkable reduction of cytokine responses as compared to the Candida infected SNP753 wild type cohort.

Conclusion: The TLR2 SNP753 appears to modulate the inflammatory cytokine response to Candida, but has no effect on biomarker patterns released during Gram-positive sepsis. SNP753 may contribute to pathogen-induced immunological non-responsiveness associated with Candida infections.

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COMPARATIVE CHARACTERIZATION OF THE IMMUNOSTIMULATORY CAPACITY OF SPORES FROM DIFFERENT FUNGAL SPECIES IN HUMAN WHOLE BLOOD

Mardas Daneshian, Mardas Daneshian, Thomas Gabrio, Thomas Hartung, Sonja von Aulock

Objectives: Fungal spores are clinically relevant contaminants as they are ubiquitous and inhalable. In addition to well-known human-pathogenic fungi, like Candida albicans and Aspergillus fumigatus, non-pathogens - particularly moulds - can become dangerous to human health. Cell wall structures such as glucans and mannans seem to play an important role in inflammatory processes but no defined common structure could yet be attributed with the inflammatory potential of fungi.

Material and Methods: The immunostimulatory capacity of spores of 44 mould species was characterized and compared in human whole blood incubations with respect to cytokine induction. As environmental exposure to fungal spores occurs mainly via the lung, we compared the response of human blood with that of a murine alveolar macrophage cell line (MH-S). Furthermore we investigated the role of TLR-2 and TLR-4 in the recognition of mould spores.

Data: The different species showed a uniform pattern regarding the induction of the proinflammatory cytokines TNF, IL-1, IL-6 and IL-8 but more variation with regard to G-CSF, IL-10 and IFNg. All of the moulds showed a partial dependency on TLR2 but not on TLR4 for cytokine induction, as assessed using cells from knock-out and mutant mice in comparison to the respective controls. The yeasts showed a partial dependency on both TLR2 and TLR4. Comparison of the response of 16 different blood donors showed a highly consistent pattern: although the absolute response of the blood donors varied, their relative response to the different spores was comparable. The difference in the absolute response of the donors could be attributed to their different monocyte counts. TNF release by murine alveolar macrophages was comparable with that of human whole blood.

Conclusion: Together, these data suggest that conserved surface structures on spore surfaces may represent common immunostimulatory epitopes that trigger inflammatory cytokine release by blood monocytes.

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EXCESSIVE ENDOGENOUS SYSTEMIC NO PRODUCTION IS NOT SUFFICIENT TO CAUSE SHOCK, MORBIDITY OR MORTALITY

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Purpose: Excessive NO production plays a key role in the systemic vasodilatation and hypotension characteristic of (septic) shock. Indeed, NOS inhibition prevents hypotension in various animal shock models and in patients. Nevertheless, is endogenous NO production sufficient to cause shock?

Methods and results: LPS signals through TLR4 and is generally used as a model for septic shock. As expected, we found that TLR4 deficiency (C3H/HeJ or C57BL/10ScNJ mice) completely abrogates LPS-induced production of TNF, IL-6 or IL-1b. In contrast, however, phenol extracted LPS (from Sigma, generally used in endotoxic animal research) induced iNOS and NO in TLR4-deficient mice to the same level as in control animals, while ultra pure LPS (from Invivogen) did not. These data clearly indicate the presence of a contaminant in phenol extracted LPS, capable of inducing high levels of endogenous NO. In addition, this contaminant has a profound synergistic effect on LPS-induced cytokine production. To identify the contaminant, we used mice (singly or doubly) deficient for different TLRs or adaptor molecules. These experiments demonstrated that the Sigma LPS contaminant is a TLR2 agonist. In addition, we are currently investigating the ability of various TLR or non-TLR agonists (e.g. synthetic lipopeptides, CpG and peptidoglycan motifs) to induce iNOS/NO, cytokines and shock in wild type mice.

Summary: Sigma LPS preparations contain a TLR2 stimulating contaminant. This contaminant induces high levels of circulating NO, and has a profound synergistic effect on LPS-induced cytokine production. Despite the induction of excessive NO, Sigma LPS does not cause any hypotension or morbidity in TLR4-deficient mice. These results indicate that, despite the pivotal role of NO in refractory hypotension during (septic) shock, NO is by itself not sufficient and requires additional factor(s) to cause hemodynamic disturbances and damage the host. The identification of these additional factors is currently under investigation

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SYNERGIC RECOGNITION OF HUMAN RHINOVIRUSES BY TOLL-LIKE RECEPTORS AND MDA-5

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The early detection of invading viruses by the host depends on their identification by pathogen sensors. These include Toll like receptors (TLRs) as well as cytoplasmic RNA helicases such as retinoic acid inducible protein I (RIG-I) and melanoma differentiation associated gene 5 (MDA-5). These pathogen sensors recognise specific molecular patterns found in viruses and trigger inflammatory and antiviral responses that result in the eradication of invading pathogens. In this study we investigated the specific recognition of Human rhinovirus 6 (HRV6) by the innate immune response. Our results show that the initial HRV-induced inflammatory response by the host is due to a synergic effect mediated through several TLRs and then by MDA-5. We established that in the first stages of HRV6 infection the TLRs play a crucial role in HRV recognition and that different constituents of HRV6 are recognised by different TLRs. The HRV6 capsid is recognized via TLR2, whereas upon HRV6 ssRNA internalisation the virus genome is recognised by TLR7 and TLR8. Once viral replication begins to generate dsRNA the type I IFN inflammatory response is augmented and mediated by MDA-5. The combined recognition of human rhinoviruses by these PAMPs and their up-regulation concurs with the huge inflammatory response seen in the common cold.

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OVEREXPRESSION OF IRAK-1, BUT NOT TLR2, PARTLY REVERSES BACTERIAL LIPOPROTEIN-INDUCED TOLERANCE

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Background: Tolerance to bacterial cell wall components including bacterial lipoprotein (BLP) represents an essential regulatory mechanism during bacterial infection. Our previous work has demonstrated that induction of BLP tolerance in vivo protects mice against both BLP or LPS-induced lethal shock and polymicrobial sepsis. In vitro we have shown that the reduced protein expression of TLR2 and IL-1 receptor-associated kinase-1 (IRAK-1) is associated with BLP tolerance. In order to further elucidate the molecular mechanisms involved in BLP tolerance, we sought to examine whether the downregulation of TLR2 and IRAK-1 expression is responsible for the development of BLP tolerance, by overexpressing either TLR2 or IRAK-1.

Methods: HEK293-hTLR2 cells, which stably overexpress human TLR2, were co-transfected with pF-kB-Luc reporter plasmids and pcDNA3-IRAK-1 expressing vector. Human THP-1 monocytic cells were also used and co-transfected with pF-kB-Luc reporter plasmids, in combination with either pcDNA3-Flag-TLR2 or pcDNA3-IRAK-1 expressing vector.

Results: BLP stimulation induces a dose-dependent NF-kB activation in HEK293-hTLR2 cells. However, BLP tolerance, as evidenced by an inhibited NF-kB activation, was observed in these cells despite their overexpression of TLR2. In contrast, overexpression of IRAK-1 partly reverse BLP tolerance, as transfection of IRAK-1 expressing vector resulted in a dose-dependent NF-kB activation in BLP-tolerised HEK293-hTLR2 cells. Similar results were also demonstrated in human THP-1 monocytic cells in which overexpression of IRAK-1, but not TLR2, activated NF-kB in BLP-tolerised THP-1 cells in an IRAK-1 dose-dependent manner.

Conclusion: Our results have shown that overexpression of TLR2 does not prevent BLP tolerance, whereas overexpression of IRAK-1 partly reverses BLP tolerance. This indicates a crucial role of IRAK-1 in BLP-induced tolerance, making it a potential target for future therapeutic strategies in bacterial infection and sepsis.

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STOCHIOMETRY OF TOLL-LIKE RECEPTOR 4 WITHIN THE LPS-SENSING APPARATUS

Kathy Triantafilou, Martha Triantafilou, Marios Mouratis, Frederick Gamper

The innate immune system utilises a trimolecular complex of receptors, comprising of TLR4, CD14 and MD2, in order to sense bacterial products. Even though the components of the mammalian LPS "sensing machinery" have been identified, our understanding of the initial events that take place as LPS engages its receptor(s) still unclear. An important question that still remains is what is the stochiometry of TLR4 in the "LPS-sensing apparatus". In the current study, we set out to investigate the specific oligomeric state of TLR4 on living cells before and after stimulation by bacterial LPS using Single particle fluorescent imaging (SPFI), which is a non-invasive biophysical method. Our data demonstrates that TLR4 exists as monomers or dimmers on unstimulated cells, and upon ligation by LPS, it is induced to form stable homodimers and tetramers that are crucial for cell activation. This study demonstrates for the first time, the stochiometry of TLR4 within the "LPS-sensing apparatus" and shows that ligand binding seems to induce dimmer or tetramer formation enabling stable ectodomain receptor-receptor interaction allowing downstream signalling to occur.

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UPTAKE OF LIPOPOLYSACCHARIDE BY HEPATOCYTES AND LIVER IS DEPENDENT ON CD11B/CD18 ACTIVATION OF P38MAPK

Melanie Scott, Hong Liao, Timothy Billiar

Introduction: The liver is the main organ that clears lipopolysaccharide (LPS), and hepatocytes (HC) are a major cell-type involved in this clearance. HC express known elements of the LPS-receptor: CD14, MD2, and TLR4. The β2-integrin, CD11b/CD18, has also recently been shown to play a specific role in LPS-signaling. In this study we investigated the role of CD11b/CD18 in LPS-uptake into mouse HC.

Methods: CD11b and CD18 expression was confirmed in HC by RT-PCR and by Western blot using RAW cells as positive controls. HC were isolated from WT and CD11bko mice and plated on collagen-coated coverslips, given 100ng/mL Alexa 488 Fluor LPS for 0-90min before fixation and visualization. WT and CD11bko mice were also given 5mg/kg Alexa 488 Fluor LPS into the portal vein in vivo. Liver was harvested, fixed and sectioned after 0-90min. Lipid raft fractions were isolated from HC before and after stimulation with 100 ng/mL LPS. Proteins were separated by SDS-PAGE and identified by Western blotting.

Results: LPS was taken up by WT HC and liver with maximal fluorescence at 60-90min. CD11bko hepatocytes and liver did not take up any significant amount of LPS. CD11bko HC also did not activate p38MAPK in response to LPS, shown by our previous studies to be crucial for HC LPS-uptake. Lipid rafts provide stable areas of plasma membrane for receptor interactions and signaling. Pretreatment with lipid raft disruptors (nystatin/filipin) blocked uptake of LPS into hepatocytes. CD14 was resident in lipid rafts, whereas TLR4 became associated early after LPS-stimulation. p38MAPK associated with lipid rafts in resting cells, but less so after LPS-stimulation. CD11b was in all fractions of resting and LPS-stimulated cells. However, CD18 was mainly in non-lipid raft fractions in resting cells, but moved to lipid rafts as early as 5min after LPS stimulation.

Conclusions: CD11b/CD18 is essential for uptake of LPS into hepatocytes. CD11b/CD18 associates with other components of the LPS-receptor in the lipid raft, and enables activation of p38MAPK for LPS-uptake.

Work supported in part by funding from NIH grant R01-GM-50441

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TOLL-LIKE RECEPTOR 4 (TLR4) MEDIATES SYSTEMIC INFLAMMATION AFTER CARDIAC TRANSPLANTATION

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Objective: Cardiac transplantation is the mainstay of therapy for patients with end-stage heart failure. Cold ischemia-reperfusion (IR) injury may lead to graft dysfunction and is a strong predictor for the development of chronic graft vasculopathy. Previous work from our laboratory and others has demonstrated that TLR4 senses endogenous danger signals and functions as a key mediator of organ injury and systemic inflammation in the setting of hepatic and cardiac warm IR. The objective of this study was to test the hypothesis that TLR4 mediates systemic inflammation in the setting of cardiac transplantation.

Methods: We performed syngeneic heart transplants in TLR4 mutant mice (C3H/HeJ mice, n=5) and TLR4 wild-type mice (C3H/HeOuJ mice, n=6). Donor hearts were procured and subjected to 2 hours of cold ischemia. The grafts were implanted heterotopically into the recipients and then retrieved after 3 hours of reperfusion. Control animals (n=3 in each group) underwent anesthesia only. Serum samples were collected and analyzed for tumor necrosis factor-alpha (TNFα), interleukin-6 (IL-6), and interferon-gamma(IFN-γ) levels by enzyme linked immunosorbent assay (ELISA).

Data: Serum TNFα levels were lower in TLR4 mutant animals compared to wild-type animals (9.07 +/- 3.97 vs. 40.5 +/- 8.75 pg/ml, $P<0.05$). IL-6 levels were also substantially lower in TLR4 mutant animals compared to wild-type animals (956 +/-239 vs. 2814+/-887 pg/ml, $P<0.05$). IFN-γ levels were not significantly elevated in either group when compared to serum from control animals.

Conclusions: TLR4 is a mediator of systemic inflammation in a murine cardiac transplant model. Further investigation is required to evaluate the effects of TLR4 signaling on the intra-graft inflammatory response, allograft rejection, and the development of graft coronary artery disease.

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MEMBRANE SORTING OF TOLL-LIKE RECEPTOR (TLR)-2/6 AND TLR2/1 HETERODIMERS AT THE CELL SURFACE DETERMINES HETEROTYPIC ASSOCIATIONS WITH CD36 AND INTRACELLULAR TARGETING

Kathy Triantafilou, Martha Triantafilou, Frederick Gamper, Rowenna Haston, Marios Mouratis, Siegfried Morath

Toll-like receptors (TLRs) are receptors of the innate immune system responsible for recognising pathogen-associated molecular patterns. TLR2 seems to be the most promiscuous TLR receptor able to recognise the most diverse set of pathogen-associated patterns. Its promiscuity has been attributed to its unique ability to hetero-dimerize with TLRs 1 and 6, and most recently to its association with CD36 in response to diacylated lipoproteins. Thus it seems that TLR2 forms receptor clusters in response to different microbial ligands. In this study we investigated TLR2 cell surface heterotypic interactions in response to different ligands as well as internalization and intracellular trafficking. Our data shows that TLR2 forms heterodimers with TLR1 and TLR6, and that these heterodimer pre-exist and are not induced by the ligand. In contrast, heterotypic associations of TLR2/6 with CD36 are not pre-formed and are ligand-induced. These receptor clusters accumulate in lipid rafts and are targeted to the Golgi apparatus. Activation occurs at the cell surface and the observed trafficking is independent of signalling.

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BACTERIAL AND VIRAL PRODUCTS INFLUENCE IMMUNE SYSTEM REPLENISHMENT

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It has long been known that hematopoietic stem cells (HSC) are quiescent most of the time and extensive proliferation by lineage restricted progenitors is thought to account for the massive numbers of new blood cells produced each day. Textbook depictions of lymphopoiesis attempt to illustrate how a sequence of binary fate decisions can produce committed progenitors from HSC. This is generally considered to be a unidirectional process and one that is antigen independent. However, we are learning that lineage restriction is more gradual and subject to change than previously imagined. For example, distinct marrow cell types can be caused to become T or dendritic cells, with some being more efficient and likely to be involved than others. There have been many demonstrations that experimental manipulation of transcription factors can result in a lineage change, with myeloid cells being a frequent default. Lineage infidelity is well known in cancer, but it is less clear if this ever happens under physiological circumstances. We recently showed that artificial stimulation of the Wnt pathway can make myeloid or lymphoid progenitors multipotential. That is, they lose some of their lineage specific characterisitics and re-acquire the ability to differentiate to other blood cell types. Other new findings indicate that the destiny of lymphoid progenitors can be dramatically influenced by microbial and viral products. While stem cells were long thought to be incapable of self/non-self discrimination, this possibility was opened by our discovery that they express a number of Toll-like receptors (TLR). Cytokines released during infection and inflammation have a substantial influence on blood cell formation in bone marrow. In addition, stem and progenitor cells can directly sense microbial/viral products via TLRs. We have demonstrated that they become reprogrammed to alternate fates when exposed to specific TLR ligands in highly defined culture conditions. The same phenomena occur in bone marrow of HSV-1 infected mice. The end result is a suppression of lymphocyte formation along with accelerated replenishment of several types of dendritic cells. While this may have survival value in life-threatening situations, it is also possible that chronic stimulation via TLR would be harmful to stem cells in some circumstances.

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DIETARY FAT IS STIMULUS FOR TLR-4 SIGNALING AND OBESITY-ASSOCIATED INFLAMMATION

Chantal Rivera, Jeff Houghton, Georg Singer, Monique Allman

Objectives: Recent clinical studies have demonstrated that morbidity and mortality among septic obese patients is significantly enhanced compared to their lean counterparts. Adiposity is attributed primarily to behaviors such as poor dietary habits. Industrialization has dramatically increased the amounts of refined grains and sugars in the typical "western diet" (WD); additionalagricultural advances have greatly enhanced the constitutive level of saturated fatty acids in domesticated animals used for food. Thus, the components typical of a WD include high amounts of saturated fat, cholesterol and sucrose. In an effort to understand how this "new" diet influences pathophysiology, the purpose of the present study was to examine the influence of feeding WD on hepatic inflammation and injury. Cholesterol and saturated fatty acids have been linked to activation of pro-inflammatory signaling cascades in cultured macrophages {8763}. These results are supported by findings in vivo of inflammation and endothelial dysfunction in baboons fed a high cholesterol/high saturated fat diet {8813}. Thus, we hypothesized that feeding WD would enhance hepatic pathology in response to infection.

Methods: Male C57BL/6 mice were fed purified control diet or the same diet supplemented with (%calories) saturated fat (35%), sucrose (50%) and cholesterol (1.2% w/w). After 3 weeks polymicrobial sepsis was induced by cecal ligation and puncture (CLP). Hepatic microvascular inflammation was accessed by intravital microscopy 6h after CLP. The direct effects of saturated fatty acid on hepatocytes were examined by culturing C3A cells in the presence of palmitate for up to 48 h. The role of toll-like receptor-4 (TLR-4) in diet-induced inflammation was addressed in wild type and TLR-4 mutant C3H/HeJ mice fed CD or WD for 3 months. The expression of inflammatory mediators in liver and C3A cells was measured by real-time PCR.

Results: After CLP, only mild histological evidence of injury was observed in livers of mice fed CD. Feeding WD for 3 weeks resulted in mild portal steatosis and was exacerbated 6h after CLP. Diffuse inflammation was observed in both dietary groups, but was more pronounced with WD. Intravital microscopy revealed that feeding WD enhanced leukocyte and platelet accumulation in terminal hepatic venules, and significantly increased mRNA levels of MCP-1, ICAM-1 and TNF-α. Hepatic expression of TLR-4 was approximately 2-fold greater in the WD+CLP group compared to CD+CLP. In support of the idea that dietary palmitate is proinflammatory, The expression of IL-8 in palmitate-treated C3A cells was increased 5-fold. Palmitate also increased mRNA levels of TLR-4, suggesting that this receptor mediates the inflammatory response to palmitate. Indeed, pre-treatment of C3A cells with an anti-TLR-4 blocking antibody blunted static adhesion of U937 macrophages to palmitate-stimulated C3A cells. Moreover, histophathological and molecular evidence of injury observed after feeding WD for 3 months were attenuated significantly in TLR-4 mutant mice.

Conclusions: In summary, these findings demonstrate the detrimental effects of the western-style diet on liver pathology directly as well as in septic subjects. Experiments in with TLR-4 blocking antibodies and mutant mice indicate that diet-induced inflammation and sensitivity to infection are likely mediated by the TLR-4 signaling pathway.

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ELUCIDATION OF THE MECHANISMS INVOLVED IN CELLULAR REPROGRAMMING

Paul Redmond

Sepsis results from the inability of the host immune system to limit bacterial spread during an ongoing infection. Massive bacterial load overrides the inhibitory mechanisms controlling inflammation. While normally helping to eradicate pathogens from a local infection, inflammation during sepsis develops into a systemic syndrome with abnormal coagulation, increased vascular permeability, ultimately septic shock and multiple organ failure. Despite more than 20 years of extensive research, sepsis continues to elude effective therapy with unacceptable high mortality rates between 30 and 70% in intensive care units.

The development of septic shock from an uncontrolled bacterial infection is characterized by the excessive release of inflammatory cytokines, due to a persistent activation of intracellular signal transduction pathways, by bacteria and their cell wall products, in inflammatory cells. Although appropriate amounts of inflammatory cytokines are essential for innate immune response to bacterial infection, exaggerated production can lead to an uncontrolled inflammatory response, tissue injury, and multiple organ failure. Therefore, a rational prophylactic and therapeutic strategy for controlling sepsis is to reprogram signal transduction pathways in inflammatory cells in order to attenuate systemic hyperinflammatory response.

Our previous work has shown that pretreatment with a sublethal dose of bacterial lipoprotein (BLP), a TLR2 agonist, protects mice against not only a subsequent lethal BLP challenge but also a subsequent lethal LPS challenge. Unlike LPS tolerance, protection against bacterial sepsis afforded by BLP tolerance was also observed in both wild-type and TLR4-deficient mice. This is closely associated with BLP-induced cellular reprogramming in phagocytes characterised by hyporesponsiveness in producing proinflammatory cytokines and simultaneously an enhanced antimicrobial activity.

Here we further elucidate the mechanisms involved in BLP-induced cellular reprogramming: 1) Despite the fact that TLR2 and TLR4 signal via the same adaptor protein MyD88, there are significant differences in intracellular signalling and antimicrobial activity between TLR2-mediated tolerance (e.g. BLP tolerance) and TLR4-mediated tolerance (e.g. LPS tolerance); 2) BLP-induced cellular reprogramming and protection against sepsis are independent of ST2, a key molecule in mediating LPS tolerance; 3) BLP-induced reprogramming of signal transduction pathways contributes to the attenuation of a late mediator of sepsis, HMGB1 release.

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MODULATION OF MACROPHAGE SIGNALLING PATHWAYS BY ANTHRAX TOXIN PROTEINS

Jin Mo Park

Bacillus anthracis, the causative agent of anthrax, produces several toxin polypeptides that interact with host signaling molecules and thereby modulate the survival and immune response of infected cells. Anthrax protective antigen (PA) binds to receptors expressed on the surface of host cells and allows cellular entry of lethal factor (LF) and edema factor (EF). LF is a metalloproteinase that specifically cleaves the NH2-terminal extension of mitogen-activated protein kinase (MAPK) kinases (MKKs), thereby preventing MAPK activation. We previously found that Incubation with B. anthracis lethal toxin (LT), a hetero-oligomer of PA and LF, renders macrophages sensitive to TLR4-induced apoptosis by preventing activation of the p38 MAPK pathway. We also found that B. anthracis secretes anthrolysin O (ALO), a cholesterol-dependent cytolysin (CDC), that specifically targets TLR4 and acts together with LT to induce macrophage apoptosis. In addition to inducing apoptosis, LF inhibition of MAPK signaling was found to result in the suppression of MAPK-dependent cytokine production in a variety of cells. In a quest for novel downstream targets of TLR4-p38 MAPK signaling in macrophages, we analyzed LPS-induced changes in the gene expression program of wild-type and p38 MAPK knockout macrophages. We identified a distinct set of LPS-inducible genes whose expression is substantially reduced in p38 MAPK knockout macrophages. These p38 MAPK-dependent genes appear to mediate both protective and pathogenic immune responses in macrophages. EF also forms a complex with PA to form edema toxin (ET). EF is a calcium/calmodulin-dependent adenylate cyclase that catalyzes the conversion of ATP to cAMP. We found that EF, with its adenylate cyclase activity, promotes motility and survival of macrophages in vitro. We also found that EF suppresses induction of certain proinflammatory cytokine genes while activating other genes whose function is related to cell migration and lymph vessel growth. Based on the functions of newly identified ET-inducible genes, we propose that EF may play a role in accelerating the migration of B. anthracis-infected macrophages to lymph nodes and spreading of the bacterium to the blood circulation.

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THE 'ETHEREAL' NATURE OF TLR4 AGONISM AND ANTAGONISM IN THE AGP CLASS OF LIPID A MIMETICS

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The discovery of toll-like receptors (TLRs) on cells of the immune system together with the identification of natural TLR ligands has led to a great deal of interest in developing synthetic TLR agonists and antagonists to manipulate innate and adaptive immune responses. Targeting TLR receptors and cognate intracellular pathways could potentially lead to more effective vaccines and novel therapeutic approaches for the treatment of immune and inflammatory diseases. For example, certain variants of lipopolysaccharide (LPS), the main cell-surface component of Gram-negative bacteria, are potent stimulators of host defense systems via their interaction with TLR4, but the pathophysiology of LPS and its active principle, lipid A, have precluded their medicinal use. Thus, considerable effort has been directed towards the development of synthetic lipid A mimetics with simplified structures and improved toxicity/activity profiles for use as vaccine adjuvants and stand-alone therapeutics. The TLR4 receptor has also been an attractive target for the development of LPS antagonists for the treatment of endotoxin-related diseases. In the course of our own structure-activity studies on lipid A, we identified a new class of potent monosaccharide immunomodulators known as aminoalkyl glucosaminide phosphates (AGPs). Among the AGPs, CRX-527 and CRX-526 have been shown to exhibit potent TLR4 agonist and antagonist activity, respectively, in both murine and human models.

Lipid A derivatives are metabolized, in part, by action of the acyloxyacyl hydrolase enzyme, which selectively cleaves the secondary fatty acyl chains that are ester-linked to the branched fatty acyl chains attached to the lipid A backbone. To overcome the chemical and metabolic instability of the secondary fatty acyl residues, and further evaluate structural modifications in the AGP series, the secondary ether lipid analogs of CRX-527 and CRX-526 were synthesized. These ether lipids and their secondary ester counterparts CRX-527 and CRX-526 were evaluated for agonist/antagonist activity in both in vitro and in vivo models. A summary of the synthetic and biological aspects of these novel glycolipids, including the striking species-specific agonist/antagonist activity of one of the molecules, will be presented.

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CHAPERONIN 10 REGULATES THE INNATE IMMUNE RESPONSE TO TOLL-LIKE RECEPTOR ACTIVATION

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Objective: In addition to its essential role in mitochondrial protein folding, we have investigated a second function of chaperonin 10 (Cpn10) as an endogenous regulator of the innate immune response induced via Toll-like receptor (TLR) activation.

Materials and Methods: Methods used in these studies include transient transfection of RAW264.7 macrophages with a luciferase reporter under the control of the Cpn10 promoter, quantitative real-time PCR of Cpn10 mRNA, Cpn10 and cytokine ELISAs. We have used FRET to assess proximity between endogenous or recombinant Cpn10 and various members of the TLR signaling complex. FACS analysis was used to determine interaction and uptake of fluorochrome-labeled Cpn10 with PBMC subpopulations. Clinical trials were approved by local ethics committees registered with the Australian Clinical Trials Registry, and performed according to ICH GCP and the Declaration of Helsinki.

Data: TLR ligation induces upregulation of the Cpn10 promoter and production of Cpn10 mRNA, resulting in the extracellular release of Cpn10. Cpn10 then interacts with antigen presenting cells via activated TLRs to modulate inflammatory signaling and cytokine production. Cpn10 administration in vivo results in modification of TLR ligand-induced cytokine production by PBMC in vitro. Phase 2 clinical trial results indicate that rCpn10 is well tolerated and efficacious, at least in the short term, in treatment of the symptoms of moderate to severely active rheumatoid arthritis and plaque psoriasis

Conclusions: We have shown that Cpn10 functions as part of an inducible negative feedback mechanism to resolve harmful inflammatory responses following TLR engagement.

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SUMMARY RESULTS OF CLINICAL TRIALS OF ERITORAN - A TOLL-LIKE RECEPTOR-4 ANTAGONIST

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Activation of toll-like receptor-4 (TLR4) has been linked to a number of pathophysiological responses triggered by a variety of ligands, the most well-characterized of which is lipopolysaccharide (LPS), a major constituent of the outer membrane of gram-negative bacteria. In mammals, the lipid A moiety of LPS is recognized by TLR4 which generates a proinflammatory responses that may prove harmful to the host. Eritoran (E5564), is a novel Lipid A analogue/antagonist that can prevent endotoxin-induced systemic inflammation in animals and humans by binding to the MD-2/TLR4 complex thereby blocking TLR4 activation.

Purpose of Studies: Phase II clinical studies have been performed to determine if eritoran has an acceptable safety profile in patients, and if inhibition of TLR-4 activation by eritoran can: 1) reduce post surgical morbidity in patients undergoing coronary artery bypass graft (CABG) surgery or 2) reduce 28-day mortality in patients with severe sepsis.

METHODS: Two studies of the safety and efficacy of eritoran in CABG include regimens of one of three dose levels or placebo administered over four hours beginning 30 minutes prior to start of surgery.

The safety and efficacy of eritoran was studied in 300 patients with severe sepsis and 20-80% predicted risk of mortality (PROM) by APACHE II. Two treatment regimens, 45 mg or 105 mg of eritoran, or placebo, were given over 6 days.

RESULTS: Following CABG surgery, even though a number of secondary endpoints suggested activity of eritoran in high-risk CABG patients, clinical benefit was not apparent for the overall population.

In sepsis patients, 28-day mortality in the intent to treat (ITT) population (N=293), was reduced by 6.7% in subjects who received high dose eritoran (26.6% vs. 33.3% for placebo; P=0.24). Furthermore, in the subset of ITT patients with baseline PROM of 51-80%, a greater reduction in mortality was observed (33.3% for eritoran 105 mg vs. 50.9% for placebo (P=0.07). Adverse events that occurred at a higher rate in patients receiving eritoran included phlebitis and an increased incidence of atrial fibrillation.

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T-REGULATORY CELL POPULATIONS UNDER STRESSFUL CONDITIONS: CHANGES IN MURINE REGULATORY T-CELL SUBSETS AND MARKERS

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The inability of present therapies to mitigate the devastating effects of sepsis indicates that a greater knowledge of the patho-physiology of septic condition is needed if we are to develop not only better, more effective interventions, but also identify whom and/or when these interventions will be most efficacious. Our laboratory along with several others have had sustained interest in not only how sepsis, as produced by cecal ligation and puncture (CLP) in the mouse, differentially effects the

immune response observed in divergent tissue sites, but to what extent these aberrations in myeloid and/or lymphoid cell functions can contribute to changes in septic morbidity/mortality. Importantly, we have observed that CLP induces marked changes in 3 key regulatory T-cell populations, i.e., $CD4^+CD25^+$ native T-regulatory (T-reg) -, γδ-T-, and NK-T-cells, within the spleen, intestine and liver, which we have found to either have the capacity to induce marked immune suppression (e.g., the septic mouse T-reg or NK-T-Cell) or have an effect on the animal's capacity to ward off septic mortality (e.g., deficiency of γδ-T-cells ↓survival; deficiency/ inhibition of NK-T-cells ↑survival; while CD25 deficiency, ↔survival) (Chung CS, et al [2006] Amer J. Physiol. 291:R1338; Rhee RJ, et al [2003] J. Surg. Res. 115:74; Winoski N, et al [2007] Shock 27:in press). Unlike the classic αβ-CD4 T-cells these various regulatory T-cell populations share a unique capacity to respond rapidly to either inflammatory, infectious and/or wound associate stimuli, i.e., innate activation. Thus, they have the capacity to serve as sentinels to changes in tissue homeostasis, and thereby potentially regulating the response to infection and/or injury. Here we discuss the potential significance of the changes in these diverse regulatory T-cell sub-populations, how they may be regulated and/or how they may modify both classic immune and/or potential non-immune targets to effect the septic animal's morbidity/mortality (supported by NIH GM-46354).

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THE EFFECT OF INJURY ON T REGULATORY CELL ACTIVITY IN MAN AND AN ANIMAL MODEL

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We recently reported increased CD4+CD25+ T regulatory (Treg) activity in the lymph nodes of mice after burn injury. The present study sought to determine if Tregs mediate the reduction in Th1 type immunity seen after serious injury in man and if the function of circulating Tregs is altered by injury in man and mouse. Peripheral blood was withdrawn from 19 consenting adult patients (35.1 ± 16.3 years of age) with injury severity scores (ISS) 36.6 ± 13.9 on days 1 and 7 after trauma and from 5 healthy individuals. CD4+ T cells were purified and sorted into Treg ($CD25^{bright}$) and Treg depleted populations. After activation of the cells with anti-CD3/CD28 antibody, production of the Th1 cytokine IFNγ, the Th2 cytokines IL-4 and IL-5 and the inhibitory cytokine IL-10 was measured using cytometric bead arrays. Treg activity was measured by in vitro suppression of autologous CD4+ T cell proliferation. Peripheral blood was obtained by cardiac puncture under anesthesia from C57BL/6 mice at 1 and 7 days after 25% body surface area burn injury. The CD4+ T cell population was isolated and sorted into CD25+ (Treg) and CD25- subsets. Treg activity was determined by suppression of isologous CD4+ CD25- T cell proliferation.

All patients survived injury. Nine (47%) developed infections post-injury. IFNγ production by patient CD4+ T cells was decreased on day 1 and day 7 in comparison with healthy controls. However, after Tregs were depleted from the CD4+ T cells, IFNγ production increased to control levels. Tregs were the chief source of IL-4 and IL-5 as well as IL-10. Treg suppression of T cell proliferation increased significantly from day 1 to day 7 after injury. In the mouse model circulating Treg activity again increased from day 1 to day 7 and day 7 Treg potency in burn animals was significantly greater than that in sham controls.

We demonstrate for the first time that human Tregs are increased in potency after severe traumatic injury. Most significantly, Tregs are important mediators of the suppression of T cell activation and the reduction in Th 1 cytokine production, commonly observed after injury. In the burn mouse model, a post-injury increase in Treg activity is not confined to lymph nodes but is also evident in the circulating T cell population in concert with injured humans.

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REGULATORY T-CELLS CONTROL INNATE AND ADAPTIVE IMMUNE RESPONSES TO BURN INJURY

James Lederer, Malcolm MacConmara, Ann McKenna, Adam Delisle, John Mannick

Severe injury disrupts immune system homeostasis causing enhanced innate immune system reactivity and suppressed adaptive immune system responses. We recently reported that a subset of CD4+ T-cells called regulatory T cells (Tregs) display enhanced regulatory function after burn injury. Tregs purified from the lymph nodes of day 7 burn-injured mice showed significantly enhance potency in inhibiting polyclonal CD4 T-cell proliferation in vitro. Moreover, sham and burn mice made deficient in Tregs showed enhanced T helper 1 (Th1) type responses when immunized with a T-cell dependent antigen. To further explore the adaptive immune regulatory activity of Tregs following injury, we used a T-cell receptor transgenic mouse model (DO-11.10 mice) to investigate whether Tregs play an active role in suppressing antigen-driven CD4+ T-cell responses in vivo. Using Treg-deficient mice as recipients of DO-11.10 CD4 T-cells, we demonstrate that Tregs in injured mice control antigen-induced CD4 T-cell expansion and Th1 reactivity. In a separate study, we reported that Tregs play an active role in controlling the inflammatory response to injury. This was demonstrated by the adoptive transfer of CD4+ T-cells or purified Tregs into sham versus burn Rag1-/- and CD4-/- mice. We have extended these observations to show that injured, Treg-deficient mice display an enhanced "two-hit" response to in vivo

LPS challenge. The results of in vivo and ex vivo experiments indicate that the mechanism responsible for Treg control of LPS reactivity involves interplay between macrophages and Tregs. In total, these results indicate that Tregs contribute to the control of injury-induced inflammation and CD4 T-cell mediated immune responses.

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CD4+CD25+ REGULATORY T-CELLS IN THE SEPTIC PATIENT

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Introduction and Objective: It is now agreed that septic shock deeply perturbs immune homeostasis by inducing an initial intense systemic inflammatory response that is accompanied by an anti-inflammatory process, acting in a negative feedback manner. These inhibitory mechanisms may become deleterious as nearly all immune functions are compromised. They may account for the majority of septic shock related death as most non-surviving patients die after initial resuscitation in a delayed immunosuppressive state. The mechanistic bases for sepsis-induced immunosuppression have not yet been clearly established. Briefly, the condition includes enhanced leukocyte apoptosis, increased circulating IL-10 levels, low lymphocyte proliferation in response to recall antigens, and deactivated functions of monocyte (usually considered as key player in sepsis). Recently, naturally occurring CD4+CD25+ T cells (Treg) have been characterized in humans as suppressor T cells. They induce T-cell anergy through a cell-contact mechanism in vitro. They inhibit the activation of Th1 cells. They produce IL-10 and TGF-b and suppress IFN-g production. Treg have also been shown to modulate the innate immune response in various murine models of infectious diseases. Given these properties (similar to sepsis-induced immunosuppression features), we hypothesized a role for these cells in sepsis pathophysiology.

Data: We observed in patients with septic shock a progressive and significant elevation of Treg (CD4+CD25+CD45RO+CD69-) percentage (among CD4+ cells) above control values. The highest values were found 5 days after the onset of shock in both survivors and non-survivors, suggesting a normal homeostatic mechanism. Afterwards, the Treg percentage remained significantly increased in non-survivors only. As septic patients present with marked lymphopenia, we then investigated Treg absolute count in an additional cohort of patients. We reported that the increased proportion of Treg was not due to their proliferation, but rather to a selective depletion of CD4+CD25- T cells. Indeed, we have shown that the number of Treg tended to remain in normal range whereas CD4+CD25- were found to be dramatically decreased. In accordance, FOXP3 (specific Treg transcription factor) mRNA expression was found in normal range whereas other T lineages transcription factors (Tbet, GATA-3) were severely suppressed. Consequently, it seemed that Treg were not affected by cell death reported during septic shock. In line with these clinical data, in a model of LPS-stimulated purified human cells, we described two important results: 1) Treg may act on monocytes by inhibiting their LPS-induced survival through a pro-apoptotic mechanism; 2) this effect is largely mediated by a soluble factor and involves the Fas/FasL pathway.

Conclusion: Collectively, these results indicate that Treg act on monocytes by inhibiting their LPS-induced survival through a pro-apoptotic mechanism involving the Fas/FasL pathway. Similar results have been recently reported in human neutrophils. The increased Treg percentage observed during sepsis may be a consequence of the marked apoptosis of other lymphocyte subsets. In parallel, Treg may also participate in an amplification loop of apoptosis processes. This may constitute a contributing mechanism to septic shock-induced immunosuppression.

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EXPRESSION-PROFILING OF TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS-1 (TREM-1) IN GRAM-NEGATIVE SEPSIS UNDERSCORES ITS DIAGNOSTIC AND POSSIBLE THERAPEUTIC POTENTIAL

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Objective: TREM-1 is a new inflammatory mediator in the inflammatory arena that amplifies Toll-like receptor (TLR)-initiated responses against microbial challenges by potentiating the secretion of proinflammatory cytokines. We aimed to characterize the expression and function of TREM-1 in sepsis caused by the Gram-negative bacterium Burkholderia pseudomallei, the causative agent of melioidosis, which the most-important cause of community-acquired sepsis in SE-Asia and northern-Australia. Materials and Methods: Subjects: Patients with sepsis caused by B. pseudomallei, healthy volunteers and male C57BL/6 mice intranasally infected with a lethal dose of B. pseudomallei.

Data: (1) 34 patients with septic melioidosis demonstrated strongly increased sTREM-1 plasma levels (ELISA), TREM-1 cell surface expression on monocytes and granulocytes (flowcytometry) and TREM-1 mRNA levels in peripheral leukocytes (Lightcycler) when compared to healthy controls. (2) WT mice were intranasally inoculated with a lethal dose of B. pseudomallei to examine TREM-1 expression over time (0-72 hrs after inoculation), in different compartments (lung / bronchoalveolar-lavage (BALF) / blood) and cell types (monocytes / macrophages / granulocytes). sTREM and TREM-1 surface expression was strongly upregulated in lung homogenates and blood. (3) To investigate the

function of the observed TREM-1 upregulation, isolated TREM-1$^+$ and TREM-1$^-$ monocytes and granulocytes obtained from blood from healthy volunteers (using cell sorter) were stimulated with LPS and HK B. pseudomallei. TREM-1$^-$ granulocytes displayed diminished pro-inflammatory responses after LPS and B. pseudomallei stimulation. There was no difference in LPS induced cytokine release between TREM-1$^+$ and TREM-1$^-$ monocytes, suggesting the existence of additional amplifiers of the TLR-cascade.

Conclusion: These data provide new information on to the regulation of TREM-1 during sepsis and underscore the potential usefulness of TREM-1 as a diagnostic target. Its therapeutic potential is under investigation.

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GRAM POSITIVE AND GRAM NEGATIVE PNEUMONIA INDUCE UNIQUE SYSTEMIC CYTOKINE PROFILES

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Serum	6h	12h	72h
IFN-γ	Pa	Pa	Pa
Eotaxin	Pa	Pa	ND
TNF-α	Pa	ND	ND
IL-6	Pa	ND	ND
MCP-1	Pa	ND	ND
MIP-α	Pa	ND	ND
IL-18	ND	Pa	ND
MIP-2	ND	Pa	ND
IL-1b	ND	ND	ND
IL-1ra	Pa	Pa	ND
TNFSR-1	ND	ND	Pa
TNFSR-2	Sp	ND	Pa
IL-10	ND	ND	ND

Background: Gram positive and gram negative bacteria initiate the host inflammatory response via different mechanisms. We sought to determine how this affects the systemic cytokine response.

Methods: FVB/N mice were intratracheally injected with Pseudomonas aeruginosa (Pa) or Streptococcus pneumoniae (Sp), both which cause 50% 3d mortality. Mice (n=7-11/group) were sacrificed at 6, 12, and 72h, and serum analyzed for cytokine concentrations using a microarray immunoassay. Statistical differences were determined by Mann-Whitney test.

Results: The table above indicates timepoints when a cytokine level was significantly ($p<.05$) higher in mice given Pa, Sp, or not significantly different (ND). Cytokine results are grouped by similar patterns, with pro-inflammatory mediators at the top, anti-inflammatory mediators at the bottom. Six of the nine pro-inflammatory mediators were significantly higher in mice given Pa than mice given Sp at six hours. Levels of IFN-g were higher at all timepoints in mice given Pa, with mean levels of 838 ,948, and 508 pg/ml, compared to 161, 146, and 214 pg/ml for mice given Sp, at 6, 12 and 72h, respectively. Overall, serum cytokine levels were higher in mice given Pa in 15 of 39 possible measurements. TNFSR-2 was the only mediator where mice given Sp had significantly higher serum levels (2034 and 992 pg/ml for Sp and Pa at 6h, respectively). These results sharply contrast with previously presented (Shock 2006) bronchioalveolar lavage data from the same animals. In BAL fluid, 10 of 13 mediators were higher in mice given Sp at 72 hours, while in serum, not a single mediator was higher at 72 hours in mice given Sp.

Conclusions: Although both models of sepsis have 50% 3d mortality, Pa induces a bigger increase in systemic inflammatory mediators, predominantly at early timepoints, with all but one mediator level higher or ND than that induced by Sp. This is in contrast to what is seen in the local, pulmonary, response, where Sp induces higher levels, predominantly at later timepoints. Gram positive and gram negative infections induce unique host responses. These differences should be considered in the evaluation of new approaches to treatment of sepsis.

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NUCLEAR TRANSLOCATION OF THE INTERLEUKIN 1 HOMOLOGUE IL 1F7B IS DEPENDENT ON CASPASE 1

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IL-1F7b is a novel homologue of the IL-1 cytokine family discovered by computational cloning. We demonstrated that IL-1F7b shares critical amino acid residues with IL-18 and binds to the IL-18-binding protein enhancing its ability to inhibit IL-18-induced interferonγ. IL-1F7b was also shown to bind to the IL-18Rα. However, IL-1F7b is rarely observed in the extracellular compartment and no IL-18Rα-dependent agonistic or antagonistic function was discovered. Recently we reported enhanced nuclear translocation of IL-1F7b after LPS-stimulation in transfected RAW264.7 cells. By using fusion constructs of IL-1F7b with GFP variants we could show that only mature IL-1F7b but not propiece IL-1F7b specifically translocates to the nucleus. IL-1F7b like IL-1α, IL-1β, IL-18 and IL-33 requires caspase-1 cleavage to generate the mature cytokine. We therefore hypothesized that caspase-1 mediated cleavage of the propiece renders mature IL-1F7b to translocate actively into the nucleus. Here we show that the addition of a specific caspase-1 inhibitor markedly reduced nuclear entry of IL-1F7b in transfected RAW264.7 cells despite enhanced cellular expression seen after LPS-stimulation which appeared completely cytoplasmic. These results indicate that caspase-1 proc-

essing is mandatory for nuclear translocation of IL-1F7b where it might also act as a transcriptional modulator.

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SPLANCHNIC ISCHEMIA AND REPERFUSION INJURY IS REDUCED BY GENETIC OR PHARMACOLOGICAL INHIBITION OF TNF-A

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In the present study, we used tumour necrosis factor-R1 knock out mice (TNF-αR1KO) to evaluate a possible role of TNF-α on the pathogenesis of ischemia and reperfusion injury of the multivisceral organs. Ischemia and reperfusion injury was induced in mice by clamping both the superior mesenteric artery and the celiac artery for 30 min, followed thereafter by reperfusion. Sixty minutes after reperfusion, animals were sacrificed for histological examination and biochemical studies. Injured wild-type(WT) mice developed a significant increase of ileum TNF-a levels, myeloperoxidase activity and marked histological injury and apoptosis. Ischemia and reperfusion injury of the multivisceral organs was also associated with a significant mortality. Reperfused ileum sections from injured-WT mice showed positive staining for P-selectin, V-CAM, ICAM-1 and E-selectin. The intensity and degree of P-selectin, E-selectin, VCAM and ICAM-1 were markedly reduced in tissue section from injured-TNF-αR1KO mice. Ischemia and reperfusion injured TNF-αR1KO mice showed also a significant reduction of neutrophils infiltration into the intestine, a reduction of apoptosis, an improved histological status of the intestine and survival. In addition, we also investigated the effect of Etanercept, a TNF-α soluble receptor construct, on ischemia and reperfusion injury of the multivisceral organs. Etanercept (5 mg/kg administered i.p. 5 min prior reperfusion) significantly reduced the inflammatory response and the ileum injury. Taken together, our results clearly demonstrate that TNF-α play an important role in the ischemia and reperfusion injury and put forward the hypothesis that modulation of TNF-α expression may represent a novel and possible strategy.

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HMGB1 (AMPHOTERIN) INTERACTIONS WITH MONOCYTES AND PLATELETS ARE INHIBITED BY GLYCYRRHIZIN

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Objective: HMGB1 (Amphoterin) is a 30-kD heparin-binding protein that mediates monocyte transendothelial migration and platelet adhesion. HMGB1 polypeptide has some proinflammatory activity by itself and it binds to bacterial substances, including lipids, which may strengthen its effects. Further, HMGB1 binds to phospho- and glycolipids of eukaryotic origin, mainly phosphatidylserine and sulfatide.

Glycyrrhizin is a sulfatide analog that has variety antiinflammatory effects. In addition, it inhibits metastasis, leukocyte adhesion to endothelium and leukocyte infiltration into tissue. Glycyrrhizin has long been used to treat chronic viral hepatitis in humans.

Glycyrrhizin, like sulfatide, binds to HMGB1. We tested whether glycyrrhizin affects monocyte and platelet adhesion to HMGB1-surface using our previously described cell binding assays.

Material and Methods: Human peripheral blood monocytes or resting prostacyclin inhibited platelets were adhered to recombinant HMGB1 coated plastic. Fibronectin and fibrinogen coatings were used as positive controls for monocyte and platelet adhesion, respectively. Glycyrrhizin was used in physiologically relevant concentrations (0.1-3 mM) to inhibit binding. After adhesion of 30 minutes non-adherent cells were washed away, and bound cells were quantified.

Data: Glycyrrhizin inhibited dose dependently monocyte and resting platelet adhesion to recombinant HMGB1 coated plastic, but did not had effect on binding to fibronectin or fibrinogen. At glycyrrhizin concentration of 1 mM monocyte binding to HMGB1-wells was 75.5±2.0% of noninhibited control and platelet binding was 30.2±20.1% of noninhibited control. Values for control proteins were 98.1±1.2% and 104.1±10.8% (fibronectin and fibrinogen, respectively).

Conclusion: Monocytes and resting platelets may use sulfatide-like structures present on their surface to bind HMGB1. Glycyrrhizin may be used to interfere HMGB1/immune cell interactions.

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CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASES (CAMK) MEDIATE MACROPHAGE LPS-INDUCED HMGB1 PRODUCTION

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Objective: HMGB1, an architectural chromatin-binding factor that bends DNA and directs protein assembly on specific DNA targets, has been demonstrated to function as a late mediator of mortality in murine endotoxemia and sepsis. Systemic levels are markedly elevated in patients that die of sepsis, and anti-HMGB1 antibodies confer a dose-dependent protection in animal models of endotoxemia. Macrophages have been demonstrated to be a primary source of HMGB1, and evidence is accumulating that production of this inflammatory medi-

ator is calcium-dependent. We have previously demonstrated that calcium/calmodulin-dependent protein kinases (CaMK) play an important role in the inflammatory phenotype of the monocyte. The broad CaMK inhibitor KN62 prevented LPS-induced mitogen-activated protein kinase (MAPK) activation and TNFa production in human monocytes. Whether this kinase family is operant during HMGB1 production is unknown. In this study we hypothesize that the CaMK family mediates macrophage HMGB1 production induced by LPS. We secondarily hypothesize that elevations in intracellular calcium concentration further induce HMGB1 production through the CaMK signaling pathway.

Materials and Methods: Human macrophage cell line RAW264.7 (American Type Culture Collection, Rockville, MD) was grown in DMEM (BioWhittaker, Walkersville, MD) supplemented with 10% fetal calf serum (Sigma), 50 U/mL penicillin, and 50 μg/mL streptomycin (Cellgro Mediatech Inc., Kansas City, MO). Selected cells were pretreated with 5uM of STO609 (Calbiochem, San Diego, CA), an inhibitor of CaMKK, the upstream kinase of CaMK I and IV, or 5uM of KN93 (Calbiochem, San Diego, CA), which has increased selectivity for CaMK II, but has been demonstrated to inhibit CaMK IV. Selected cells were then treated with LPS (100 ng/mL) or with the calcium ionophore ionomycin (2uM). Supernatant was harvested and assayed for HMGB1 by western blot. Relative concentrations were determined by densitometry.

Data: LPS induced the release of HMGB1. Pretreatment with STO609 inhibited HMGB1 production by 68%, and KN93 inhibited HMGB1 release by 44%. Ionomycin, when applied after LPS stimulation, induced a two-fold elevation in HMGB1 that was completely inhibited by STO609, such that concentrations were similar to that of unstimulated control cell populations. KN93 had no effect on ionomycin-induced HMGB1 production.

Conclusion: The family of CaMKs are important in mediating HMGB1 production consequent to LPS stimulation. Elevations in intracellular calcium concentration induce even greater release of HMGB1, which appears to be mediated through the CaMKK signaling pathway and does not involve CaMK II.

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LPS INDUCED IL-10 PRODUCTION REQUIRES CD14 IN MACROPHAGES. TNF-ALPHA PRODUCTION CAN OPERATE THROUGH AN ALTERNATE PATHWAY

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Objective: CD14 along with associated toll receptors is the accepted signaling pathway involved in the responses of macrophages to LPS. Since LPS binding to CD14 requires interaction with a serum factor (LPS binding protein, LBP) there is a controversy regarding the fixed macrophages of the liver (Kupffer cells) as a number of studies have shown that these cells can respond to LPS in the absence of serum. The purpose of this study was to determine if alternate pathways for LPS mediated activation are present in Kupffer cells but not in other (peritoneal) macrophages.

Materials and Methods: Rat Kupffer cells were isolated from livers by collagenase perfusion. Resident peritoneal macrophages were isolated by gentle saline lavage of rat peritoneal cavities. Macrophages were incubated with LPS at 1ug/ml in the presence and absence of serum and cytokines (IL-10 and TNFa) were measured in the supernatants by ELISA, 3,6,12 and 24 hours later. mRNA levels for the cytokines were measured using a competitive PCR method (Ouantikine Cytoexpress Kit). Treatments were also carried out in the presence of the beta-adrenergic receptor agonist terbutaline (10^{-7}M).

Data: Stimulation of both Kupffer cells and peritoneal cells resulted in secretion of TNF-alpha in both serum and serum free conditions. However, the response was greater in the presence of serum. Pre-incubation with terbutaline caused a significant ($P<0.05$) down regulation of the TNF-alpha response. Neither cell type produced IL-10 in the absence of serum with or without terbutaline treatment. In the presence of serum IL-10 was secreted however terbutaline caused a significant increase ($P<0.05$) in IL-10 production. Message levels were increased and were reflective of the amount of protein expressed except in the case of TNF-alpha following terbutaline treatment. In both cell types while TNF-alpha decreased, message levels did not significantly change.

Conclusions: The data show that TNF-alpha can be produced by both cell types using a CD-14 dependant and a CD-14 independent mechanism. IL-10 production however depends entirely on the CD-14 pathway. The effects of the beta adrenergic receptor agonist terbutaline, is to increase IL-10 production causing inhibition TNF-alpha secretion. The data also suggests that separate signaling pathways are involved in the regulation of expression of these cytokines and that expression is influenced by cross talk with the beta-adrenergic receptor system. The interactions between these pathways are important in controlling circulating levels of TNF-alpha during injury and sepsis.

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DECREASED EXPRESSION OF CX3CR1 IN SEPTIC SHOCK: TRANSCRIPTIONAL REGULATION BY LPS, CORTISOL AND SOLUBLE FRACTALKINE

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Objective: Based on microarray study, we recently described a list of 28 genes whose peripheral blood (PB) expression could efficiently discriminate survivor from non-survivor septic shock patients after the early proinflammatory reaction (1). Among the genes up-regulated in survivors, the single receptor of the fractalkine chemokine CX3CR1 showed the highest fold change. The objective of the present study was first to confirm the microarray data at the mRNA and protein level and second to investigate the underlying mechanisms of CX3CR1 regulation.

Material and Methods: 58 patients with septic shock were enrolled in the study. PAXgene blood samples were obtained regularly in the course of the syndrome for CX3CR1 gene expression analysis using qRT-PCR. In parallel, blood collected into EDTA tubes was used for monocyte CX3CR1 cell-surface analysis using flow cytometry and serum soluble fractalkine measurement by ELISA. 21 healthy donors served as control. For in-vitro experiments, PBMC from healthy donors were treated in triplicate in the presence or absence of LPS, soluble fractalkine, IL-10 (10 ng/mL each) or dexamethasone (10^{-6} M) for 24h.

Data: As compared to healthy controls, septic shock patients showed a strong and persistent decrease in PB CX3CR1 mRNA expression. Similarly, a marked decrease in PB CX3CR1 cell surface expression was observed. Both at the onset of shock and later in the course of the syndrome, survivor patients showed higher level of PB CX3CR1 mRNA than non-survivors. Regarding the time-course analysis of paired-samples, a significant decrease in PB CX3CR1 mRNA level was observed in non-survivors, while it remained stable in survivors. Serum soluble fractalkine levels were increased in septic shock patients compared to the controls. The levels were higher in non-survivors compared to survivors, although the difference did not reach statistical significance. We showed that LPS decreases CX3CR1 mRNA in healthy PBMC in vitro (5.26±1.06 fold decrease). In addition, soluble fractalkine could slightly inhibit CX3CR1 mRNA expression (1.62±0.07 fold decrease). Finally, using two factors that have been implicated in the anti-inflammatory response in septic shock, we showed that corticosteroids but not IL-10 had a negative transcriptional effect on CX3CR1 in vitro (2.71±0.09 fold decrease).

Conclusion: These data illustrate both at the mRNA and protein level that PB CX3CR1 is decreased in septic shock. Given that CX3CR1 is mainly expressed by proinflammatory and cytotoxic cells (2,3), this finding might be considered as a new feature of sepsis-induced immunosuppression. Our results suggest that soluble fractalkine, LPS and corticosteroids could contribute to the decreased expression observed in septic shock.

Literature: (1) Pachot et al. Immunol Lett 2006; 106:63-71. (2) Nishimura et al. J Immunol 2002; 168:6173-80. (3) Ancuta et al. J Exp Med 2003; 197:1701-7

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LIPOPOLYSACCHARIDE INDUCES INTERLEUKIN-10 MRNA STABILITY THROUGH A POST-TRANSCRIPTIONAL MECHANISM

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Cytokine expression is regulated at the level of transcription as well as by post transcriptional mechanisms. Interleukin-10 (IL-10) is a potent anti-inflammatory cytokine that is central in the overall inflammatory response after insult. Stability of mRNA directly contributes to protein expression and p38 MAPK has been shown to mediate this stability. Data from animal models and immortal cell lines has demonstrated LPS modulates mRNA stability separate from transcription. We hypothesized that LPS stabilizes IL-10 mRNA in human monocytes through a p38 MAPK pathway and this mechanism is distinct from transcriptional control.

Methods: Peripheral blood mononuclear cells (PBMC's) were isolated from healthy human donors by Ficoll-Hypaque centrifugation. PBMC's were plated in RPMI 1640 supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 mg/mL streptomycin and monocytes were selected by adherence. After incubation of 16 hours, monocytes were stimulated with LPS for 4 hours. Cells were then placed in media alone, media with LPS, media with Actinomycin D to arrest transcription, media with LPS and Actinomycin D, or media with LPS, Actinomycin D, and a p38 MAPK inhibitor (SB203580). Cells were then harvested at 0.5, 1, 2, and 4 hours after addition of the above media cocktails. Total RNA was isolated using the RNeasy kit (Qiagen, Valencia, CA) and a cDNA library constructed. IL-10 and GAPDH (internal control) mRNA levels were measured using quantitative real-time polymerase chain reaction. Quantification of mRNA levels was achieved using the $2^{-\Delta\Delta Ct}$ method. Levels of IL-10 expression and mRNA levels were compared amongst groups.

Results: GAPDH mRNA levels did not change through out groups or time points. Over 4 hours IL-10 mRNA degraded to less than 50% of control. The addition of LPS, however, stabilized IL-10 mRNA and slowed the rate of mRNA degradation. When the p38 inhibitor SB203580 was added, this affect disappeared and mRNA degraded faster.

Table

Fold Change in IL-10 mRNA Level			
Time	Actinomycin D	Act D + LPS	Act D + LPS + p38 inhib
0.5	1	0.9	0.8
1	0.5	0.2	0.6
2	0.6	0.7	0.2
4	0.3	0.4	0.1

Conclusions: LPS induces IL-10 mRNA stability in human monocytes. This increased stability disappears with p38 MAPK inhibition. These data suggest a p38 MAPK dependent mechanism for LPS induced IL-10 mRNA stability that is distinct of any signaling through the nucleus. These findings suggest the IL-10 component of the inflammatory response can be modulated at the post-transcriptional level.

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CD73-DERIVED ADENOSINE AMELIORATES IMMUNE DYSFUNCTION AND ORGAN INJURY IN SEPSIS

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Objective: Adenosine is produced in response to cellular stress and damage and elevations in extracellular adenosine are found in sepsis. The dominant pathway leading to high extracellular adenosine levels during sepsis is release of precursor adenine nucleotides (mostly ATP) from the cell followed by extracellular catabolism to adenosine by a cascade of ectonucleotidases. CD73 is a cell surface protein with ecto-5′-nucleotidase enzyme activity that catalyzes the dephosphorylation of 5′-AMP to adenosine. Therefore, this molecule plays a key role in the generation of extracellular adenosine by catalyzing the last step in the cascade of ATP breakdown. To investigate the role of CD73-derived adenosine in sepsis, we subjected CD73 wild type (WT) and knockout (KO) mice to cecal ligation and puncture (CLP).

Materials and Methods: The immunological status of animals was assessed by measuring cytokine levels and bacterial counts from blood and peritoneal lavage fluid, and thymocyte apoptosis. Tissue damage was examined using hematoxylin and eosin staining.

Data: CD73 KO mice exhibited a significantly higher bacterial burden and higher cytokine levels than their WT counterparts. CD73 deficiency increased immune cell apoptosis, measured as increased levels of cleaved caspase-3 in the thymi of CD73 KO mice relative to those of CD73 WT animals. CLP-challenged CD73 KO mice showed signs of gut, liver, and kidney injury, whereas organs of CLP-induced WT mice were normal.

Conclusion: CD73-derived adenosine decreases bacterial load, cytokine levels, and immunocyte apoptosis, and organ injury during sepsis. This work was supported by the National Institutes of Health (NIH) Grant R01 GM66189 and by a grant from the Hungarian Research Foundation (OTKA T049537).

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REGULATORY EFFECTS OF ESTROGEN IN LUNG INFLAMMATORY RESPONSES

Peter Ward

Objective: To assess effects of gender and ovarian hormones on lung inflammatory injury.

Materials and Methods: Male and female young adult C57BL/6 mice were used. Oophorectomized (OVX) females were also employed. Where indicated, estradiol (50 μg/kg body weight) was injected intraperitoneally 1 hr before induction of acute lung injury (ALI). ALI was induced by intratracheal administration of E. coli lipopolysaccharide (LPS, 1mg/kg body weight). Endpoints were leak of ^{125}I-albumin from the vascular compartment into lung, injury was measured as a ratio of ^{125}I ratio activity in lung (after perfusion with saline) to that radioactivity present in serum. Other endpoints were neutrophils (PMNs) in bronchoalveolar lavage (BAL) fluids, lung content of myeloperoxidase (MPO), and BAL content of TNFα, IL-6 or IL-1β, as assessed by ELISA. ICAM-1 content in lung homogenates was also assessed by ELISA.

Data: When ovary-intact female and male littermates of C57Bl/6J mice were evaluated for intensity of LPS-induced ALI, males consistently had more intense ALI, greater PMN content in BAL fluids, and increased lung content of MPO when compared to females. Bronchoalveolar lavage (BAL) fluids from males showed higher levels of IL-1β, and lung extracts had higher levels of ICAM-1 as compared to females. When OVX and ovarian intact mice were compared in the lung model, the inflammatory responses and injury parameters were greatly exaggerated in OVX mice. Treatment of OVX mice with estradiol resulted in greatly attenuated parameters of lung inflammation and injury, with values close to those of ovary-intact mice. In OVX mice treated with

estradiol, the enhanced levels of IL-1β (in BAL fluids) and lung content of ICAM-1 were reduced to levels found in ovarian-intact mice. In vitro exposure of wild-type macrophages to LPS showed that the co-presence of estradiol substantially suppressed release of IL-1β.

Conclusions: ALI induced in mouse lungs after airway injection of LPS, the intensity of which is much greater in males compared to females. OVX females also showed greatly enhanced ALI responses to LPS, which could be reversed if OVX mice were treated with estradiol. The intensity of ALI seems to be closely correlated with BAL levels of IL-1β and tissue levels of ICAM-1. These data suggest that ovarian hormones such as estradiol modulate the lung inflammatory responses and protect females from excessive inflammatory injury.

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GENDER DIMORPHISM IN MYOCARDIAL ISCHEMIA AND REPERFUSION INJURY

Daniel Meldrum

Heart failure from ischemic heart disease remains the leading cause of death in the industrialized world. Women experience an overall lower incidence of heart failure and higher heart failure survival than men. Tumor necrosis factor-alpha (TNF) is increased in myocardial tissue after ischemia and reperfusion (I/R). TNF contributes to postischemic myocardial dysfunction and induces proinflammatory signaling, which may be mediated by the 55-kDa TNF receptor (TNFR1). In humans, there is a direct correlation between functional capacity, survival, and circulating TNF levels. Although decreasing the TNF level in animals was beneficial after myocardial ischemia, simply decreasing the bioavailability of TNF in humans with heart failure was not beneficial. This led to the important appreciation that TNF may have beneficial or deleterious effects in the heart, depending on which of its receptors is activated. Females have a lower incidence of heart failure and a higher heart failure survival than males. Based on this, our global hypothesis is that TNFR1 signaling resistance occurs in the female myocardium during ischemia through estrogen mediated upregulation of SOCS3 signaling in females. This talk will: 1) review the important original data from Dr. Chaudry 's group that led to this hypothesis; 2) present the findings from males and females that support this hypothesis; 3) explore the implications for future translational studies on anti-TNF therapy in heart disease.

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AGE DIFFERENCES IN THE INFLAMMATORY AND HYPERMETABOLIC RESPONSE POST BURN

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Introduction: According to the World Health Organization, the highest mortality in severely burned children occurs in patients less than 4 years of age. The aim of the present study was to identify contributors to mortality in this age group.

Patients and Methods: Severely burned pediatric patients were divided into three age groups: 0-3.9 years, 4-9.9 years and 10-18 years of age. Resting energy expenditure (REE) was measured by oxygen consumption, body composition was determined by dual x-ray absorptiometry (DEXA), liver size and cardiac function by ultrasound, and inflammatory markers, hormones, and acute phase proteins by laboratory chemistry.

Results: One-hundred eighty-eight children were included into the study. N=55 were 0-3.9 years, n=70 were 4-9.9 years and n=63 were 10-18 years. Measured REE and percent predicted REE was highest in the 10-18 years group followed by the 4-9.9 year and lowest in the 0-3.9 year, $p<0.05$. Children 0-3.9 years maintained lean body mass and body weight during acute hospitalization while children >4 years lost body weight and lean body mass, $p<0.05$. DEXA analysis reveals distinct differences in body composition between the three groups, $p<0.05$. The inflammatory cytokine profile showed no differences between the three age groups. Liver size significantly increased in the 10-18 years group (+160%), followed by the 4-9.9 years (+135%) and was lowest in the group 0-3.9 years (+83%), $p<0.05$. Acute phase proteins and urine cortisol were significantly decreased in the toddler group compared to the older children, $p<0.05$. Cardiac data indicate increased cardiac work and impaired function in the 0-3.9 years group compared to the other two age groups, $p<0.05$.

Conclusions: Increased mortality in young children is associated with increased cardiac work and impaired cardiac function, but not with the inflammatory and hypermetabolic response. Improving cardiac function in children younger than 4 years of age may improve morbidity and mortality of these patients.

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ESTROGEN, HEAT SHOCK PROTEINS AND CARDIOVASCULAR SYSTEM

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Hormones have acute, single-dose effects, as well as chronic effects on gene expression in the cardiovascular system. Estrogen influences the expression of the heat shock proteins (HSPs), a family of protective proteins, in the cardiovascular system. Female rats have higher levels of HSP72 in their hearts than males. Ovariectomy will reduce female levels to that of males after 9 weeks. Estrogen increases the expression of the cardioprotective HSP72 in isolated, adult male rat cardiac myocytes. A similar, but lesser effect, is seen with progesterone and high dose dexamethasone. Pretreatment with estrogen or dexamethasone will protect against injury from simulated ischemia. Endothelial cells show similar responses, but are more sensitive to estrogen, showing an increase in HSP72 with a much lower concentration of estrogen. The mechanism(s) of these effects remains to be completely defined. One possibility is that binding to the intracellular estrogen (ER) or glucocorticoid (GR) receptor alters the equilibrium amongst HSP90 and the intracellular proteins it binds. HSP90 binds ER,GR and heat shock factor (HSF)-1, the transcription factor activating HSP expression in response to stress. Geldanamycin, which inhibits all binding by HSP90, induces increased HSP72 expression. A second possible mechanism is through the activation of NFkB by membrane-localized ER, which leads to HSF-1 activation and an increase in HSP72. Inhibiting the action of NFkB with binding decoys blocks the increase in HSP72. The mechanism for differing HSP72 expression in the "chronic" setting of gender difference remains to be determined. Here our understanding may have been confused by the use of models ranging from juvenile through adult. As hormonal changes are not complete until adulthood, it is important to understand observed changes in the context of the model used. Aging introduces further changes in hormone levels that can blunt the expression of HSPs and other genes. Loss of estrogen, independent of aging, can be proinflammatory and adversely effect the response to injury. Understanding changes in the expression of HSPs and other genes in response to estrogen has the potential to be applied in the clinic setting as a powerful treatment.

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GENDER AND POST-INJURY IMMUNE AND CARDIAC FUNCTIONS

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Several clinical and experimental studies demonstrate a gender dimorphism of the immune and organ responsiveness in the susceptibility to and morbidity from shock, trauma, and sepsis. In this respect, cell-mediated immune responses have been shown to be depressed in males following trauma-hemorrhage, whereas they were maintained/enhanced in proestrus females. Sex hormones have been shown to be responsible for this gender specific immune response following adverse circulatory conditions involving signal transduction pathways, i.e. p38 MAP kinase. Specifically, studies indicate that androgens cause immunodepression following trauma-hemorrhage in males. In contrast, female sex steroids appear to exhibit immunoprotective properties following trauma and severe blood loss.

Altered cardiovascular responses and diminished organ blood flow has been shown to decrease tissue oxygenation in males following trauma-hemorrhage resulting in depressed immune responses. In proestrus females, however, cardiovascular responses are maintained under those conditions. In addition, modulation of androgen- and estrogen-synthesizing enzymes appears to contribute to gender specific immune responses.

Recently, sex hormones, i.e. DHEA have been shown to modulate PBMC function also in surgical patients. Thus, the immunmodulatory properties of sex hormones/receptor antagonists/sex steroid synthesizing enzymes following trauma-hemorrhage might represent novel therapeutic strategies for the treatment of immunodepression in surgical patients.

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CONSIDERATION OF SEX AND SEX-STEROIDS IN PERIOPERATIVE IMMUNOMONITORING USING A WHOLE BLOOD APPROACH

Heiko Trentzsch, Melanie Wasmuth, Siegfried Zedler, Eugen Faist

Gender-related differences in the inflammatory response are supposed to affect occurrence of septic complications following tissue trauma in favor of female sex. This observational clinical study investigated effects of sex and sex-steroids on immune function under physiologic conditions in a whole blood approach.

A cohort of 41 patients undergoing elective open abdominal surgery had perioperative immunomonitoring. Patients were divided by sex (male= m; female= f) and clinical course (uneventful= CON, mCON=12, fCON=18; sepsis according to Bone Criteria= SEP; mSEP=9, fSEP=2). Groups were subdivided by age: <50yrs (mCON=4, fCON=5 - premenopausal) and >50 yrs (mCON=8, fCON=13 - postmenopausal).

Blood was drawn before surgery (D-1) and on postOP day 1,3,5 and 7 (D1,D3,D5,D7). Serum was analyzed for sIL2-R, sTNF-RI (p55) and RII (p75), IL-6, CRP, PCT, testosterone (TES), estrogen (EST) and progesterone (PRO). We performed white blood-cell count and meas-

ured mean signal expression of HLA-DR on CD14+ cells by FACS. Cellular immune-function was tested by incubating whole blood for 24h with LPS (1μg/ml). LPS-induced TNF-α, IL-10, IL-4 and INF-γ levels were measured in supernatants by multiplex analysis. Data was analyzed using ANOVA (Kruskal-Wallis or One-Way ANOVA with suitable post hoc tests) or non-parametric testing (t-test or Mann-Whitney-test) as appropriate. Linear regression was used to detect correlations between sex-steroid levels and immune parameters. Statistical significance was accepted at $p<0.05$.

Sepsis occurred more often in males (fischer's exact test, $p= 0.032$). There was no epidemiological difference in mCON and fCON. In males, TES decreased on D1 returning to baseline on D7, m<50 recovered faster ($p<0.05$). EST and PRO were unchanged. In females, after surgery, EST seemed to drop in fCON<50 and PRO seemed to drop in fCON, both without statistical significance. There was no effect on TES. Interestingly, immune parameters did not correlate with sex-steroid levels at all. Only few gender-differences were observed: In mCON HLA-DR significantly drops with surgery, returning to baseline on D3. However, in fCON this drop is not significant. LPS-induced INF-γ levels show decreased levels after surgery with significant reduction in mCON on D1 and D3. Although there was no difference in absolute levels between mCON and fCON, females still had low levels on D7, thus recovering more slowly. All other parameters showed no gender-difference. Stratification by gender confirmed known markers associated with septic complications for male patients.

Our data supports the dogma of females being less susceptible to sepsis. Although, sex-steroids have profound pharmacologic effects, plasma-protein-borne sex-steroids in the blood do not affect immune function. In deed there are no marked differences in the inflammatory response of males and females with an uncomplicated postoperative course. In conclusion, gender-differences, that protect females from sepsis, are likely to be a consequence of individual risk-factors and thus can only be observed when data is not stratified by clinical course.

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A 150

VASCULITIS AND PULMONARY HEMORRHAGE: PATHOGENESIS, TREATMENT AND OUTCOMES

Ulrich Specks

Diffuse alveolar hemorrhage (DAH) occurring in the context of a vasculitis syndrome is usually caused by pulmonary capillaritis. Pulmonary capillaritis is defined as a histopathologic pattern of alveolar wall inflammation that leads to the disruption of the integrity of alveolar-capillary basement membranes and flooding of the alveoli with blood. The underlying immune-mediated process causing capillaritis is systemic in nature, and alveolar hemorrhage is but one clinical manifestation. Rarely, pulmonary capillaritis occurs in isolation. This presentation outlines a systematic diagnostic approach to the management of patients presenting with DAH leading to treatment based on pathomechanisms. Pulmonary capillaritis with DAH is most commonly caused by the vasculitides affecting predominantly small vessels, Wegener's granulomatosis or microscopic polyangiitis. These disorders have detectable autoantibodies reacting with intracellular neutrophil enzymes (proteinase 3 or myeloperoxidase). These anti-neutrophil cytoplasmic antibodies (ANCA) have been implicated in the pathogenesis of capillaritis by exerting a variety of pro-inflammatory effects that contribute to endothelial cell destruction. Aggressive immunosuppression remains the primary mode of therapy. Once remission has been induced, the focus shifts towards remission maintenance with less toxic regimens. The role of glucorticoids, cytotoxic agents, plasma-exchange and novel biologic agents in the therapy of pulmonary capillaritis will be reviewed in light of recent trial results.

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MECHANISMS OF RAPIDLY PROGRESSIVE RENAL FAILURE IN THE SETTING OF WEGENER'S GRANULOMATOSIS (WG), MICROSCOPIC POLYANGIITIS (MPA) AND CHURG STRAUSS SYNDROME (CSS)

Cees Kallenberg

Within the spectrum of idiopathic systemic vasculitides, WG, MPA, and CSS belong to the group of small-vessel vasculitides and are strongly associated with the presence of anti-neutrophil cytoplasmic auto-antibodies (ANCA). ANCA directed to proteinase 3 (PR3-ANCA) are more frequently seen in WG, and ANCA to myeloperoxidase (MPO-ANCA) more frequently in MPA and CSS. Rapidly progressive glomerulonephritis (RPGN) is a clinical hallmark of the ANCA-associated vasculitides (AAV), histopathologically manifested as necrotizing crescentic glomerulonephritis (NCGN). RPGN occurs in Goodpasture's syndrome in association with autoantibodies to the glomerular basement membrane (GBM) resulting in a linear fluorescence along the GBM when a renal biopsy is stained for IgG and complement. It also occurs in immune complex mediated renal diseases, such as SLE and infection-related renal disease, in which a granular staining for IgG and complement is seen along the GBM. In AAV, immune complexes are, however, generally lacking in cases with RPGN (pauci-immune NCGN). Nevertheless, ANCA, in particular MPO-ANCA, appear to be pathogenic as shown in experimental models in which MPO-ANCA alone are able to induce systemic vasculitis including NCGN. This is far less clear for PR-ANCA in experimental models. In vitro, both PR3-ANCA and MPO-ANCA are able to activate (primed) neutrophils towards the release of lytic enzymes

and the production of reactive oxygen species. In the presence of endothelial cells ANCA-activated neutrophils adhere to and damage these endothelial cells. Taken together, experimental data strongly suggest a direct pathogenic role for MPO-ANCA in AAV-associated renal disease, probably by their potential to activate neutrophils. This is, presently, less clear for PR3-ANCA associated (renal) disease in which other (exogenous?) factors may be operative as well. Despite the experimental evidence, it should be stated that the clinical association between levels of ANCA and (renal) disease activity is not perfect.

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VASCULITIS AND RENAL TRANSPLANTATION - SHOULD SUCH PATIENTS BE TRANSPLANTED?

Kirsten de Groot

Anti-neutrophil cytoplasmic antibody-associated systemic vasculitis (AASV) is characterized by a necrotizing small vessel inflammation, frequently involving the kidneys presenting with a rapidly progressive glomerulonephritis. This scenario leads to end-stage renal failure in 10-20% of the patients referred to renal care centers. Up to 25% of these patients regain dialysis-independent renal function subsequently. For patients remaining on chronic maintenance hemodialysis, renal transplantation is one option of renal replacement therapy. However data is on the outcome of AASV patients after transplantation is scant, as patients with AASV as the underlying cause of end stage renal failure may have been under-recognized in renal units before ANCA testing was available and thus patients may have been classified variably. The retrospective analysis of several patient series reveals that patient and graft survival in AASV patients is not inferior to that of patients with nonsystemic inflammatory conditions. However, the median age of patients with AASV is significantly higher compared to that of other patients cohort at transplantation, such as diabetics or patients with IgA nephropathy. The overall risk of relapse after renal transplantation is 17% including recurrent glomerulonephritis, but these relapse rates per patient and year are lower compared to AASV patients on maintenance dialysis. Median time from transplantation to relapse was 31 months. AASV patients undergoing renal transplantation should be in complete or at least stable partial remission, ANCA titer or time on dialysis do not seem to affect outcome after transplantation. In onclusion, renal transplantation is a realistic option in patients with end-stage renal disease and AASV with a reasonable patient and graft survival and lower recurrence rate as compared to maintenance dialysis.

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ACCELERATED ATHEROSCLEROSIS IN SYSTEMIC LUPUS AND RHEUMATOID ARTHRITIS

Susan Manzi

Women with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are at significant risk for premature and accelerated atherosclerosis. The reasons for the increased risk in these inflammatory autoimmune conditions is not clear but cannot be fully explained by traditional cardiovascular risk factors alone. There are many interesting parallels between pathogenesis of SLE and RA and atherosclerosis that are currently under investigation, including the role of systemic inflammation, infectious agents, immune-mediated vascular damage and autoantibody production. This presentation will examine the burden of cardiovascular disease in SLE and RA, discuss the current wisdom regarding pathogenesis, and offer practical approaches to management.

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PATHOGENIC MECHANISMS IN GIANT-CELL ARTERITIS

Maria Cid

Giant-cell arteritis (GCA) is a granulomatous vasculitis involving large and medium-sized vessels. It is currently believed that inflammatory lesions develop as a consequence of an antigen-specific immune response but the triggering agent(s) have not been identified. Activation of vascular dendritic cells may be an important early step in the development of inflammatory lesions. It has been proposed that dendritic cell activation can be triggered by stimuli sensed by toll-like receptors, supporting a role for innate immunity in the early events leading to GCA. Activated dendritic cells efficiently recruit, present antigens and activate T cells which subsequently undergo clonal expansion and a Th1 functional differentiation with vigorous production of IFNg. IFNg is a powerful stimulator of macrophage activation and granuloma formation, which configure the typical histopathologic pattern of GCA. At this point, activated T cells and macrophages release cytokines and growth factors with powerful local and systemic effects. Most of the clinical manifestations and complications of GCA may be attributable to the potent effects that these mediators exert on the vessel wall and on distant tissues. The strong acute phase response characteristic of GCA appears to be related to the production of pro-inflammatory cytokines IL-1b, TNFa and IL-6 in lesions. Proteolytic enzymes such as MMP2, MMP9 and MMP12, as well as reactive oxygen species produced by activated macrophages, have a high destructive potential and disruption of the vessel wall integrity may lead to deleterious consequences such as aneurysm formation, an increasingly recognized compli-

cation of GCA. Activated macrophages also produce angiogenic factors such as VEGF, FGFs, PDGF, and IL-6, among others, which elicit a prominent neovascularisation in lesions. Myointimal cell proliferation, migration, and matrix production, stimulated by growth factors such as PDGF and TGFb, leads to the development of intimal hyperplasia, resulting in lumen occlusion and ischemia of supplied tissues. A strong angiogenic response is associated with low risk of ischemic events, suggesting that angiogenesis may have a compensatory role for ischemia at distal sites. Patients with GCA usually experience a dramatic relief of their symptoms with corticosteroid treatment. Corticosteroids may abrogate the production of many inflammatory mediators critical in determining GCA symptoms and complications. However, while some patients easily enter sustained remissions, others suffer from relapsing disease, requiring long corticosteroid treatment. Mechanisms involved in persistence of disease activity are not known. Patients who develop an intense acute phase response are usually more refractory to treatment. These patients have increased production of pro-inflammatory cytokines which are able to maintain and amplify inflammatory cascades. Increased TNFa and CCL2 production in lesions have been demonstrated, indeed, to be associated with higher corticosteroid requirements. However, blocking TNFa is not sufficient to abrogate disease activity, as demonstrated by a recent clinical trial, and increased production of TNFa may be only a footprint of other events more relevant in determining the fate of the disease. Investigating mechanisms leading to persistence of inflammatory activity are crucial to identify new therapeutic targets and improve the outcome of patients with GCA.

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ADJUNCTIVE THERAPY TO CORTICOSTEROIDS IN GIANT CELL ARTERITIS

Gary Hoffman

In Northern Europe and North America, Giant Cell Arteritis (GCA) has been estimated to have an annual incidence of 19-32 cases per 100,000 in people older than 50 years. In Mediterranean countries the annual incidence appears to be lower, occurring in 6-10 cases per 100,000. Treatment with corticosteroids (CS) dramatically alters the symptoms and disease course, reducing the likelihood that the patient will develop blindness. However, relapses usually occur when CS doses are tapered, resulting in retreatment, CS dependency and toxicity. About 80% of patients will experience at least one adverse event attributable to CS, ~60% will have >2 adverse events. Patients with GCA have an increased risk of fractures and CS-related cataracts. There is an unmet need for adjuncts to the treatment of GCA that would effectively reduce the dose and duration of CS and provide more durable remissions. Prior publications have evaluated the utility of cytotoxic and anti-inflammatory agents in GCA. However, these reports have either been anecdotal, uncontrolled, or if controlled, generated conflicting results with regard to efficacy.

Interleukin (IL)-1, IL-6, tumor necrosis factor-a (TNF-a), and interferon-g have each been implicated in contributing to vascular injury in patients with GCA. Previously published case studies reported that some patients with GCA or polymyalgia rheumatica who received the anti-TNF-a agent infliximab sustained remission and became CS-independent. We have conducted the first randomized, placebo-controlled, double-blind, multicenter trial of standardized treatment with glucocorticosteroids and adjunctive treatment with either placebo or infliximab in patients who were newly diagnosed with GCA.

The results of this study failed to demonstrate that infliximab improved the duration of remissions or reduced CS requirements. Additional support of these findings comes from a study of PMR. Considered to be a forme fruste of GCA, the results of this trial also showed no significant therapeutic benefit from infliximab. Thus, these two studies indicate that the addition of infliximab to CS do not markedly diminish relapse rates or cumulative CS requirements.

These results are contrary to our observations in Takayasu's arteritis (TAK), another form of large vessel vasculitis that affects predominantly young women of reproductive age. The annual incidence of TAK in the USA has been reported as 2.6/1,000,000. While up to 20% of TAK patients have a monophasic illness, most have a relapsing/remitting course, typically requiring prolonged treatment with CS and additional immunosuppressive agents. However, most of TAK patients are unable to achieve adequate disease control on these therapies as well.

In TAK patients refractory to other immunosuppressive therapies, anti-TNF therapy led to complete or partial remission in 79% of patients. Anti-TNF therapy allowed prednisone to be discontinued or tapered in 60% and 28% respectively. Of those patients taking concurrent immunosuppressive drugs other than prednisone, anti-TNF therapy allowed reduction of the dose of these agents in 50%. Why our results differ in these two forms of granulomatous vasculitis is uncertain. Nonetheless, these findings provide further support for the rationale for the randomized controlled trial of anti-TNF therapy in TAK.

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SURFACTANT TREATMENT: NEW INTERPRETATIONS OF EVIDENCE

Wolfgang Strohmaier, Giuseppe Marraro

The treatment of the Adult Respiratory Distress Syndrome (ARDS) was and still is a major challenge in an ICU. A long list of papers described successful experimental work following the concept of surfactant

administration for the treatment of established ARDS. But there is only a short list of papers describing clinical success. In this presentation we will argue that this discrepancy is the consequence of overdrawn extrapolations and simplifications. We will select three representative aspects and discuss an alternative interpretation of evidence.

First, the use of surfactant is based on biological evidence and has proven to be highly effective in preterm neonates. At this point the first overdrawn extrapolation was made: If it is effective in preterm babies to administer a high dose (200 mg/kg) of surfactant as a bolus it must work likewise in adults. Support to this concept came from findings showing that protein-rich edema fluid inhibits surfactant. The conclusion: to give more surfactant to overcome inhibition and to replenish the pool. The results of most experimental studies seemed so convincing, that the increasing knowledge about the striking pathophysiologic differences between the neonate and the adult situation was neglected. The second aspect is an overdrawn extrapolation again: virtually all types of lung injury models responded to the above established approach, namely pouring down the trachea a big amount of surfactant. This led to the simplified belief that all types of lung injury can clinically be treated with surfactant in the same way. The third aspect is directly linked to the diagnosis of ARDS in clinical trials: the current definition does neither take into account different etiologies nor PEEP nor the bias estimating LIS. But where is the basic mistake? We believe that it is the initiation of therapy: in experimental studies treatment starts within minutes, sometimes hours after lung injury. In contrast, in clinical trials treatment is started hours or days after the – questionable –diagnosis of established ARDS.

Newer experiments brought up a new treatment modality: the therapeutic BAL with diluted surfactant. This BAL is done as usual almost immediately after lung injury and proved as effective although much less surfactant remained in the lungs. At the same time the clinical trials with surfactant failed. Analysis revealed that patients with direct lung injury showed better outcome than others. And in all of these studies established ARDS was treated, meaning that treatment was started days after lung injury and not minutes as in experimental studies.

Our interpretation of present experimental and clinical evidence is that in cases of direct lung trauma BAL with diluted surfactant is a potent therapeutic intervention in the ICU, since direct lung injury often allows a precise estimate of the time of occurrence and has a well described sequelae. Future treatment must focus on the prevention of ARDS known to carry a high potential to harm the patient. Since many triggers for ARDS are well-known, early surfactant treatment should be considered to be more than a theoretical option in more clinical situations.

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LUNG INFLAMMATION AND INFLAMMATORY GENE EXPRESSION PROFILING UPON BRONCHIAL INSTILLATION OF LIPOPOLYSACCHARIDE AND LIPOTEICHOIC ACID IN HUMANS

Jacobien Hoogerwerf, Alex de Vos, Paul Bresser, Christian Draing, Sonja von Aulock, Tom van der Poll

Objectives: Toll-like receptors (TLRs) play a crucial role in the recognition of 'pathogen-associated molecular patterns' in the lung, which is considered to be important for an appropriate immune response against pathogens that enter the lower airways. Here, we studied the effects of two different TLR agonists relevant for respiratory infections in the human alveolar space: lipoteichoic acid (LTA, TLR2 agonist) and lipopolysaccharide (LPS, TLR4 agonist). To determine the specific contribution of alveolar macrophages in vivo, this study investigated the effects of LTA and LPS on inflammatory gene expression patterns of isolated macrophages.

Materials and Methods: A dose-finding pilot experiment was performed with bronchial LTA instillation (4-100 ng/kg body weight). In an additional experiment sixteen healthy non-smoking male subjects were given either LPS (E. coli, 4 ng/kg body weight) or LTA (S. aureus, 100 ng/kg body weight): by bronchoscope sterile saline was instilled into a lung subsegment followed by instillation of either LTA or LPS into the contralateral lung. Six hours later a bronchoalveolar lavage was performed and inflammatory reactions were determined in the fluid obtained. RNA from alveolar macrophages was isolated and analyzed by multiplex ligation-dependent probe amplification. CD71 was measured by flow cytometry.

Data: The pilot study showed a significant dose-dependant increase of neutrophil counts and cytokine release upon LTA instillation compared to saline. In the additional experiment both LTA (100 ng/kg) and LPS mounted an inflammatory response, as reflected by increased neutrophil counts. Moreover there was an increased release of pro-inflammatory cytokines and chemokines upon LTA or LPS instillation compared to saline. LPS enhanced alveolar macrophage expression of mRNAs encoding the pro-inflammatory mediators IL-6, IL-8, MCP-1 and MIP-1ß. However, no significant increases were observed after instillation of LTA and the expression levels of IL-18 were decreased. LPS induced activation of macrophages as shown by increased surface expression of CD71 on macrophages, whereas LTA showed a trend towards an increased expression ($P = 0.06$).

Conclusion: This is the first study to report that instillation of LTA in a subsegment of the lung induces a significant inflammatory response in human volunteers. LTA and LPS differentially affect inflammatory gene expression profiles in human alveolar macrophages in vivo. The specific contribution of macrophages should therefore be further investigated. This novel human model may be used to evaluate pathogenetic mechanisms and new interventions.

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A 158

STREPTOCOCCAL M1 PROTEIN CAUSES SEVERE LUNG DAMAGE VIA ACTIVATION OF AN INNATE IMMUNE CASCADE: A CENTRAL ROLE FOR NEUTROPHIL GRANULE PROTEINS

Oliver Soehnlein, Heiko Herwald, Lennart Lindbom

Objective: Severe infections with Streptococcus pyogenes are associated with massive inflammatory reactions in man, and recent data have demonstrated a critical role of complexes formed by streptococcal M1 protein and fibrinogen in the inflammatory response (Herwald et al., Cell, 2004). In the current study we aimed at defining the importance of blood neutrophils (PMN) and their secretion products as well as alveolar macrophages in the lung damage caused by M1 protein.

Materials and Methods: M1 protein was intravenously injected into Balbc mice and allowed to circulate for 30 min. The lung damage was assessed by electron microscopy, comparison of wet weight/dry weight ratios and by analysing the bronchioalveolar lavage (BAL) fluid with respect to protein content and leukocyte count. Ablation of alveolar macrophages was induced by intranasal application of clodronate liposomes and depletion of circulating PMN was performed by intravenous injection of RB6-8C5 monoclonal antibody. Neutrophil granule proteins were restored to the circulation in neutropenic animals through intravenous administration of neutrophil secretion obtained after beta2-integrin cross-linking. Measurements of intracellular Ca^{2+} and assays quantifying protease activity and release of reactive oxygen species after challenge with neutrophil granule proteins were used to asses the activation of macrophages.

Results: Injection of M1 protein caused severe lung damage as characterized by PMN accumulation, haemorrhage, tissue destruction, vascular leakage and edema formation. Depletion of either PMN or alveolar macrophages completely abolished lung tissue injury. Injection of neutrophil granule proteins restored the inflammatory reaction in neutropenic mice, but not in mice that were depleted of both PMN and alveolar macrophages revealing a critical cross-talk between the two cell populations in the pathogenesis of M1 protein-induced lung damage. In-vitro stimulation of murine macrophages with neutrophil granule proteins caused a strong activation of macrophages as indicated by intracellular Ca^{2+} mobilisation. This activation was associated with a rapid exocytosis of proteases and extracellular release of reactive oxygen radical species which may contribute to the lung damage.

Conclusion: Our data demonstrate a central role of the neutrophil - alveolar macrophage axis in severe streptococcal infections. Of particular importance are neutrophil granule proteins which activate resident macrophages in the lung.

A 159

BLOCKAGE OF THE BETA-ADRENERGIC RECEPTOR AUGMENTS LIPOPOLYSACCHARIDE-INDUCED LUNG INFLAMMATION IN MICE

Ida Giebelen, Tom van der Poll

Objective: β2 adrenergic receptors are expressed on different cell types in the lung including respiratory epithelial cells, smooth muscle cells and macrophages. β2 receptor agonists are widely used by patients with obstructive pulmonary disease because of their bronchodilatory properties. We recently established that β2 agonists also exert strong anti-inflammatory effects in the lung (Am J Respir Crit Care Med. 2005; 172: 878). The aim of the current study was to determine the role of β adrenergic receptors in the regulation of lung inflammation induced by lipopolysaccharide (LPS).

Material and Methods: To investigate the effect of blockage of the β receptor, C57BL/6 mice inhaled nebulized propranolol, a non selective β antagonist, after which LPS (10 μg/mouse) was administered intranasally. At 3 and 6 hours after LPS administration bronchoalveolar lavage (BAL) was performed and lungs were harvested.

Data: LPS induced a profound inflammatory response in the lungs as reflected by influx of neutrophils and release of proinflammatory cytokines (TNFα, IL-6) and chemokines (MIP-2, KC) into BAL fluid (all $P < 0.05$ versus saline). Blockade of endogenous β adrenergic receptors in the pulmonary compartment resulted in enhanced LPS-induced lung inflammation which especially was reflected by a stronger secretion of TNFα, IL-6 MIP-2 and KC into BAL fluid and lung homogenate ($P < 0.05$ versus LPS only).

Conclusion: Hence, these data indicate (1) that lung cells secrete products that stimulate β2 receptors, and (2) that β2 adrenergic receptors, which are widely expressed in the lungs, serve as negative regulators of lung inflammation.

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UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR DEFICIENT MICE SHOW REDUCED LUNG INFLAMMATION DURING HYPEROXIA

Marieke AD van Zoelen, Jennie Pater, Tom van der Poll

Background: Patients with respiratory failure often require supplemental oxygen therapy and mechanical ventilation. Although both supportive measures are necessary to guarantee an adequate oxygen uptake, they can also cause lung inflammation and injury. Hyperoxia induced lung injury is characterized by infiltration of neutrophils into the lung. Mechanisms underlying the enhanced recruitment of neutrophils to the lung during hyperoxia have not been fully elucidated. Urokinase plasminogen activator receptor (uPAR) is expressed at the surface of several cell types and has been implicated to be important for leukocyte trafficking.

Objective: To determine the role of uPAR in pulmonary inflammation induced by hyperoxia.

Material and Methods: C57Bl/6 wild type and C57Bl/6 uPAR knock-out mice were exposed to either hyperoxia (O_2>80%) or room air. After 4 days, pulmonary cell suspensions were obtained and analyzed by flow cytometry or bronchoalveolar lavage (BAL) was performed.

Data: First, we established in C57Bl/6 wild type mice that exposure to hyperoxia (O_2>80%) during 4 days induces migration of uPAR positive granulocytes into the lungs compared to healthy control mice (exposed to room air). Subsequently, we compared C57Bl/6 wild type with C57Bl/6 uPAR knock-out (KO) mice during hyperoxia. Hyperoxia elicited a pulmonary inflammatory response as reflected by a profound rise in the number of total cells recovered from BAL fluid and lung cell suspensions which was primarily due to a rise in the number of neutrophils (both $P < 0.05$ versus room air). In addition, hyperoxia induced an enhanced release of proinflammatory cytokines and chemokines (IL-6 and KC) and increased total protein and alkaline phosphatase (AF) levels in BAL fluid (all $P < 0.05$ versus room air). uPAR KO mice had lower numbers of total cells and neutrophils in BAL fluid and lungs compared to wild type mice ($P < 0.05$). Furthermore, bronchoalveolar IL-6 and KC concentrations, total protein and AF concentrations were decreased in uPAR KO mice (all $P < 0.05$ versus wild type mice).

Conclusion: These findings suggests that endogenous uPAR contributes to a detrimental inflammatory response during hyperoxia induced lung injury and that inhibition of uPAR may improve the outcome.

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PNEUMONITIS ASSOCIATED WITH SIROLIMUS: CLINICAL CHARACTERISTICS, RISK FACTORS AND OUTCOME

Peter Schenker, Stefan Weiner, Oliver Vonend, Lars Rump, Richard Viebahn, Michael Schöffer

Objective: The introduction of sirolimus as an immunosuppressive drug for renal transplantation has lead to an increase of unexplained interstitial pneumonitis.

Methods. Out of 111 patients receiving sirolimus for prophylaxis of renal transplant rejection 10 patients with interstitial pneumonitis were indentified. Patients underwent computed tomography scans of the lungs and bronchoalveolar lavage (BAL). Medical records were reviewed and sirolimus trough levels were measured to identify risk factors associated with the occurrence of pneumonitis.

Data: Pneumonitis occured within one and eight months after initiation of sirolimus treatment. The patients presented with cough, dyspnea, low-grade fever, fatigue or weight loss. Leucocyte counts varied markedly (mean 7227 / µl, range 1100 - 12000 / µl) and C-reactive protein levels were elevated in all patients (mean 10.7 mg/dl, range 1.1 - 23.5 mg/dl).Chest radiography was unconspicuous in 4 patients. CT scans of the lungs revealed bilateral interstitial infiltrates with ground glass opacities in all 10 patients. Blood cultures and cultures obtained from bronchial aspirates were negative. Cells obtainted from BAL were analysed in three patients, showing lymphocytic alveolitis in 2 patients and alveolar hemorrhage in 1 patient.. In 3 out of 8 patients PCR detected sequences of Mycoplasma pneumoniae, Influenza virus A, and Cytomegalovirus. However, antibiotic and antiviral treatment did not lead to improvement of the condition. The mean sirolimus trough level at presentation was 16. µg/L (range: 6.2 - 38.7 µg/L). Transplant renal function was severely impaired in all patients with pneumonitis (pneumonitis (mean serum creatinine: 3.1 mg/dl, range: 1.9 to 5.3 mg/dl). Two patients needed hemodialysis shortly before pneumonitis was diagnosed.

Sirolimus was discontinuated in 5 patients and the dose of sirolimus reduced in the remaining 5 patients. Antibiotic treatment was given in 9 out of 10patients, without an obvious benefit. Pneumonitis resoluted within 14 - 28 days in all patients. Patient 4 continued sirolimus at a low blood level, but relapsed after five months. At this time, the sirolimus-level was elevated again. After discontinuation of sirolimus pneumonitis regressed within two weeks.

Conclusion: The frequency of interstitial pneumonitis appears to be increased in renal transplant patients receiving sirolimus. Risk factors were a sirolimus blood through level above 12 µg/L, a serum creatinine of 1.9 mg/dl or higher. Pneumonitis may recur in patients with continued sirolimus treatment, especially when the sirolimus blood levels increases again. Discontinuation of sirolimus may be the safest treatment option for these patients.

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COAGULATION FACTOR XIII IS AN IMPORTANT MEDIATOR IN THE COAGULATING PLASMA INDUCED IL-8 PRODUCTION BY HUMAN LUNG EPITHELIAL CELLS

Marcel Schouten, Karin von Eije, Tom van der Poll, Cornelis van 't Veer

Objective: Activation of the coagulation system induces inflammation. In conditions of pulmonary damage, like in pneumonia or adult respiratory distress syndrome (ARDS), plasma leakage into the alveolar compartment leads to coagulation activation and fibrin formation. This results in a local inflammatory response, which could contribute to pulmonary damage, e.g. by the recruitment and activation of neutrophils. We investigated the potential of coagulating plasma to induce the release of the neutrophil chemoattractant IL-8 by alveolar epithelium and sought to elucidate the underlying mechanism.

Material and methods: Human alveolar epithelial cells (A549) were grown to confluence in a 96 wells plate. Citrate plasma obtained from normal human volunteers was diluted eightfold, recalcified and thereafter put immediately on the cells, as were heat killed bacteria, pro-inflammatory mediators, various compounds of the coagulation system and their inhibitors. Cells were incubated for three hours at 37°C and 5% CO_2 after which supernatant was removed for IL-8 measurement.

Data: Coagulating plasma induced IL-8 production as potently as IL-1β and TNF-α, while heat killed bacteria did not induce any IL-8 production and thrombin only elicited a small IL-8 production. The anticoagulant proteins activated protein C (APC) and tissue factor pathway inhibitor (TFPI) inhibited the plasma induced IL-8 production, but had no effect on the responses elicited by Il-1β and TNF-α, indicating involvement of the coagulation process in the plasma induced IL-8 production. Plasma fibrinogen depletion potently inhibited IL-8 production, but clots generated by incubation of purified thrombin and fibrinogen failed to drive IL-8 production, indicating a role for a fibrin dependent factor in the coagulation induced IL-8 production. The coagulation factor XIII inhibitor monodansylcadaverin inhibited plasma induced IL-8 production to the same extent as APC and TFPI. Consistently, factor XIII deficient plasma failed to induce coagulation dependent IL-8 production, although clots were still formed.

Conclusion: Coagulating plasma induces IL-8 production by human lung epithelial cells to the same extent as IL-1β and TNF-α. This process is dependent on coagulation factor XIII. Activated factor XIII is known to crosslink fibrin, but the exact mechanism by which factor XIII induces IL-8 release by alveolar epithelium still has to be elucidated. Prevention of clot formation with anticoagulant proteins like APC or TFPI or more specific inhibition of factor XIII could counteract the coagulation induced factor XIII dependent induction of inflammation in conditions in which plasma leakage occurs, like in pneumonia and ARDS.

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POLYMORPHISMS IN THE IL-8 AND IL-6 GENES ARE ASSOCIATED WITH PNEUMONIA IN BURN PATIENTS

Fernando Rivera, Agnes Burris, Bret Arnoldo, Jureta Horton, Ming-Mei Liu, Joseph Minei

Previous studies have shown that IL-6 and IL-8 levels are associated with acute lung injury [ALI] and adult respiratory distress syndrome [ARDS]. We have previously demonstrated that IL-6 -174 G→C and IL-8 -251 T→A respectively affected plasma IL-6 and IL-8 protein levels. Therefore, we want to determine if polymorphisms on these genes are associated with ALI and ARDS. We analyzed a cohort of 328 burn injured patients. We did not find any association between IL-6 and IL-8 SPNs with ALI or ARDS. However, both genes were associated with an increased risk for the development of pneumonia; IL-6 -174 C-allele (adjusted odds ratio = 1.53, 95% CI = 1 - 2.35 p 0.04) and IL-8 T-allele (adjusted odds ratio = 2.34, 95% CI = 1.5 - 4 p 0.01). This increased risk associated was independent of other known clinical risk factors.

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ROLE OF NEURONAL NITRIC OXIDE SYNTHASE IN OVINE LUNG INJURY

Fiona Saunders, Martin Westphal, Frank Schmalstieg, Robert Cox, Daniel Traber

Objective: One major factor contributing to acute lung injury and systemic inflammation after burn and smoke inhalation injury is excessive production of nitric oxide by neuronal nitric oxide synthase (nNOS, NOS-1). We hypothesized that the use of 7-nitroindazole (7-NI), a selective nNOS inhibitor, blocks molecular mechanisms in the pathogenesis of ovine acute lung injury.
Material and Methods: Eleven adult ewes were chronically instrumented with a Swan-Ganz™, a femoral arterial, a left atrial catheter and a lung lymphatic fistula to determine cardiopulmonary hemodynamics and pulmonary transvascular fluid flux. After seven days of recovery, sheep were randomly allocated to either an injured untreated control group (n=6), or an injury group treated with 7-NI (n=5). The injury was induced by a 40% total body surface area flame burn in association

with 48 breaths of cotton smoke. 7-NI (1 mg/kg/h) was continuously infused from 1 h post injury to the end of the 24-h study period.
Data: The combination injury was associated with systemic inflammation and oxidative stress, as evidenced by a 2.5-fold increase in plasma nitrite/nitrate (NOx) levels, as well as 6-fold, 2-fold and 3-fold increases in interleukin-8 (IL-8), myeloperoxidase (MPO) and malondialdehyde (MDA) lung tissue concentrations, respectively. These molecular changes were linked to severe pulmonary dysfunction. Compared to untreated controls, 7-NI significantly reduced NOx plasma levels (8.4±1 vs. 26±10 μmol/L) and decreased IL-8, MPO (3.9±0.2 vs. 5.8±0.7 U/g tissue) and MDA lung tissue content (2.7±0.3 vs. 6.6±1.1 nmol/mg protein), thereby improving pulmonary obstruction (12.4±2.2 vs. 28.7±5.2 obstruction score) and PaO_2/FiO_2 ratio(456±40 vs. 313±56, each $p<0.05$).
Conclusion: These data show that nNOS-derived NO plays a crucial role in the pathophysiology of combined burn and smoke inhalation injury and suggest that selective nNOS inhibition may represent a useful approach to attenuate the degree of pulmonary damage.

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MECHANICAL VENTILATION ACTIVATES RENAL ENDOTHELIAL CELLS IN A RAT MODEL OF ACUTE RESPIRATORY DISTRESS SYNDROME

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Objective: Mechanical ventilation (MV) strategy influences survival in critically ill patients and has been implied to play a role in multiple organ failure including acute renal failure. We hypothesize that MV with high tidal volume activates renal endothelium, initiating inflammation and coagulation and thereby contributes to acute renal failure (ARF).

Material and Methods: Male rats were anesthetized, a tracheotomy was performed and 0.4 ml/kg hydrochloric acid was dispersed intra-tracheally. Subsequently, rats were randomized to a lung protective strategy, tidal volume 6 ml/kg and 5 cm H_20 positive end expiratory pressure (PEEP) (LVt) or a lung injurious strategy, tidal volume 15 ml/kg, 0 PEEP (HVt). Urine samples were taken and after 4 hrs animals were sacrificed, blood samples were taken and organs harvested. Serum cytokines were measured by ELISA. Tissue factor (TF) activity, active plasminogen activator inhibitor type 1 (PAI-1) and intercellular adhesion molecule 1(icam-1) were measured in serum and kidney homogenates.

Data: MV with high tidal volume significantly increased peak inspiratory pressures ($P<0.05$), decreased oxygenation ($p<0.05$) and increased lung wet tot dry weight ratio ($p<0.001$). Serum TNF-α, MIP-2 and IL-6 levels were non-significantly increased in the HVt group. No differences were observed in creatinine clearance and fractional sodium excretion after 4 hrs. In serum levels of ICAM-1 and PAI-1 no differences were observed, however TF activity was significantly decreased ($p<0.01$). In kidney homogenates no differences were observed in ICAM-1 and TF activity. In contrast PAI-1 levels decreased significantly in kidney homogenates ($p<0.05$).

Conclusion: High tidal volume MV significantly worsened acid induced lung injury and showed a trend to increase serum cytokines. This was associated with alterations in coagulation and fibrinolysis in serum and kidneys, but not with changes in ICAM-1 levels in serum or kidney.

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THE MURINE PULMONARY ENDOTHELIAL CELLÆS RESPONSE TO PRO-INFLAMMATORY STIMULI ASSOCIATED WITH TRAUMATIC SHOCK

Joanne Lomas-Neira, Doreen Wesche-Saldato, Chun-Shiang Chung, Fabienne Venet, Mario Perl, Alfred Ayala

The development of sepsis/septic shock in the critically ill patient or animal is associated with an increased probability for the development of acute lung injury (ALI). Characteristic of ALI are increased pulmonary vascular permeability, inflammation, pulmonary edema and PMN accumulation. Endothelial cells (EC) function as a critical and selective interface for neutrophil adhesive interactions and for the propagation of the inflammatory response via interaction with circulating levels of pro-inflammatory cytokines/chemokines. In this respect, understanding the contribution of ECs to the pathogenesis of ALI is of critical importance. This has been especially difficult in the mouse (an important genetic model system) were there are few fully functional pulmonary micro-vascular endothelial cell lines and were the isolation and culture of lung pulmonary ECs has been of limited success.

Given that, the recent development of a transgenic (Tg) mouse strain with GFP cDNA fused to the promoter/enhancer region of an EC specific gene, TIE2 (TgTIE2GFP)287Sato/J, Jackson Laboratory), now offers the possibility of isolating ECs from the lung for ex vivo culture experiments. For this study, lungs from TIE2 Tg mice (male, 8-9 weeks old) were perfused with Hank's Balanced Salt Solution, harvested and incubated in enzymatic digest solution for 45 min in a 37°C water bath. Lung digest cells were then centrifuged, washed and counted. GFP^+ cells were sorted from the mixed pulmonary cell population using a BD-FACSVantage SE, flow cytometer and cultured in T25 tissue culture flasks coated over night with 0.1% gelatin (4 lungs yield approx. $3.4x10^6$ GFP^+ EC cells). A confluent monolayer (resistance

>1200ohms) displaying characteristic EC morphology and expressing GFP (as assessed under fluorescent microscopy) was observed after 5 days. Cells were passed at day 5, $2.5x10^6$ cells were re-cultured and the remaining were plated at $1x10^6$ cells/well on glass slides (0.1% gelatin coated) in 6 well culture plates. To establish the functional characteristics of these cells, they were incubated with TNF-a (10ng/ml) for 4 hours. The supernatants were aspirated and stored for cytokine/chemokine assays, the ECs were trypsonized and lysed for assessment by Western Blot.

Our data showed that TNF-a stimulation increased the production of neutrophil chemoattractant proteins, MIP-2 and KC (mouse homologues of IL-8), and pro-inflammatory cytokine, IL-6, by murine pulmonary ECs. Subsequently, we found using the EICS system to assess membrane integrity in response to pretreatment with TNF-a, followed by co-culture with leukocytes from septic mice, that along with exhibiting a loss of membrane integrity, there was a significant increase in IL-6 production.

Taken together, these findings describe a straightforward method for obtaining mouse pulmonary ECs which exhibit general characteristics and responsiveness comparative to other EC, but are also distinctive to the mouse. (NIH RO1-HL73525)

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MOLECULAR DIAGNOSTICS IN ACUTE LUNG INJURY

J Perren Cobb

Molecular markers of use in optimizing diagnosis and therapy for acute lung injury remain elusive. This presentation will review recent progress in the application of genomics, transcriptomics, and proteomics to determine genetic predisposition to acute lung injury and to gauge the type and severity of insult. Data from both humans and mouse models will be discussed.

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NF-K-BETA AS A KEY LINK BETWEEN CHRONIC INFLAMMATION AND CANCER

Florian Greten

In a mouse model of colitis-associated cancer we were recently able to gather molecular evidence that point to the IKKbeta dependent NF-kappaB activation pathway as the major link between inflammation and cancer. Mice deficient for IKKbeta in enterocytes showed a massive reduction in tumor incidence without decreasing the severity of inflammation. A significant induction of apoptosis in enterocytes was responsible for the decrease in tumor numbers, but led also to more tissue damage and a more severe course of acute colitis. Conversely, mice that lacked IKKbeta in myeloid cells (macrophages and neutrophils) were not only able to decrease tumor incidence but also to reduce tumor size, which was due to a diminished production of pro-inflammatory cytokines that can stimulate proliferation of enterocytes in a paracrine manner. In addition to inflammation-associated tumorigenesis, also in a mouse model for sporadic colorectal cancer epithelial IKKbeta positively regulates the cell cycle machinery. Thus, IKKbeta represents an interesting target for the treatment and prevention of colorectal cancer.

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EXTRACELLULAR REDOX AND ENDOGENOUS DANGER FACTORS IN TUMOR PROGRESSION

Anna Rubartelli

Molecules derived from the dying cells, also called damage-associated molecular pattern molecules (DAMPs), have a crucial role in activating the innate immune system. DAMPs include leaderless secretory proteins, a group of cytosolic or nuclear proteins that have intracellular functions but can also be secreted by cells of the innate immunity or passively released by any type of injured cells. On delivery, leaderless secretory proteins move from a reducing (intracellular) to an oxidizing (extracellular) milieu which may cause their oxidation-mediated inactivation. The rapid extracellular inactivation of oxidation-sensitive DAMPs can be advantageous to limit and solve the inflammatory response. In case of chronic inflammation, a situation often associated to tumours, inflammation is sustained by the pathologic microenvironment through mechanisms only partially understood. Here I will discuss some results obtained on primary human non small cell lung cancers and on lung cancer cell lines in vitro and in vivo, that point to a crucial role of the oxide-reductases/leaderless proteins/DAMPs MIF and thioredoxin in the alteration of the microenvironment sustaining inflammation and promoting tumor progression. Overexpression of Thioredoxin and MIF is accompanied by release of the two proteins (by infiltrating inflammatory cells or by necrotic tumour cells) and of large amounts of non-protein thiols that generate a reducing microenvironment. This altered microenvironment may sustain inflammation by prolonging the extracellular activity of oxidation-sensitive DAMPs and concurs to increase the invasiveness of the tumor cells by altering the expression of adhesion molecules.

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THERMAL STRESS-RELATED MODULATION ON IMMUNE RESPONSE AND THE ROLE OF HEAT SHOCK PROTEINS

Valeria Milani, Gabriele Multhoff, Rolf Issels

Fever is a ubiquitous response to infection and generally accompanies immune responses. It has shown strong evolutionary conservation despite the significant metabolic demands on the individual. This suggests a pronounced beneficial role for survival. Coley was the first that experimented with intentional induction of fever and inflammation as treatment of cancer. In this setting, which is considered as the first "cancer vaccine", a strong correlation between a patient's cancer regression and high fever was observed. The cellular stress response under hyperthermic conditions is the basis for innovative concepts to treat malignancies. During clinical hyperthermia temperatures between 40°C and 44°C are reached at the tumor site. Within this temperature range various effects are being observed including direct cytotoxicity (local necrosis) inducing release of HSPs (extracellular HSP) and potentially activating the immune stimulatory activity, and survival of cells with elevated levels of intracellular HSP that potentially enacts cytoprotection and escape from immune destruction. Clinical hyperthermia affects various aspects of innate and adaptive immune responses. Thermal stress influences antigen presentation, maturation and migration of dendritic cells (DCs), as well as homing of lymphocytes to lymph nodes thereby facilitating T cell priming. At the tumor site heat may enhance antigen presentation via MHC I ligands for CTL recognition and induces activating ligands for natural killer cells (NK). In addition, heat upregulates the heat shock proteins, which are highly conserved intracellular proteins. Some HSPs, in particular Hsp70, the major stress-inducible member of the HSP70 family, can be released in the extracellular milieu by necrosis where they gain new immune stimulatory functions.Our previous results have shown that Hsp70 is able to activate the adaptive immune system by mediating the cross-presentation of tumor antigens via receptor-mediate uptake, thereby inducing a tumor-specific T cell response. In addition inducible Hsp70 concomitantly activates the innate immune system by promoting the proinflammatory reaction, inducing DC maturation and activating natural killer cells. In particular, we identified the surface expression of Hsp70, which is restricted to tumor cells as a recognition structure for the cytolytic attack mediated by NK cells. We have also shown that Hsp70 high-expressing tumor cells are killed significantly better by NK cells as compared with their low-expressing counterparts. Moreover incubation of NK cells with soluble Hsp70 protein or with the Hsp70-peptide TKD (aa450-463) (multimmune GmbH) plus low-dose IL-2 further enhanced the cytolytic activity of NK cells in vitro and in vivo. These properties of heat and of the heat shock proteins are the rationale for using them for immunotherapeutic strategies against cancer.

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AN UNIQUE KUPFFER CELL RECEPTOR SYSTEM: INVOLVEMENT IN COLORECTAL CANCER METASTASIS TO THE LIVER

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Objective: The heterogeneous RNA binding protein M4 (hnRNP M4) exhibits a unique function in Kupffer cells. It acts as a cell surface receptor for proteins that contain the penta-peptide motif Proline-glutamic acid-Leucine-Proline-Lysine (PELPK). These molecules include members of the carcinoembryonic antigen (CEA) gene family. Activation of this receptor results in production of pro-inflammatory cytokines that up-regulate adhesion molecules on the sinusoidal epithelium leading to implantation of tumor cells within the liver and also protects the tumor cells from the effects of hypoxia. Thus CEA producing tumors have a greater proclivity to grow in the liver than those that do not. In this abstract we describe some of the effects of CEA on signaling pathways in Kupffer cells and differentiated THP-1 cells as a convienient model system for this pathway. **Material and Methods**: Kupffer cells were isolated from rat livers by collagenase perfusion. THP-1 cells were grown in culture and differentiated with PMA resulting in expression of hnRNP on their cell surface. Cells were treated with CEA (1ug/ml) for various time points. Subcellular fractionations were carried out followed by SDS-PAGE and western blotting for hnRNP M4. Cell lysates were probed by western blotting for c-jun, phospho c-jun, Rho-B and nucleoporin. We also used immunoprecipitation with anti hnRNP M4 antibodies in CEA treated and untreated Kupffer cells to identify associated molecules.

Data: Subcelluar fractionation of differentiated THP-1 cells showed two isoforms of hnRNP M4 in the membrane fractions, amounts were significantly lower in undifferentiated cells. CEA treatment reduced the amount of membrane bound protein suggesting internalization of hnRNP M4 following CEA binding. Immunoprecipitations also brought down CEA and beta-actin along with hnRNP M4. Rho-B expression also increased. In CEA treated Kupffer cells decreases were seen in nucleoporin expression and similar results were seen using differentiated THP-1 cells. The transcription factor c-jun decreased in expression with an increase in c-jun phosphorylation.

Conclusions: These data imply that CEA binding is followed by internalization of the CEA/hnRNP-M4 complex by a process involving actin and this may be regulated by Rho B. Changes in nucleoporin suggest translocation into the nucleus of hnRNP-M4 and there is then involvement of the c-jun pathway in activation of the cells to produce cytokines. These pathways may be potential targets for intervention to prevent implantation of CEA producing colorectal cancer cells in the liver.

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INDUCING T CELL AND B CELL RESPONSES WITH IMMUNOSTIMULATORY RNA

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Recognition of pathogens by the innate immune system is mediated through patternrecognition receptors that recognize distinct microbial components. Nucleic acids from pathogens are recognized by several classes of receptors, including Toll-like receptors (TLR) and cytoplasmic receptors. Microbial DNA, in particular DNA rich in unmethylated CpG motifs, is detected by TLR9 in the endosome. Long double-stranded RNA (more than 30 nucleotides), a replicatory intermediate for some viruses, is detected by TLR3, by the serinethreonine kinase PKR, and by the cytoplasmic helicase proteins RIG-I and MDA5. The 5′- triphosphate end of RNA generated by viral polymerases directly binds to RIG-I (Hornung et al. 5′-Triphosphate RNA is the ligand for RIG-I. Science 2006). Singlestranded RNA (ssRNA) from ssRNA viruses has been shown to be detected through TLR7 and TLR8. Furthermore, we have recently described double-stranded, short interfering RNA (siRNA) molecules that interact with TLR7 in a sequence-specific manner to induce IFN-α production in dendritic cells (Hornung et al. Sequence-specific potent induction of IFN-alpha by short interfering RNA in plasmacytoid dendritic cells through TLR7. Nat Med 2005). The stimulatory activity on DC was also observed with the corresponding single-stranded RNA oligoribonucleotides.

We investigated the effect of isRNA on the development of an immune response. We show that isRNA activates dendritic cells and induces production of Th1-type cytokines both in vitro and in vivo. Cytokine production led to bystander activation of T and B cells. We further demonstrate that isRNA triggers the generation of antigen-specific cytotoxic T cells and of an IgG2a-biased antibody response to antigen in a sequence-dependent manner. In summary, we provide evidence for the first time that isRNA oligonucleotides can simultaneously activate the innate and adaptive arms of the immune system (Bourquin et al. Immunostimulatory RNA oligonucleotides trigger an antigen-specific cytotoxic T cell and IgG2a response. Blood 2006)

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NUCLEIC ACID RECOGNITION IN PLASMACYTOID DENDRITIC CELLS - ROLE FOR GENERATION OF AUTOIMMUNITY

Anne Krug, Emina Savarese, Hans-Joachim Anders, Christian Steinberg, Wolfgang Reindl

Plasmacytoid dendritic cells (PDC) are major producers of type I interferons (IFNs) in acute viral infection and during autoimmune diseases such as systemic lupus eythematosus . Triggering of TLR9 and TLR7 by DNA- and RNA-viruses in PDC leads to rapid production of type I IFNs and other proinflammatory cytokines and chemokines. TLR7 and TLR9 also recognize intracellularly delivered mammalian nucleic acids, which may promote autoimmunity. We found that autoimmune complexes containing U1 small nuclear ribonucleoproteins which occur in patients with systemic lupus erythematosus (SLE) trigger IFN-a production in PDC in a TLR7-dependent, TLR3-independent manner. The U1snRNA component of RNP-containing autoimmune complexes acts as an endogenous ligand for TLR7 in PDC, in addition to the TLR9-mediated recognition of self DNA within autoimmune complexes. In the pristane-induced model of SLE TLR7-deficient mice failed to produce U1snRNP autoantibodies and showed significantly less deposition of immune complexes and complement in glomerular capillaries leading to the pathological changes of glomerulonephritis than wildtype mice. Immune complexes containing U1snRNP simultaneously act as autoantigens and autoadjuvants in TLR7-expressing antigen presenting cells, such as B- cells and dendritic cells promoting the generation of autoreactive T- and B-cell responses in SLE.

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UPAR-MEDIATED APOPTOSIS IN MELANOMA

Robert Besch, Carola Berking, Claudia Kammerbauer, Klaus Degitz

The urokinase-type plasminogen activator receptor (uPAR) is involved in several biological processes, including proteolysis, adhesion, migration, and inflammation. Increased expression of uPAR is associated with metastasis in several tumor types. We studied the biological role of uPAR in melanoma and found that inhibition of uPAR via RNA interference induced massive death in three different metastatic cell lines. Annexin-V staining and caspase activation analysis revealed induction of the mitochondrial apoptotic pathway. The expression of members of the Bcl-2 family (Bax, Bcl-2, Bak, Bcl-x_L) was changed in a pro-apoptotic manner. uPAR inhibition induced the expression of the tumor suppressor p53 and of its downstream target gene p21. Inhibition of p53 rescued cells from apoptosis indicating that p53 was critical for apoptosis induction.

Apoptosis was observed in melanoma cells carrying activating BRAF mutations and occurred in the presence of extracellular signal-regulated kinase (ERK) phosphorylation. uPAR can activate focal adhesion kinase (FAK), which is implicated in adhesion-dependent tumor cell survival. However, inhibition of FAK did not induce apoptosis. Our data suggest a new function of uPAR acting as a survival factor for melanoma by downregulating p53. Inhibition of uPAR induces a pro-apoptotic signalling pathway via p53 that is independent of ERK or FAK signalling. These findings may offer new treatment strategies for metastatic melanoma.

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COMBINING IMMUNOTHERAPY AND CHEMOTHERAPY IN EXPERIMENTAL PANCREATIC CANCER

Marc Dauer, Christian Bauer, Franz Bauernfeind, Max Schnurr, Stefan Endres, Andreas Eigler

Objective: Tumor-specific immune responses can be induced in cancer patients by vaccination with tumor-antigen-loaded dendritic cells (DC). However, clinical responses in patients with solid cancer are usually poor. Combination with other treatment modalities may overcome immunoresistance of solid tumors. Using a human in vitro model for DC-based immunotherapy, we were able to show that gemcitabine sensitizes pancreatic cancer cells to CTL-mediated cytotoxicity. Based on these results, we used a murine model of pancreatic cancer to evaluate whether combination with gemcitabine can increase the therapeutic efficacy of DC-based vaccination in vivo.

Material and Methods: DC derived from bone marrow of C57BL/6 mice by in vitro culture with GM-CSF and IL-4 were loadad with snygeneic, apoptotic Panc02 pancreatic carcinoma cells and stimulated with LPS and IFN-γ. For tumor induction, 1x 10E6 Panc02 cells were injected s.c. into the flanks of C57VL/6 mice. Therapeutic vaccination was started when the subcutaneous tumors formed a palpable nodule. For vaccination, 0.3 x 10E6 DC were administered s.c. into the contralateral flank in weekly intervals. Gemcitabine was injected i.p. twice weekly in a concentration of 50 mg/kg. For prophylactic vaccination, mice were vaccinated three times prior to tumor challenge. To evaluate the induction of a specific CTL response, an in vivo cytotoxicity assay based on the detection of p15E-peptide specific CTL was established.

Data: The p15E peptide was exclusively expressed in pancreatic carcinoma cells, but not in normal tissue of C57BL/6 mice. P15E peptide-specific CTL could only be detected in animals vaccinated with tumorantigen-loaded DC. Prophylactic immunization with tumor-loaded DC completely prevented tumor development as compared to vaccination with apoptotic tumor cells only or with unloaded DC. At day 35 after tumor challenge, tumor size was 79 mm2 in the untreated control group vs. 0 mm2 in the DC group. DC vaccination also induced immunological memory:mice that had received prophylactic DC vaccination were rechallenged with Panc02 cells after 95 days. These mice were still completely protected from tumor development. In the therapeutic setting, combination of DC vaccination and gemcitabine improved survival of tumor-bearing mice as compared to immunotherapy or chemotherapy alone. Survival at day 58 after tumor challenge was 0 % in the control group, 13 % in the DC group, 17 % in the gemcitabine group and 50 % in the combined treatment group. Using an i.v. tumor injection model leading to development of pulmonary metastatic disease, we were able to show that DC vaccination completely prevents metastatization: while all untretaed mice died after injection of 25 x 10E4 Panc02 cells into the tail vein, all mice receiving prophylactic DC vaccination survived.

Conclusion: DC-based vaccination induces specific CTL and prevents local tumor growth as well as development of metastatic disease in a murine model of pancreatic cancer. As compared to DC-based vaccination or chemotherapy alone, the combination of the two approaches significantly increased survival of mice bearing experimental pancreatic tumors. A phase II trial investigating - combined treatment of patients with advanced pancreatic carcinoma with autologous DC and gemcitabine is currently performed at our institution.

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IMMUNOTHERAPY OF HEPATOCELLULAR CARCINOMA

Tim Greten, Firouzeh Korangy

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world with a five year survival rate of less than 5 % and an incidence of at least one million new patients per year. In most cases the development of HCC is the result of a chronic viral hepatitis, which progresses into liver cirrhosis and finally into liver cancer. For a small group of HCC patients, surgical or local ablative therapy might be suitable. However, these therapies are limited by tumor size, hepatic functional reserve and intra-hepatic metastases. Therefore, for the majority of patients with advanced disease, these treatments with curative intent are no longer possible and alternative treatments are urgently needed. Local ablative therapies are a possible therapeutic option for patients with less advanced tumors, however no therapeutic options are available for patients with advanced disease. Immunotherapy represents a possible therapeutic alternative for patients with advanced HCC. A number of preclinical studies and smal clinical trials have been performed to develop potent immunotherapies. Due to the special situation of these patients with an underlying chronic liver disease, a chronic viral infection in many cases antigen-specific, humoral and innate immune responses can be impaired. Spontaneous tumor specific

CD4+ as well as CD8+ tumor-specific immune responses can be detected in patients with HCC. However these responses are weak and cannot stop tumor progression. In addition, we have found that patients with HCC have an increase in the requency of CD4+CD25+ regulatory T cells, which have been shown to impair tumor specific immune responses. Therefore, we propose that future immunotherapeutic approaches should not only increase the frequency of tumor-specific T cells in patients with HCC, but also impair the function or number of CD4+CD25+ regulatory T cells.

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IMMUNOTHERAPY OF CANCER BY TRIFUNCTIONAL ANTIBODIES

Horst Lindhofer

Trifunctional monoclonal antibodies (trAbs) represent a new concept of immunotherapeutic treatment for cancer. TrAbs are intact, hybrid-hybridoma derived, bispecific monoclonal antibodies. Via their variable regions they bind to a tumor-associated antigen and CD3 on T cells; in addition, due to their unique combination of immunoglobulin heavy chains, they selectively bind to and activate accessory cells expressing Fcg receptors type I (CD64) and III (CD16). Therefore, this antibody format is referred to as "trifunctional". It is hypothesized that in cancer patients, trAbs redirect Tcells and Fcgreceptor-positive accessory cells to tumor cells, resulting in the formation of a socalled "tricell complex". This leads to (i) the exchange of costimulatory signals between the different immune cells necessary for their physiological activation and (ii) the efficient killing of tumor cells by various mechanisms including accessory cell-induced phagocytosis and Tcell-mediated lysis. As a consequence of this concerted attack, even apoptosis-resistant tumor cells or tumor cells with low expression of the target antigen can be eliminated.

The ability of trAbs to kill tumor cells has been demonstrated in various in vitro and in vivo model systems using tumor cell lines from different tumor types. Remarkably, in immunocompetent mouse tumor models, trAbs were able to induce a protective antitumor immunity in contrast to control antibodies.

As of March 2007, over 550 patients with different types of carcinomas have been treated in several clinical phase I, II and III trials with the trifunctional antibodies catumaxomab (anti-EpCAM x anti-CD3) and ertumaxomab (anti-Her2 x anti-CD3).

Furthermore, only recently a randomized phase II/III clinical trial demonstrated that ovarian cancer patients with malignant ascites significantly benefited from catumaxomab therapy regarding the primary endpoint of the study (puncture-free survival).

In addition, the clinical observations suggest a therapeutic potential of trAbs even in patients with advanced disease. Taken together, the encouraging results obtained in the completed studies warrant the further development of trifunctional antibodies in various indications.

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LINKING INNATE AND ADAPTIVE IMMUNITY BY VACCINATION WITH ADJUVANT-FORMULATED TUMOR ANTIGEN

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Objective: Tumor vaccination aims at inducing cytotoxic T cells capable of killing tumors. However, immune responses are severely impaired in cancer patients. Overcoming tumor-induced immune suppression is a challenge for new generation tumor vaccines. Immune stimulatory complexes (ISCOMs), a formulation of cholesterol, phospholipids and saponin, have been successfully used for the delivery of protein antigen to APC in vivo. We developed a mouse model for studying ISCOM vaccines for the treatment of orthotopic pancreatic cancer.

Material and Methods: For tumor induction, OVA-expressing pancreatic carcinoma cells (Panc02-OVA) were injected either s.c. or directly into the pancreas of anaesthetized mice. Tumor growth was closely monitored. An ISCOM vaccine containing OVA was injected s.c. with or without CpG before (prophylactic) or after (therapeutic) tumor induction. OVA-specific CD8+ T cell responses were analyzed in peripheral blood by intracellular IFN-gamma staining. Antigen uptake, activation and cytokine production of dendritic cells (DC) in draining lymph nodes was analyzed by FACS.

Data: Vaccine draining lymph nodes increased significantly in size and cellularity. The ISCOM vaccine was effectively taken up by DC, resulting in the upregulation of activation markers, production of IL-12 and potent T cell stimulation. Two vaccinations with OVA/ISCOM resulted in a 100% protection from subsequent Panc02-OVA tumor challenge. However, established tumors were not affected. Interestingly, the T cell response was severely impaired in tumor bearing mice in an antigen-specific manner. By combining the ISCOM vaccine with CpG, the T cell response was completely restored and most mice were cured from their cancer. Three months later these mice were rechallenged with Panc02-OVA or wildtype tumors. Panc02-OVA tumors were rejected in 100%, indicating long-term memory induction. A significant proportion of wild type tumors were also rejected. T cell analysis showed evidence of epitope spreading in mice which rejected their tumor.

Conclusion: ISCOM vaccines combined with CpG activate innate and adoptive immune effector cells in vivo and are capable of breaking tumor-induced immune

suppression leading to the cure of orthotopic pancreatic cancer.

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VACCINATING LYMPHOPENIC LUNG CANCER PATIENTS FOLLOWING RECONSTITUTION WITH AUTOLOGOUS PBMC: FIRST RESULTS OF A PILOT-PHASE I CLINICAL TRIAL

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Objective: Given the considerable toxicity and modest benefit of adjuvant chemotherapy for non-small cell lung cancer (NSCLC), there is clearly a need for new treatment modalities in the adjuvant setting. Active specific immunotherapy, i.e. therapeutic vaccination, may represent such an option. However, the available results of vaccination strategies in lung cancer patients are sobering. More recently, combination treatment modalities using chemotherapy and vaccination have led to a resurgence of active specific immuntherapeutic approaches in lung cancer patients. To evaluate feasibility and safety of an irradiated, autologous tumor cell vaccine, we are conducting a Pilot-Phase I clinical trial in patients with resected non-small cell lung cancer (NSCLC) following induction of lymphopenia by chemotherapy and reinfusion of autologous PBMCs.

Methods: For this trial, NSCLC patients stages IIB/IIIA are recruited. Vaccines are generated from their resected lung specimens. Patients undergo leukapheresis to harvest their peripheral blood mononuclear cells (PBMC) prior to or following the surgical procedure. Furthermore, patients receive preparative chemotherapy (cyclophosphamide 350 mg/m2 and fludarabine 20 mg/m2 on 3 consecutive days) for induction of lymphopenia followed by reconstitution with the autologous leukapheresis product. Intradermal vaccine injections are administered on day 1 following reconstitution and every two weeks for a total of up to five vaccine cycles. Dosage is based upon tumor cell yield and ranges from 5 x 106 to 300 x 106 tumor cells/vaccine. GM-CSF is given continuously (at a rate of 50ug/24h) at the site of vaccination via minipump for six consecutive days after vaccination. Two additional leukaphereses (pre chemotherapy and post vaccination) are taken for immune monitoring purposes. The study design received institutional review board approval.

Preliminary data: To date, vaccines were successfully manufactured for 4 of 4 patients. The most common toxicity was a local injection-site reaction. No delayed type hypersensitivity (DTH) skin reactions were observed so far. Immune responses to chemotherapy+reconstitution+vaccination are measured by vaccine site and DTH skin reactions and the induction of tumor-specific T cells. Here, we demonstrate preliminary results of the conducted immune monitoring. Immunohistochemical assessment (CD3, CD4, CD8, CD1a) of punch biopsies taken in the area of the local injection-site reaction 48 hours following the third vaccine is also presented. **Conclusion**: Thus far, all vaccines were well tolerated. Only grade 1/2 vaccine site reactions and constitutional symptoms were observed. Up to this point, the overall trial design seems safe and feasible. The timing of lymphopenia/reconstituion/vaccination necessary to induce a homeostasis-driven proliferation of T cells is possible in this adjuvant clinical setting. Therefore, to exploit homeostasis-driven T cell proliferation for the induction of a specific anti-tumor response might represent a promising novel approach in the fight against lung cancer.

Acknowledgement: This work is supported by grants from the Chiles Foundation, Portland, Oregon, USA and the "Programm zur Foerderung von Forschung und Lehre (FoeFoLe)" of the Ludwig-Maximilians-University Munich, Germany. The authors represent the Munich NSCLC vaccine study group (H. Winter, N.K. van den Engel, M. Schlemmer, H. Pohla, S. Gruetzner, B. Wagner). We wish to thank Smiths Medical for providing the minipump and Dr. S. Arbogast for technical assistance.

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POLYPROPYLENE GLYCOL IS AN INHIBITOR FOR LTA AND OTHER TLR-2 AGONISTS

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Objectives: Polypropylene glycol (PPG) is often used in growth media for bacteria to avoid foaming. Lipoteichoic acid (LTA) isolated from S. aureus grown in the presence of PPG lacked immunostimulatory potency. However, LTA from control cultures without PPG induced cytokine release.

Material and Methods: In this study we investigated the inhibitory properties of polypropylene glycol on cytokine induction by different TLR-2 and TLR-4 ligands in human whole blood.

Data: First we tested the blocking efficacy of several glycol compounds such as PPG of different molecular weights, polyethylene glycol (PEG) and polybutylene glycol (PBG). We stimulated human whole blood with LTA from S. aureus, added increasing concentrations of these glycols and measured the induced TNF release by ELISA. PPG 1200 was found to be the best inhibitory structure tested (IC_{50}=51 ng/ml). This was also true for the release of other cytokines (for 1 mg/ml LTA: IC_{50} for IL-1b induction was 72 ng/ml, for IL-6 83 ng/ml and for IL-8 161 ng/ml, n=4, all $p<0.01$). We were also interested whether cytokine release induced by other TLR-2 agonists is affected in the same way by PPG 1200. TNF release by LTA from six different bacterial species, Pam_3 Cys as well as LPS from P. gingivalis was similarly

inhibited by PPG 1200 resulting in very low IC_{50} values for all stimuli tested. Beside the TLR-2 agonists we also investigated TLR-4 agonists like lipopolysaccharide (LPS) from six different bacterial strains. The IC_{50} values after stimulation with LPS were 100 fold higher than with LTA from S. aureus. The TLR-2 and TLR-4 agonist Zymosan was also only inhibited at high PPG 1200 concentrations. To clarify whether PPG interferes with ligand or receptor, LTA was coated to polystyrene plates and PPG was added. After washing away unbound PPG, whole blood was added. LTA induced TNF release was inhibited in the presence of PPG, suggesting binding of PPG to LTA. This was supported by the observation that LTA from S. pneumoniae R6 migrated slower on a thin layer chromatography plate in the presence of PPG.

Conclusion: We report and characterize a novel inhibitor of cytokine induction by TLR-2 agonists.

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HSP60 RELEASE AFTER LPS TREATMENT AND ITS EFFECTS ON CARDIAC MYOCYTES

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Objective: Heat Shock Proteins (HSP) are a family of cardioprotective, intracellular proteins; however, some studies suggest that extracellular HSP60 can be a danger signal for toll-like receptor (TLR) 4. It has been shown that lipopolysaccharide (LPS) induces an inflammatory response, myocardial depression and apoptosis via TLR4. We hypothesized that LPS induces release of HSP60 from cardiac myocytes and that extracellular HSP60 binds to a cell surface receptor on cardiac myocytes (CM) inducing apoptosis and amplifying the cardiodepressive effects of LPS in a paracrine fashion.

Material and Methods: CM isolated from adult male Sprague-Dawley rats were treated for 6h with 0, 5, 10, 100, 500 ng/mL LPS. HSP60 release was measured in the media by western blotting. Cell damage was monitored by LDH activity in the media. For binding assays, CM were incubated with Oregon-green labeled recombinant human HSP60 (OG-HSP60) in increasing concentrations up to 0.2 μM (n=3). For competition assays, CM were incubated with 0, 0.07 and 0.35 μM HSP60 followed by incubation with 0.07 μM OG-HSP60 (n=3). Endotoxin was cleared from HSP60 preparations with polymyxin B (PMX). Activation of NFκB was measured with a binding activation kit. Apoptosis in CM was determined by caspase-3 activity and CDD ELISA (DNA fragmentation) after 16 and 19h of incubation, respectively, with HSP60 (1 μg/mL) or TNF (10 ng/mL). HEK293-mTLR4/MD2-CD14 were treated with HSP60 for 24h and then the media was assayed for IL-8. Heat-inactivation, incubation with anti-HSP60 antibody and trypsinization of HSP60 served as control.

Data: HSP60 was released from CM after LPS treatment up to 10 ng/mL in a dose-dependent manner. Increased LDH release occured with 100 ng/mL LPS treatment. Binding and competition assays with OG-HSP60 showed a saturable, specific binding of HSP60 to the surface of CM. Dissociation constant (K_D) was calculated with 0.10 μM. NFκB was maximally activated after 30 minutes (16438 ± 4487 relative light units (RLU), $p<0.001$ vs control) and showed a marked decrease at 40 and 90 min. There was a significant caspase-3 activation and DNA fragmentation after HSP60 treatment ($p<0.05$). HSP60 preparations did not induce IL-8 release in HEK293-TLR4/MD2-CD14.

Conclusions: LPS induces HSP60 release from cardiac myocytes in a dose-dependent manner. Extracellular HSP60 induces apoptosis in cardiac myocytes possibly through NFκB activation. Cardiac myocytes have a specific receptor for extracellular HSP60. However, TLR4 is not activated by HSP60 in TLR4/MD2-CD14 transfected HEK293 cells. Further studies will be needed to identify the HSP60 receptor on cardiac myocytes.

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HSP70 EXPORT FORM TUMOR CELLS - A DANGER SIGNAL FOR THE IMMUNE SYSTEM

Gabriele Multhoff, Mathias Gehrmann

Depending on their intra- and/or extracellular localization heat shock proteins (HSP) either mediate protection against stress-induced cell damage or act as danger signals stimulating the adaptive and innate immune system (1-3). However, it remained elusive how cytosolic HSPs become externalized and thus communicate with immunocompetent effector cells. Several groups demonstrated that a variety of different cell types, including tumor cells, have the capacity for an active release of Hsp70 in detergent-soluble vesicles (4,5). Biophysical properties including floating properties (1.17g/ml) correlating with a maximum acetylcholine esterase activity characterized them as exosomes (3-5). Profiling of luminal proteins revealed that tumor-derived exosomes contain cytosolic proteins but lack ER-residing proteins. An exosomal enrichment of the small GTPase Rab-4 documented their intracellular transport route from the early endosomal compartment to the plasma membrane. A proteomic analysis of the exosomal membrane by flow cytometry and immunoelectron microscopy revealed that exosomal and plasma membranes of tumors are identical with respect to their protein composition and protein topology. In particular exosomes originating from Hsp70 plasma membrane-positive tumors present Hsp70 on their exosomal membrane. In line with these findings only Hsp70 surface-positive exosomes but not their negative counterparts had the capacity to stimulate the innate immune system in a similar manner like soluble Hsp70 protein (3). These data provide an explanation how the

innate immune system might become activated by tumor-derived exosomal Hsp70 in vivo.

Recently, a direct interaction of HSP70s with phosphatidylserine in pre-apoptotic cells could be identified by the group of Antonio DeMaio (6). In order to characterize the localization of Hsp70 in plasma membranes and exosomes generated from viable tumor cells that differ in their capacity to present Hsp70 on the surface we currently perform lipid analysis by mass spectrometry.

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MYCOBACTERIUM BOVIS BCG INDUCES HIGH MOBILITY GROUP BOX 1 PROTEIN RELEASE FROM MONOCYTIC CELLS

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Objective: High mobility group box 1 protein (HMGB1), a nuclear protein is a critical cytokine that mediates the response to infection, injury and inflammation.

The aim of our study was to elaborate a reliable in vitro model to investigate whether Mycobacterium bovis BCG is able to induce HMGB-1 secretion from the monocytic U-937 cells.

Material and Methods: Western blot technique was applied for the detection of HMGB-1 from supernatants of cells, following induction with LPS, Staphylococcus aureus, and Mycobacterium bovis BCG. HMGB1 was subjected to MALDI-TOF mass and PSD analysis. Quantitation of the secreted HMGB1 was performed by ELISA.

Data: The BCG strain resulted in a higher amounts of secreted HMGB-1 than that of LPS or Staphylococcus aureus. The translocation of the HMGB1 towards the cytoplasm following infection of cells with BCG was demonstrated by immunofluorescence examinations.

Conclusion: Our pilot experiments draw attention the HMGB1- inducing ability of Mycobacterium bovis. Assesment of the pathophysiological role of this late cytokine in mycobacterial infections demands further in vitro and in vivo examinations.

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UNCONVENTIONAL BIOLOGICAL PROPERTIES AND STRUCTURE OF LTA FROM LISTERIA MONOCYTOGENES

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Objectives: Listeria monocytogenes (Lm) are human pathogens that multiply and spread intracellularly in host cells including monocytes and liver cells. Like other Gram-positive bacteria, their lipoteichoic acid (LTA) is considered the major immune stimulatory component and its structural and biological peculiarities were investigated.

Methods: LTA was prepared from Lm by butanol extraction and the hydrophilic portion was separated by HIC. LTA content was determined in the fractions via measurement of phosphate. The immune stimulatory capacity of LTA from Lm, S. aureus (Sa) and Streptococcus pneumoniae (Sp) was analyzed by human whole blood incubations and subsequent measurement of inflammatory mediators by ELISA. The toll-like receptor (TLR) dependency was investigated using bone marrow cells from knockout mice. Complement activation was examined by measuring the binding of L-ficolin and cleavage of C4. Structural data were obtained by NMR and MS-analysis.

Data: Butanol extraction of Lm resulted in the isolation of two LTAs. Both LTAs induced the release of a broad variety of cytokines from blood leukocytes. The immunostimulatory potency of LTA1 was comparable to that of LTAs from Sa or Sp, while LTA2 was weaker. Cytokine induction by both Lm LTAs was completely TLR2 and TLR1 dependent, but TLR4 and TLR6 independent, thereby reflecting the TLR-dependence of whole Lm bacteria. In contrast to LTA of Sa or Sp, LTA1 and LTA2 of Lm induced the proliferation of mouse spleen cells. For LTA from Sa, the induction of the lectin-binding pathway of complement activation via binding of L-ficolin followed by cleavage of C4 was shown. When whole Lm, LTA1 or LTA2 were incubated with L-ficolin, the L-ficolin binding activity of LTA1 was comparable to LTA from Sa, while LTA2 showed only very weak L-ficolin binding. Furthermore, LTA1 potently induced cleavage of C4, while LTA2 failed to do so. The complement

activating capacity of whole Lm was comparable to that of whole Sa. According to chemical analysis, both LTA1 and LTA2 consist of a polyglycerophosphate (PG) backbone substituted with D-alanine and glucose and bound to a glucose-galactose dissacharide. However, the number of PG repeating units is smaller in case of LTA2, and the lipid anker of LTA2 carries four instead of two fatty acids, which are usually found in LTA.

Conclusion: Lm contain two LTAs, which show structural peculiarities and possess unusual immunostimulatory activities. LTA1, rather then LTA2, seems to reflect the biological properties of the whole bacteria.

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PRESENTATION OF LIPOTEICHOIC ACID POTENTIATES ITS INFLAMMATORY ACTIVITY

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Objective: Lipoteichoic acid (LTA) is a strong inducer of chemoattractants but a weak inducer of proinflammatory cytokines. In the present study we investigated how adhesion of LTA to a surface modulates its immunostimulatory potency.

Material and Methods: Highly purified LTA was preincubated with different materials. After washing, human whole blood was added and cytokine release was measured by ELISA.

Data: Adhesion of LTA to a polystyrene surface drastically increased its immunostimulatory potency in human whole blood in comparison to soluble LTA, although only 1% of the LTA had bound, as determined using rhodamine-labeled LTA. The release of the proinflammatory cytokines IL-1β, TNFa and IL-6 and the chemokines IL-8 and G-CSF was increased two to ten-fold, but IL-10 release was unaltered. This presentation effect was not shared by LPS or other TLR2 agonists and was less pronounced in polypropylene vessels. LTA did not induce cytokine release in silicone-coated borosilicate vessels, which should not bind LTA, indicating that presentation on a surface is in fact essential for the immune recognition of LTA. In line, covalent coupling of LTA to polystyrene beads restored cytokine induction in borosilicate.

Conclusions: This novel aspect of presentation as a factor in the recognition of LTA fits the physiological situation in the bacterial cell wall, where LTA projects through the peptidoglycan. These observations support suggestions that LTA is the immunostimulatory principle of peptidoglycan (PGN). In practical terms, contamination of medical devices with components of Gram-positive bacteria may pose an underestimated inflammatory risk.

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ACUTE PHASE RESPONSE IMPAIRS HOST DEFENSE AGAINST ENTEROCOCCUS FAECIUM PERITONITIS IN MICE

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Objective: Multiresistant Enterococcus faecium is an important cause of hospital-acquired infections. In particular patients with a pre-existing illness are susceptible to infections with E. faecium. Such predisposing diseases are almost invariably associated with an acute phase protein response (APR). We here tested the hypothesis that a sterile acute phase response renders the host more vulnerable to E. faecium infection. Therefore, we used two well-established models to induce a sterile APR, namely subcutaneous injection of either turpentine or casein.

Material and Methods: One day prior to intraperitoneal infection with 10^8 CFU E. faecium C57BL/6 mice were injected subcutaneously with either turpentine in both hind limbs, or casein in the back/neck region. Control mice were injected with saline, or bicarbonate respectively. Mice were sacrificed at different time points up to one week after infection to determine immune responses and bacterial loads in blood, peritoneal lavage fluid, liver and lung.

Data: At the sites of the subcutaneous turpentine injections abscesses were formed, which was not the case for the casein injection sites. Both turpentine and casein induced an acute phase protein response as reflected by a transient weight loss and strong increases in the plasma levels of serum amyloid P and C3. Additionally, these mice had less circulating granulocytes and less peritoneal granulocyte influx compared to the control mice. Both turpentine and casein injected mice showed a significant delay in clearing the enterococci from all tested organs. It could not be demonstrated whether this was accompanied by reduced cyto- and chemokine responses, since E. faecium does not cause impressive levels of these immune proteins. All differences relative to controls were more profound in the turpentine-injected mice.

Conclusion: These data suggest that a pre-existing sterile acute phase protein response, such as occurs after trauma or major surgery, impairs host defense against E. faecium peritonitis.

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METABOLIC MODULATION OF VASCULAR FUNCTION WITH CONSEQUENCES FOR COAGULATION AND INFLAMMATION

Marcel Levi

Considerable cross talk between inflammation and coagulation is important in the pathogenesis of vascular disease. Inflammation not only leads to activation of coagulation, but coagulation also considerably affects inflammatory activity. The intricate relationship between inflammation and coagulation may not only be relevant for vascular atherothrombotic disease but has also major consequences for the pathogenesis of microvascular failure and subsequent multiple organ failure, as a result of severe infection and the associated systemic inflammatory response.

Major players in the interaction between inflammation and coagulation are tissue factor (the main initiator of coagulation), the protein C pathway, and plasminogen activators and their inhibitors. At the cellular site, endothelial cells and mononuclear cells are important.

Interestingly, recent studies show a marked modulation of the inflammatory-coagulation axis by metabolic pathways. Dyslipidemias, in particular those associated with decreased HDL, lead to enhanced responses to endotoxin and other pro-inflammatory stimuli. Hyperglycemia affects tissue factor-dependent activation of coagulation, whereas hypoerinsulinemia has a major impact on plasminogen activator inhibitor-type-1.

Metabolic pathways therefore may contribute to the development of (micro)vascular thrombosis in inflammatory states, which may result in tissue ischemia and subsequent organ damage. Hypothetically, the beneficial effect of strict metabolic regulation (for example by intensive insulin therapy) may be partly related to this modulatory effect on inflammation and coagulation.

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EFFECT OF STRICT GLUCOSE REGULATION ON CLINICAL OUTCOME, INFLAMMATION AND DOWNSTREAM PATHWAYS

Jan Wernerman

Tight glucose control is beneficial for mortality and morbidity in long staying ICU-patients. This has been demonstrated from Leuwen for patients in the surgical ICU, mainly patients undergoing open-heart surgery. It has also been demonstrated by the same group for patients with medical diagnoses. However, more detailed analysis of the published data together with preliminary findings reported at congresses lately, makes the picture more complicated.

Among medical ICU-patients, short stayers randomized to tight glucose control may have an elevated mortality rate, or at least they blunt the positive effect for long stayers when the material was analysed on an intension to treat basis. In addition a German multi-center study of sepsis patients, the VISEP study, failed to demonstrate any beneficial effect. An in addition a European multi-center study, the GLUCONTROL study, also fails to demonstrate any beneficial effect. Booth these studies were prematurely halted due to safety. Reason being the high rate of hypoglycaemia. In the electronic appendix to the second Leuwen study on medical ICU-patients also reports a high incidence of hypoglycaemia. None of the studies have revealed any direct connection between hypoglycaemia in tight glucose control and mortality using regression analysis. For the GLUCONTROL study the safety concern for hypoglycaemia was not the only argument to halt the study, the other reason was protocol violations. That is interesting because a discussion has come up how the tight glucose control should be documented. The method per preference would be to have the area under the curve for the plasma glucose measurements. This has so far not been presented in any published or presented study. The two studies published from Leuwen show a good separation of the two groups, while the VISEP study and the GLUCONTROL study show a considerable overlap. If this is a real difference between the four studies or if this is attributable to different ways of presenting data is not clear.

So today we stand with conflicting data. The key questions seam to be how to administer tight glucose control without hypoglycaemia and secondly if this should start on the day of ICU admittance also in medial ICU patients or should be postponed a few days in this group of patients. More documentation is needed on these points.

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CLINICAL IMPLICATIONS OF THE BI-DIRECTIONAL RELATIONSHIP BETWEEN COAGULATION AND INFLAMMATION

Steven Opal

The cellular and humoral elements that compromise the human innate immune response share a common evolutionary substrate with the coagulation system and remain highly interlinked processes. Innate immunity and coagulation function as a rapid response system to protect the host from loss of the internal milieu from hemorrhage following tissue trauma and to defend invasion of omnipresent risk of microbial invasion from breaks in the integument. Local or generalized activation of the coagulation system is nearly a uniform finding in acute inflammatory states. Coagulation parameters are often measured by clinicians in patients with severe sepsis, even in the absence of demonstrable clinical abnormalities in the coagulation system. Coagulation activation, consump-

tion and loss of coagulation inhibitors have made global markers of coagulation and fibrinolysis (thrombin generation, platelet count wave form analysis, fibrin degradation products and specialized coagulation measures (prothrombin fragment F1.2, antithrombin and protein C levels), routine clinical assessments of severe sepsis. These coagulation and fibrinolytic parameters are clearly appropriate indicators for the experimental and therapeutic use of anti- coagulants in sepsis. These same coagulation markers could be utilized for specific anti-inflammatory mediators in future clinical trials (anti-cytokines, anti-LPS strategies, intracellular signal inhibitors) as consumptive coagulopathies are independent predictors of mortality in severe sepsis. DIC scores have additive predictive value over standard risk prediction systems such as SAPS and the APACHE scoring system.

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ATHEROSCLEROSIS IN TYPE 2 DIABETES: WHAT IS SO SPECIAL ABOUT IT?

Nikolaus Marx

Patients with type 2 diabetes mellitus exhibit an increased propensity for the development of atherosclerosis with its clinical sequelae, acute myocardial infarction or stroke. Associated metabolic disorders like dyslipidemia, hyperglycaemia as well as the presence of advanced glycation endproducts, endothelial dysfunction and hypercoagulability have been implicated in atherogenesis in these patients. In addition, atherogenesis in diabetic patients shows various differences compared to non-diabetic subjects, all contributing to the increased risk of these patients. The lecture will focus on the aspects of lesion development in type 2 diabetic patients with respect to the different phases and stages of atherogenesis as well as the role of inflammation for lesion development.

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BURN PATIENTS AS MODELS FOR INFLAMMATION INDUCED DIABETES

David N. Herndon, Ludwik Branski, William Norbury, Marc Jeschke

Severe burns serve as a model for inflammation induced diabetes. Burn-induced hyperglycemia is caused by post-receptor insulin resistance in the skeletal muscle and the liver, and is associated with elevated insulin levels. Some of the involved pathways include an impaired insulin-stimulated Akt/PKB activation; alterations in the phosphorylation of key proteins in the insulin signaling cascade, including IRS-1, and changes in stress kinases also contribute to insulin resistance. The consequences are reduced glucose uptake and skeletal muscle wasting.

The liver of the burned patient shows resistance to insulin. The classic insulin-regulated pathways (PEP carboxykinase, glucokinase) and the PI3K-pathway are downregulated, leading to increased blood glucose levels. Glucose levels are further increased due to burn injury-induced dysfunction in mitochondrial respiration and gluconeogenesis.

In clinical studies in insulin-resistant severely burned children, we found that insulin sensitivity correlated with the basal rate of palmitate oxidation, and that there was a lack of downstream insulin signaling and a significant increase in PKC -b activation in response to the acute elevation of insulin concentration. We will show that insulin, fenofibrate and metformin attenuate hyperglycemia and increase muscle protein synthesis as measured by stable isotope techniques, thereby indicating a metabolic link between hyperglycemia and muscle loss following severe injury. We will furthermore show that a hyperinsulinemic/euglycemic clamp through hospital course leads to increased lean body mass, accelerated wound healing, stimulated constitutive protein synthesis, and decreased inflammation and acute phase response.

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CLINICAL RELEVANCE OF THE LINK BETWEEN INFLAMMATION AND INSULIN RESISTANCE

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The prevalence of the metabolic syndrome has risen dramatically in western societies. This state is characterized by a clustering of metabolic cardiovascular risk factors (visceral adiposity, insulin resistance, low HDL cholesterol and a systemic pro-inflammatory state) that confer an increased risk of developing both type 2 diabetes and atherosclerotic cardiovascular disease (CVD). Innate immune receptors can be activated by a variety of endogenous signals that are generated in insulin resistance, including oxidized lipoproteins and C reactive protein (CRP). Macrophage infiltration of adipose may in turn promote insulin resistance. In fact, cytokines (monocyte derived inflammatory mediators) induce insulin resistance and endothelial dysfunction, while insulin and adipokines (adipose derived signaling molecules) modulate macrophage and endothelial inflammatory responses. Indeed, plasma levels of innate immune inflammatory markers as well as adipokines and measures of insulin resistance are independent predictors of CVD events. This presentation will focus on three aspects of potential clinical importance in studying the links between inflammation and insulin resistance/metabolic syndrome; (1) the use of human inflammatory models in studying the pathophysiology of insulin resistance; (2) the utility of inflammatory markers in predicting type 2 diabetes and its complications; and (3) therapeutic targeting of innate

Table

Parameter	Diabetes (N = 188)	Non-Diabetes (N = 642)	**P**-value
PT (s)	20.0 ± 7.0	20.7 ± 7.8	0.31
APTT (s)	46.7 ± 23.1	45.8 ± 16.8	0.24
Platelets (10^9/l)	230 ± 131	195 ± 116	<0.01
TATc (µg/l)*	1013.0 ± 4457.0	18.8 ± 23.1	0.93
F1+2 (nmol/l)*	27.1 ± 111.0	2.2 ± 1.8	0.99
D-dimer (µg/ml)	6.4 ± 7.8	7.4 ± 8.8	0.12
PAI-1 (AU/ml)*	39.3 ± 28.6	44.7 ± 28.2	0.26
Protein C (%)	55.7 ± 28.4	51.0 ± 27.8	0.05
Protein S (%)	45.8 ± 26.1	40.5 ± 24.2	0.02
Antithrombin (%)	65.5 ± 22.3	59.1 ± 23.5	<0.001
IL-6 (pg/ml)	5935 ± 2.40E4	1.10E4 ± 4.70E4	0.11
TNF-α (pg/ml)*	67.9 ± 170.0	53.0 ± 86.7	0.98

immunity in insulin resistance and the metabolic syndrome.

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DIABETES DOES NOT ALTER MORTALITY OR HEMOSTATIC AND INFLAMMATORY RESPONSES IN PATIENTS WITH SEVERE SEPSIS

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Objective: Diabetes patients have an increased risk for infections and a higher mortality due to infection. In addition, diabetes is associated with an imbalance between coagulation and fibrinolysis resulting in a tendency towards a procoagulant state. We hypothesized that the presence of diabetes negatively influences the course of sepsis due to (among others) changes in hemostasis and inflammation.

Material and Methods: 830 patients admitted to the ICU for severe sepsis (placebo treated patients from the PROWESS study) were stratified according to the presence or absence of diabetes (pre-admission diagnosis on their case report form). Clinical and laboratory outcome parameters were compared between patient groups using analysis of variance and Chi-Square test. Data are means ± SD.

Data: Diabetes was overrepresented in septic patients (188/830 = 22.7%) relative to comparable non hospitalized age cohorts (10-15%). Plasma glucose levels of diabetes patients were higher at baseline (239 ± 126 vs. 152 ± 76.6 mg/dl, $P < 0.0001$) and throughout the 28-day observation period. Diabetes patients in the study population were older than patients without diabetes (64.4 ± 12.8 vs. 59.5 ± 17.3 years; $P < 0.01$). Contradicting our hypothesis, 28-day mortality did not differ between diabetes (31.4%) and non-diabetes patients (30.5%); 90-day mortality was also equal (39.1 vs. 39.0%). Causative organisms (gram-positive, gram-negative, fungal) did not differ between groups. Except for slightly elevated platelet counts, protein S and antithrombin levels at baseline, diabetes patients did not differ from non-diabetes patients regarding coagulation, fibrinolysis, anticoagulation and inflammation markers throughout the 28-day study period (see table for baseline data; * indicates that parameter has been measured in a subpopulation).

Conclusion: Though Diabetes is a risk factor for sepsis, once established, the outcome of severe sepsis does not appear to be significantly influenced by the presence of diabetes nor the hyperglycemia associated with it in the PROWESS trial.

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ASSOCIATION OF LIPOPOLYSACCHARIDE-BINDING PROTEIN AND CORONARY ARTERY DISEASE IN MEN

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Background: The mechanisms by which the innate immune recognition of pathogens could lead to atherosclerosis remain unclear. Lipopolysaccharide binding protein (LBP) is the first protein to encounter Lipopolysaccharide and to deliver it to its cellular targets, toll-like receptors. Thus its presence might be a reliable biomarker that indicates activation of innate immune responses. In this study we tested the hypothesis that LBP might be able to be used as a biomarker for coronary artery disease (CAD).

Methods and Results: 247 men undergoing elective coronary angiography were studied and the extent of coronary atherosclerosis was assessed by two established

Table

Group	IL-6 (pg/ml)	GMCSF (pg/ml)	TNF-a (pg/ml)	IL-8 (pg/ml)	IL-1B (pg/ml)
LVG	1097 ±259	9.2 ±2.8	6.6 ±3.3	28.6 ±7.5	3.6 ±1.6
HVG	642* ±132	24.9* ±4.2	26.8* ±7.4	34.4 ±6.9	21.7* ±11.4

scores: 'Extent Score' and 'Severity Score'. Serum LBP concentration was significantly increased in 172 patients with angiographically confirmed CAD compared to 75 individuals without coronary atherosclerosis (20.6 ± 8.7 vs. 17.1 ± 6.0 pg/mL; P=0.002). Odds ratios for CAD in the first, second, and third quartiles for serum LBP were 5.444 (95% confidence interval (CI), 2.018 to 14.691), 2.138 (95% CI, 0.954 to 4.343), and 1.593 (95% CI, 0.749 to 3.387) compared with the fourth quartile. Moreover in multivariate regression analyses, adjusted for established cardiovascular risk factors and markers of systemic inflammation, LBP was a significant and independent predictor of prevalent CAD (P=0.016).

Conclusions: LBP might serve as a novel marker for CAD. Our results underline the importance of innate immune mechanisms for the initiation and development of CAD, possibly even for later adverse cardiovascular events such as plaque fissure and rupture with the consequence of myocardial infarction.

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INCREASED VARIABILITY DURING TIGHT GLUCOSE CONTROL ALTERS THE CYTOKINE PROFILE IN BURN PATIENTS

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Glucose control in patients in the ICU has been implemented in most burn centers in the USA improving outcomes. However, while mean glucose control may be maintained within a target range, variability of the glucose level may be indicative of a poor outcome. The hypothesis has been put forward that tight glucose control attenuates the cytokine response to trauma. We proposed that while a tight mean glucose may be maintained a high variability will be associated with an increase in the plasma levels of inflammatory cytokine.

Methods: Burn patient with an expected ICU stay of greater than 3 days were enrolled within 24 hours of injury. All patients were treated with insulin to maintain plasma glucose (G) at >80 and <110 mg/ml following a standard protocol. Patients were grouped as to variability of the glucose measurement: >50% out of control range high variability (HVG) and <50% low (LVG). Plasma cytokine levels were measured on days 0, 1, 3, 5, and 7 following injury.

Results: Nineteen subjects were enrolled in this study, 7 LVG and 12 HGV. G levels upon admission were not different between groups. Mean glucose concentrations over the period of treatment were similar 97±5 versus 101±11 mg/dl for 122±43 measurements per patient. The percentage of measurements out of range was 41± 5 % for LVG and 61±7% for HVG ($p<0.05$). There was no difference between groups in %TBSA, %full thickness, incidences of inhalation injury or ISS. Mean plasma levels of TNF-a, IL-1B and GMCSF were increased ($p<0.05$) with HVG, while IL-6 was reduced (Table; values are means ± SEM).

There was no difference ($p>0.05$) in the lengths of ventilation, ICU stay or hospitalization stay. Survival rates were 71% for LVG compared to 58% for HGV ($p>0.05$).

Conclusion: Alterations in the cytokine profile is observed in severely burned patients who have tight glucose control but highly variable glucose. While not demonstrated significantly in the present study HGV has been associated with poor outcomes. Alteration in the cytokine profile may contribute to this observation. Tighter control of glucose can be achieved by more measurements via computerized inline systems reducing variability.

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PLATELETS IN ATHEROGENESIS

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Platelets play a critical role in haemostasis and thrombosis. However, it is increasingly recognized that platelets are involved in atherogenesis and atheroprogression. Atherosclerosis is characterized by a chronic inflammation resulting in vascular remodelling mechanisms that are critically involved in progression of the disease. Platelets represent an important linkage between inflammation, thrombosis and atherogenesis. Inflammation is characterized by interactions between platelets, leukocytes, and endothelial cells. These interactions trigger autocrine and paracrine activation processes leading to leukocyte recruitment into the vascular wall. Platelet-induced chronic inflammatory processes at the vascular

wall result in development of atherosclerotic lesions and atherothrombosis. Inhibition of platelet adhesion to the vessel wall results in substantial attenuation of atherosclerosis in mouse models. Evaluation of platelet-mediated processes of atherogenesis may lead to novel therapeutic strategies in patients with atherosclerotic diseases.

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IMAGING OF INFLAMMATION IN ATHEROSCLEROTIC PLAQUES

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Most clinical events due to atherosclerosis occur as a result of plaque inflammatory cell activity causing plaque rupture. It is also recognised that silent rupture events contribute to plaque growth and that 'statins' reduce clinical events by modifying plaque composition more than size. Indeed, there is a poor correlation between risk of plaque rupture and angiographic appearance. There is therefore an urgent need for a diagnostic test that can predict clinical events, that can target 'culprit' lesions for intervention and that can monitor the effects of medical therapy on plaque composition. ^{18}fluorodeoxygucose positron emission tomography (^{18}FDG-PET) is emerging as a technique that can identify inflamed atherosclerotic lesions. Our group has demonstrated ^{18}FDG uptake by macrophages into symptomatic carotid artery lesions with significantly less uptake into asymptomatic lesions. By co-registering ^{18}FDG-PET with high resolution magnetic resonance imaging (MRI), we have been able to obtain anatomical and functional data on angiographically visible and invisible lesions and to correlate these findings with clinical events[2]. These studies, and studies by other groups, strongly suggest that that ^{18}FDG-PET may be able to identify 'culprit' lesions in patients with symptomatic carotid artery disease. Studies in experimental models of atherosclerosis suggest that changes in ^{18}FDG uptake correlate closely with experimentally manipulated plaque macrophage content indicating that ^{18}FDG-PET may be able to monitor and quantify plaque macrophage content in vivo. However, the poor anatomical resolution of PET mandates co-registration with another imaging modality. Whilst our experience is predominantly with MRI, other groups are reporting successful imaging approaches with combined PET/CT scanners.

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This work was supported by the British Heart Foundation

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IMMUNE PATHOGENIC AND REGULATORY PATHWAYS IN ATHEROSCLEROSIS

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Atherosclerosis is a chronic disease of the arterial wall where both innate and adaptive immuno-inflammatory mechanisms are involved. Inflammation is central at all stages of atherosclerosis. It is implicated in the formation of early fatty streaks, when the endothelium is activated and expresses chemokines and adhesion molecules leading to monocyte/lymphocyte recruitment and infiltration into the subendothelium. It also acts at the onset of adverse clinical vascular events, when activated cells within the plaque secrete matrix proteases that degrade extracellular matrix proteins and weaken the fibrous cap, leading to rupture and thrombus formation. Cells involved in the atherosclerotic process secrete and are activated by pro-inflammatory cytokines. Recent advances in our understanding of the mechanisms of atherosclerosis provided evidence that the immuno-inflammatory response in atherosclerosis is modulated by regulatory pathways involving the two anti-inflammatory cytokines IL-10 and TGFβ, which play a critical role in counter-balancing the effects of pro-inflammatory cytokines. Interestingly, IL-10 and TGF-β are also the two cytokines that mediate the immune regulatory functions of a sub-population of T cells, named regulatory T (Treg) cells. We recently demonstrated that natural CD4+CD25+ Treg cells play an important role in the control of atherosclerosis in apoE-/- mice. It is therefore believed that atherosclerosis may result from an imbalance between pathogenic T cells, either Th1 or Th2, producing pro-atherogenic mediators, and Treg cells with immunosuppressive properties, and that promotion, expansion or exogenous administration of Treg cells might limit disease development and progression.

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PLATELET ACTIVATING FACTOR RECEPTOR IMPROVES THE EARLY ANTIMICROBIAL HOST RESPONSE TO SEVERE PSEUDOMONAS AERUGINOSA PNEUMONIA

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Background: Pseudomonas (P.) aeruginosa is a common pulmonary pathogen and is a leading cause of nosocomial pneumonia in the United States and Europe. Lung injury associated with P. aeruginosa infection results from both the direct destructive effects of the organism on the lung parenchyma as well as from exuberant host immune responses. Because of the high incidence of pneumonia and the increasing antimicrobial resistance, further understanding of the non-specific host defense is necessary. Platelet activating factor (PAF) has been implicated as a crucial mediator of lung inflammation.

Objective: To determine the role of the PAF receptor (PAFR) during bacterial pneumonia caused by P. aeruginosa.

Material and Methods: C57Bl/6 wild type and C57Bl/6 PAFR knock-out mice were intranasally inoculated with P. aeruginosa and sacrified after 6 hrs.

Data: PAFR KO mice had more bacteria in their lungs, blood, spleen and liver homongenate (all $P < 0.05$ versus wild type mice). Myeloperoxidase activity and scores for granulocyte staining in lungs were increased in the PAFR KO mice ($P < 0.05$ versus wild type mice). Furthermore, PAFR KO mice had higher levels of the pro- and anti-inflammatory cytokines TNF-α, IL-6 and IL-10 in lung homogenate and plasma. In addition, concentrations of the chemokine MIP-2 were increased in lung homogenate in the KO mice. In the liver homogenates only IL-6 and MIP-2 levels were increased. Activation of coagulation and fibrinolysis was increased in the KO mice as reflected by higher TATc and D-dimer plasma concentrations, respectively. Finally, the PAFR KO mice had more lung damage as reflected by an increased relative lung weight, enhanced total protein BALF concentrations and increased histopathology inflammatory scores.

Conclusion: PAFR mice displayed an impaired defense against P. aeruginosa pneumonia as reflected by an enhanced bacterial outgrowth at the site of the infection and an increased dissemination to distant organs, which was accompanied by increased lung inflammation and damage. These data indicate that endogenous PAFR plays a protective role during severe P. aeruginosa pneumonia.

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ACTIVATION OF COAGULATION AND INHIBITION OF FIBRINOLYSIS IN THE LUNG UPON BRONCHIAL INSTILLATION OF LIPOTEICHOIC ACID IN HEALTHY VOLUNTEERS

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Objectives: Pneumonia is characterized by an acute inflammatory response in the lung but is also frequently associated with changes in coagulation and fibrinolysis in the bronchoalveolar space. Immune cells are important in the initiation of coagulant pathways, while various inflammatory mediators are capable of altering haemostasis. Here, we studied the effects of lipoteichoic acid (LTA), a major outer cell wall component of gram-positive bacteria and relevant for respiratory infections in the human alveolar space.

Material and Methods: To determine the effect of LTA on the haemostatic balance in the human lung, sixteen healthy non-smoking male subjects were given LTA (S. aureus) at a dose of either 4, 20 or 100 ng/kg body weight. In addition, eight volunteers received LPS (E. coli, 4 ng/kg body weight) as positive controls. By bronchoscope sterile saline was instilled into a lung subsegment followed by instillation of either LTA or LPS into the contralateral lung. Bronchoalveolar lavage fluid (BALF) was obtained six hours thereafter.

Data: Besides a significant inflammatory response as shown by neutrophil influx and cytokine production, bronchial instillation of LTA induced soluble tissue factor and thrombin-antithrombin complexes compared to instillation of saline in the contralateral lung ($P<0.001$). Additionally decreased levels of antithrombin and activated protein C were measured after LTA instillation ($P<0.001$). The induction of coagulant factors and inhibition of anti-coagulant factors was paralleled by a decrease in fibrinolysis as quantified by a decreased plasminogen activator activity, which was associated with an increased plasminogen activator inhibitor type I ($P<0.001$).

Conclusion: For the first time this study shows that - besides eliciting an inflammatory response - instillation of LTA in a subsegment of the lung induces coagulation and inhibition of fibrinolysis in human volunteers. This novel human model may be used to study pathogenetic mechanisms involved in bronchoalveolar coagulation during lung inflammation.

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SEPSIS FROM PSEUDOMONAS AERUGINOSA PNEUMONIA DECREASES INTESTINAL EPITHELIAL PROLIFERATION AND SLOWS MIGRATION

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Objective: The entire intestinal epithelium turns over in 3-5 days, with cells proliferating in the crypts of Lieberkuehn, migrating and differentiating along the villus, and being eliminated by apoptosis and/or exfoliation at the villus tip. We have previously shown that intestinal epithelial apoptosis is increased in pneumonia-induced sepsis and that proliferation is decreased in a 90% lethal pneumonia model. Other investigators have studied proliferation and apoptosis in other models, but no one has looked at migration in any model of sepsis. The **Objective** of this study was to determine the effect of sepsis on proliferation and migration in a more clinically relevant 50% mortality model.

Material and Methods: FVB/N mice were given intratracheal injections of 20 µl of 0.1 McFarland Pseudomonas aeruginosa (50% mortality) or 20 µl of normal saline (0% mortality). To evaluate gut epithelial proliferation, animals were sacrificed at 24 hours postoperatively (n=7-8) and were given an intraperitoneal injection of 200 µl of 5 mg/ml 5-bromo-2-deoxyuridine (BrdU) 1.5 hours prior to sacrifice to label cells in S-phase. Proliferation was quantified by BrdU staining in 100 contiguous intestinal crypts. Based upon the results obtained, animals were injected with BrdU at the time of operation (n=4-8) to assess the effect of sepsis on migration of cells with basal levels of crypt proliferation (since BrdU was injected at the same time that sepsis was induced, there was not sufficient time for sepsis-induced changes in proliferation to take effect). An additional group was injected with BrdU 22.5 hours after surgery (n=3-8) to assess the effect of sepsis on migration in the presence of a sepsis-induced decrease in proliferation. All mice used to assess migration were sacrificed 48 hours after the induction of sepsis. Intestinal sections were stained for BrdU and migration was quantified by measuring the distance between the base of the crypt of Lieberkuehn and the most distal BrdU-positive nucleus within the crypt-villus structure. Migration was measured in twelve crypt-villus units per mouse and data were normalized for villus length. Data from septic and sham mice were compared by t test. Data: At 24 hours, septic mice had significantly decreased proliferation compared to sham (798 ±60 vs. 1081 ±57 S-phase cells, $p<0.005$). In mice injected with BrdU at the time of operation when proliferation was the same in septic and sham mice, migration at 48 hours was significantly slower in septic mice compared to sham (62 ±1 vs. 84 ±2 percent villus length, $p<0.0001$). In mice injected with BrdU 22.5 hours following the induction of sepsis when proliferation was significantly decreased in septic mice, migration at 48 hours was significantly slower (24 ±1 vs. 30 ±1 % villus length, $p<0.0001$).

Conclusion: Gut epithelial proliferation is decreased and migration is significantly slower in a clinically relevant model of pneumonia-induced sepsis. This represents a fundamental change in the gut's regenerative capacity during sepsis which could potentially have functional importance in survival and thus be a target for future sepsis research.

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ADRENOMEDULLIN REDUCES VASCULAR HYPERPERMEABILITY AND IMPROVES SURVIVAL IN RAT SEPTIC SHOCK

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Objective: Current therapies of sepsis and septic shock imply an extensive volume supply to maintain hemodynamic stability. The vasoregulatory peptide adrenomedullin (AM) has been shown to prevent the transition to the fatal hypocirculatory septic state by poorly understood mechanisms. We tested the hypothesis that therapeutic application of adrenomedullin would reduce vascular hyperpermeabilty thereby contributing to improved hemodynamics and survival.

Material and Methods: Prospective randomized controlled animal study, male Spraque-Dawley rats, monitoring of hemodynamics, organ permeability. We used 4.800 U/kg of Staphylococcus aureus a-toxin, a poreforming exotoxin, to induce vascular leakage and circulatory shock in rats. Infusion of 24 µg/kg*h adrenomedullin was started 1 hour after a-toxin application.

Results: Infusion of a-toxin in rats induced cardiocirculatory failure resulting in a 6-hour mortality of 53 %. Alphatoxin provoked massive vascular hyperpermeability, which was indicated by an enrichment of Evans blue dye albumin in the tissues of lung, liver, ileum and kidney. Plasma fluid loss yielded in a significant hemoconcentration. Hemodynamic impairment as observed after a-toxin infusion was closely correlated to vascular hyperpermeability. Therapeutic administration of 24 µg/kg*h adrenomedullin reduced 6-hour mortality from 53 % to 7 %. Stabilization of the endothelial barrier by adrenomedullin was indicated by a reduced extravasation of albumin and plasma fluid and might have contributed to hemodynamic improvement.

Conclusion: These data suggest that adrenomedullin related reduction of vascular hyperpermeabilty might represent a novel and important mechanism contributing to its beneficial effects in sepsis and septic shock.

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CONTRIBUTION OF CD44 TO THE HOST DEFENSE AGAINST KLEBSIELLA PNEUMONIAE

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Objective: Pneumonia is a major health problem and the most frequent cause of sepsis. CD44 is a glycoprotein involved in inflammation and cell-cell/cell-matrix interactions. The aim of our study was to determine the contribution of CD44 to the immune response to gram-negative pneumonia and sepsis caused by the common respiratory pathogen Klebsiella pneumoniae.

Methods: Wild type C57BL/6J mice (WT) and CD44 gene deficient ($CD44^{-/-}$) mice were intranasally inoculated with K. pneumoniae. At 24 and 48 hours after infection blood, lungs and spleen were harvested for measurement of colony forming units (CFUs), cytokines and chemokines. Survival analysis was performed over a period of 10 days.

Results: $CD44^{-/-}$ mice displayed significantly reduced CFUs in blood and spleen, and showed a trend towards reduced CFUs in lung as compared to WT mice at both 24 and 48 hours after intranasal infection with K. pneumoniae. There were no differences in cytokine and chemokine levels in lungs from both mouse strains, whereas IL-6 levels were significantly reduced in plasma from $CD44^{-/-}$ mice as compared to WT mice at both time-points. Survival did not differ between groups.

Conclusion: The presence of CD44 impairs host defense against K. pneumoniae pneumonia, facilitating dissemination of the infection to blood and distant body sites. These data suggest that Klebsiella pneumoniae abuses CD44 to accomplish severe pneumonia and subsequent sepsis.

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MOLECULAR SIGNATURES OF TRAUMA HEMORRHAGIC SHOCK-INDUCED LUNG INJURY: HEMORRHAGE- AND INJURY-ASSOCIATED GENES

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Objective: The etiology of trauma-hemorrhage shock-induced acute lung injury has been difficult to elucidate due, at least in part, to the inability of in vivo studies to separate the non-injurious pulmonary effects of trauma-hemorrhage from the tissue injurious ones. To circumvent this in vivo limitation, we utilized a model of trauma-hemorrhagic shock (T/HS) in which T/HS-lung injury was abrogated by dividing the mesenteric lymph duct. In this way, it was possible to separate the pulmonary injurious response from the non-injurious systemic response to T/HS by comparing the pulmonary molecular response of rats subjected to T/HS which did and did not develop lung injury as well as to non-shocked rats.

Material and Methods: Four groups of male Sprague-Dawley rats (n=3) were studied in order to identify changes in pulmonary gene expression associated with T/HS, both in the presence and absence of lung injury. These included T/HS (laparotomy plus MAP 35 mm Hg x 90 min shock), trauma-sham shock (T/SS; laparotomy), T/HS with mesenteric lymph duct ligation (LDL) and T/SS with LDL. Three hours after reperfusion, the rats were sacrificed and whole lung tissue was harvested for genechip analysis and histology. Total RNA was isolated, labeled and hybridized to Affymetrix RG-U34A GeneChip. Robust microarray analysis and multiclass significance analysis of microarray (SAM) with a median false discovery rate of zero were used to identify gene expression signatures.

Data: Using the rat Affymetrix U34A GeneChip, 139 of the 8,799 assessed genes were identified by SAM. Hemorrhage without the secondary effects of lung injury modulated the expression of 21 genes such as interleukin-1β (IL-1β), metallothionein-2 and myeloctomatosis oncogene (c-myc). In response to injury, 42 genes were identified to be differentially expressed. Upregulated genes included the L1 retroposon and guanine deaminase whereas downregulated genes included catalase (CAT) and superoxide dismutase (SOD1). Realtime PCR confirmed the differential expression for selected genes. PathwayAssist analysis identified IL-1β as a central regulator of two sub-pathways of stress response related genes (c-myc and SOD1/CAT) as well as several unrelated genes such as lipoprotein lipase.

Conclusion: Our model system, exploiting the use of LDL, provided a unique opportunity to distinguish the molecular changes associated with T/HS-induced acute lung injury from the systemic molecular response to shock and thereby yields novel information on the pathogenesis of T/HS-induced acute lung injury.

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INTERACTION OF INDUCIBLE NITRIC OXIDE SYNTHASE AND HEME OXYGENASE-1 IN THE LUNG DURING LIPOPOLYSACCHARIDE- AND CROSS TOLERANCE

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Objective: Pretreatment with low-dose LPS protects cells and organs against a subsequent lethal gram-negative (LPS tolerance) or gram-positive (cross tolerance) stimulus. We determined whether this phenomenon also occurs in vivo in the rat lung, using Toll-like receptor (TLR)4 'specific' LPS. Furthermore, the potential

involvement of inducible nitric oxide synthase (iNOS) and the antioxidant stress protein heme oxygenase (HO)-1 was evaluated.

Materials and Methods: After ethical approval, rats were injected ip with a low-dose of LPS (1 mg/kg) or saline. At different time points, lung tissue was taken to determine mRNA, protein and activity of iNOS and HO-1. In additional experiments, iNOS and HO-1 inhibition was investigated using 1400W and SnPPIX. 24h later, animals were subjected to 6h of Gram-negative (LPS; 6 mg/kg, iv) or Gram-positive shock (LTA/PepG; 3/10 mg/kg, iv). After 6h, myeloperoxidase (MPO) activity, wet-dry weight ratio, HO-1 western blot analysis and HO activity was determined in lung tissue. Data are presented as mean±SEM of n observations. Comparisons between groups were made using Prism 4.0 (GraphPad Software Inc., USA) using the t-test or ANOVA followed by Bonferroni's post-hoc test. Probability values of *$P<0.05$ were considered statistically significant.

Data: In the rat lung, LPS treatment (1 mg/kg) induced a significant increase in iNOS protein at 8h with a corresponding rise in plasma nitrate/nitrite at 8-16h. Simultaneously, HO-1 mRNA transcripts were observed at 8-16h and maximal expression of the protein followed (24h). Pretreatment with low-dose LPS reduced myeloperoxidase activity (neutrophil infiltration) and wet-dry ratio (pulmonary edema) in the lungs of animals subjected to Gram-negative or Gram-positive shock, thereby demonstrating the existence of LPS tolerance or cross tolerance. Pretreatment with low-dose LPS and the selective iNOS inhibitor 1400W reduced HO-1 protein expression, and lung protection was abolished. SnPPIX did not affect HO-1 expression, but HO activity and lung protection were significantly reduced.

Conclusion: We propose that NO (likely iNOS derived) regulates the induction of HO-1 during both LPS tolerance and cross tolerance. In turn, the lung protective properties of HO-1 could account for the protective effects afforded by low-dose LPS pretreatment. Our finding may have therapeutic importance for patients suffering from lung injury, e.g. during sepsis or surgery.

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CARDIOPROTECTION BY ECTO-5′-NUCLEOTIDASE (CD73) AND A2B ADENOSINE RECEPTORS

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Ecto-5′-nucleotidase (CD73)-dependent adenosine generation has been implicated in tissue-protection during acute injury. Once generated, adenosine can activate cell-surface adenosine-receptors ($A_1AR/A_{2A}AR/A_{2B}AR/A_3$ AR). Here, we define the contribution of adenosine to cardioprotection by ischemic preconditioning (IP). Based on initial observations of CD73 induction by IP, we found inhibition or targeted gene deletion of *cd73* abolished infarct size-limiting effects. Moreover, 5′-nucleotidase treatment reconstituted $cd73^{-/-}$-mice and attenuated infarct sizes in wildtype mice. Transcriptional profiling of adenosine receptors suggested a contribution of the $A_{2B}AR$ as it was selectively induced by IP. Specifically, *in situ* IP conferred cardioprotection in $A_1AR^{-/-}$, $A_{2A}AR^{-/-}$, or $A_3AR^{-/-}$-mice, but not in $A_{2B}AR^{-/-}$-mice or in wildtype mice following inhibition of the $A_{2B}AR$. Moreover, A_{2B} AR-agonist treatment significantly reduced infarct sizes following ischemia. Taken together, pharmacological and genetic evidence demonstrate the importance of CD73-dependent adenosine generation and signaling through $A_{2B}AR$ for cardioprotection by IP and suggest 5′-nucleotidase or $A_{2B}AR$-agonists as therapy for myocardial ischemia.

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INJECTION OF SOLUBLE BONE MARROW COMPONENTS IN INJURED SOFT TISSUE CAUSES SYSTEMIC INFLAMMATION

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Purpose: Patients with bilateral femur shaft fractures are known to be at high risk for systemic complications, possibly mediated by immunologic changes (inflammatory state). The cause of systemic inflammation and its association to long bone fractures is not well understood. Bone marrow possesses immunogenic cells and the effect of released bone marrow components in the area of injured soft tissue is unknown. To determine whether bone marrow components play an important role in the induction of systemic inflammation following long bone fractures, we examined the systemic inflammatory response of male HeOuJ mice after injection of bone marrow components in injured soft tissue of both thighs. Pro- and anti-inflammatory cytokines served as marker to monitor the degree of inflammation.

Material and Methods: Bone marrow was harvested from sacrificed HeOuJ mice and centrifuged to separate bone marrow cells and soluble bone marrow components (supernatant). Mice, 6-8 weeks old, were anesthetized with pentobarbital (70mg/kg IP). Both lower limbs were exposed to soft tissue injury by trapping the tissue around the femur with a hemostat for 30 sec. and either PBS (phosphate buffer solution), bone marrow cells or soluble bone marrow components (supernatant) were injected (0.1ml/leg). Control mice have been anaesthetized without exposure to soft tissue injury or injections. Mice were sacrificed 6 hours following trauma and serum levels of IL-6, IL-10, TNF-α and IFN-γ were measured.

Results: We found significantly higher IL-6 levels in mice that were injected soluble bone marrow components as compared to controls and to injection of PBS or bone marrow cells in injured soft tissue ($p<0.05$). There were

Table

	IL-6	IL-10	IFN-γ	TNF-α
Controls	29,97	-	3,26	-
STI + PBS	25,74	0,5	3,35	1,97
STI + BMC	42,73	0,96	3,37	15,24
STI + SBMC	70,98	0,09	3,16	5,5

cytokine serum levels; STI (soft tissue injury), PBS (phosphate buffer solution), BMC (bone marrow cells), SBMC (soluble bone marrow components)

no differences in serum levels of IL-10, TNF-α or IFN-γ (figure 1). No significant differences in any cytokine levels comparing soft tissue in combination with PBS or bone marrow cell injection to control animals were found. IL-6 levels of soluble bone marrow components were just above detection level.

Conclusion: These results indicate that soluble bone marrow components injected in injured soft tissue are capable to induce systemic inflammation. The soft tissue injury itself did not provoke any significant inflammation and mainly served to create a hyperaemic area. Interestingly, the injection of immunogenic bone marrow cells did not elicit an inflammatory response. However, the systemic inflammatory response in our model does not completely reflect the response of HeOuJ mice after bilateral femur fracture, where higher IL-6 levels and also elevated IL 10 levels were observed. This may be due to the absence of bone cells and bone debris as part of a "normal" fracture or because of a too small soft tissue injury. We conclude that soluble bone marrow components are capable to induce systemic inflammation. Whether this inflammation is caused by cytokines or other forms of danger signals is subject to further studies.

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SINGLE CELL TRACKING OF INTERSTITIALLY MIGRATING LEUKOCYTES UPON LOCAL MICROINJECTION OF CHEMOATTRACTANTS IN VIVO

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The mechanisms mediating leukocyte interstitial migration are poorly understood. In this study, we extended the intravital microscopic technique to allow single cell tracking of migrating leukocytes upon local microinjection of relevant chemoattractants in vivo. In mice, local inflammation was induced in the cremaster muscle by paravenular microinjection of MIP-1α or PAF using a microinjection system. In an additional group, diffuse inflammation was induced by intrascrotal injection of PAF. Leukocyte migration was analyzed within 60 min after microinjection using RLOT intravital microscopy and an imaging software. Microinjection of chemoattractants induced leukocyte adherence, transmigration, and interstitial migration, preferentially on the vessel side ipsilateral to microinjection. Leukocyte migration was target-oriented toward chemotactic stimuli as shown by significantly increased curve/straight line velocity, curve/straight line distance, straightness, and linearity as compared to saline microinjection. Leukocyte motility did not significantly differ between the groups receiving MIP-1α or PAF via microinjection. In contrast, leukocyte migration was rather chaotic upon intrascrotal stimulation as shown by lower migration straightness, straight line migration distance, and velocity. Finally, we tested this approach by verifying the role of Rho-kinase for leukocyte migration in vivo. Tissue superfusion with the Rho-kinase inhibitor Y-27632 blocked MIP-1α-induced leukocyte motility. Thus, we have designed a new technique for the quantitative analysis of target-oriented interstitial migration of single leukocytes in vivo and show that i) local microinjection of chemoattractants induces directional leukocyte locomotion in the interstitium toward the chemotactic gradient, whereas intrascrotal injection leads to chaotic leukocyte migration and ii) inhibition of Rho-kinase attenuates leukocyte motility in vivo.

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CHARACTERIZATION OF LYMPHOCYTE SUBPOPULATIONS DURING DIFFERENT GRADES OF MURINE SEPSIS

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Objective: It is well known that sepsis is associated with reduced peripheral lymphocyte numbers which is due to apoptosis. Programmed lymphocyte death involves mainly thymocytes but also peripheral T cells and dramatically appears in the immature thymocyte population (CD4+CD8+ and CD4-CD8-). Little is known about B cell apoptosis during sepsis, primarily late stages seem to be involved. The significance of lymphocytes to the survival is documented by the markedly reduced capacity of recombination activating gene RAG-/- (lymphocyte-deficient) mice to survive sepsis. However, previous reports are conflicting with respect to numeral changes of CD8+ cells whereas numbers of CD4+ cells consistently decrease in the course of murine sepsis. The aim of this study was to describe the changes of lymphocyte

subpopulations during different grades of sepsis in the mouse.

Material and Methods: Upon anesthesia induction with isoflorane sepsis was initiated by cecal ligation and double puncture in 3 groups of 3 C57BL/6J-mice per group [18G, 22G, 26G] (CLP). Control mice underwent laparatomy and manipulation of the cecum only (Sham). Subsequently, mice received 1ml 0.9% NaCl s.c. once and buprenorphine 25ng/g s.c. twice a day. 48 hrs and 96 hrs post-surgery single cell suspensions of thymus and spleen were analyzed by means of cell surface staining and flow cytometry. Fluorescence-labeled antibodies included CD3, CD4, CD8, B220, IgM, IgD, CD69.

Data: Similar to previous results, thymi primarily demonstrated a time-dependent reduction of CD4+CD8+ double-positive cells which was more pronounced during severe sepsis (22G). As 18G-treated mice died within 48hrs, this pattern could only be partially detected. Interestingly, thymus cellularity was mostly reduced in 26G-treated animals at 96hrs post-CLP. Previously, this group was shown to recover fastest with highest survival rates of about 90%. In contrast, in all groups significant proportions of peripheral CD4+ and CD8+ cells could be detected. They were similarly reduced to about 70% after 48hrs and to 50% in 22G and 26G mice compared to sham mice. In 18G-treated mice, reduction was more pronounced. As far as B cells are concerned no significant differences between the groups could be detected. Notably, IgD+ splenic B cells similarly increased in all groups from 48hrs on. Relative numbers of peripheral T cells expressing the early activation marker CD69 were clearly more pronounced at 48hrs compared to 96hrs in all groups including sham mice. Again, no clear difference could be seen between the groups.

Conclusion: A mild CLP model is more appropriate to study lymphopoiesis during murine sepsis. The rapid occurrence of peripheral activated T cells suggest a very early function of the adaptive immune system during sepsis. Considering a milder disease course of 26G mice they seem to more efficiently use their T cells to fight the infection. B cells are not likely to play a major role in polymicrobial murine sepsis. Further studies have to be performed to elucidate the turnover and the homing of lymphocytes during sepsis.

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RHAPC IMPROVES GLOBAL HEMODYNAMICS AND VISCERAL MICROVASCULAR BLOOD FLOW IN OVINE SEPTIC SHOCK

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Objective: Recombinant human activated protein C (rhAPC) has been shown to reduce mortality in patients with severe sepsis. The aim of this study was to investigate in the effects of rhAPC on regional microvascular blood flow in our model of hyperdynamic septic shock following acute lung injury (ALI) resulting from smoke inhalation (1). We hypothesized that the systemic anticoagulant effects of rhAPC might improve microcirculation.

Material and Methods: Fifteen sheep (33-38 kg) were operatively prepared for chronic study. After 7 days of recovery, sheep were randomly allocated to either the sham (group 1), control (group 2), or treatment group (group 3, n=5 each). After a tracheostomy was performed, ALI was produced in the 2nd and 3rd group by insufflation of 48 breaths of cotton smoke. Then a 30 mL suspension of live **Pseudomonas aeruginosa** bacteria (containing $2\text{-}5x10^{11}$ cfu) was instilled into the lungs according to an established protocol (1). The sham group received only the vehicle. The sheep were studied for 24h in the awake state and were ventilated with 100% oxygen. In group 3, rhAPC (24μg/kg/h) was intravenously administered, beginning 1 h post injury until the end of the experiment (2). The animals were resuscitated with Ringer's Lactate Solution to maintain filling pressures. Colored microspheres were injected at baseline (BL), and 24h. Systemic hemodynamics was determined intermittently. Tissue samples for microsphere analysis were obtained after the experiment during necropsy. Statistical analysis: two-way ANOVA and Student-Newman-Keuls post hoc comparisons. Data are expressed as mean ± SEM. Significance $P<0.05$.

Data: Cardiovascular variables and regional microvascular blood flow (mL/min/g tissue) in trachea, spleen, and kidney cortex remained stable in sham animals. In the control group, cardiac index (CI in $L/min/m^2$) increased significantly after 24h vs. BL (BL: 5.0±0.4 vs. 24h: 7.9±0.4), associated with a significant drop in systemic vascular resistance index (SVRI in $dynes/cm^5/m^2$, BL: 1450±70 vs. 24h: 525±50, each $p<0.05$). RhAPC stabilized CI (BL: 5.1±0.3 vs. 24h: 6.1±0.3) and SVRI (BL: 1460±80 vs. 24h: 920±80) did not fall to the same extent as in controls ($p<0.05$ each). After 24 hours, renal blood flow significantly decreased in controls (80±11% of BL) compared to the rhAPC group (110±11% of BL). In control sheep, the regional microvascular blood flow in trachea (820±115% of BL) and spleen (140±11% of BL) significantly increased over time and was significantly attenuated for rhAPC treated animals in tracheal blood flow (400±50% of BL) as well as in blood flow to spleen (102±13% of BL, each $p<0.05$).

Conclusion: RhAPC significantly improved hemodynamic variables and visceral microvascular blood flow in this ovine model of septic shock and ALI resulting from smoke inhalation injury. RhAPC might be a useful treatment for patients with smoke inhalation injury associated with sepsis.

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D9-THC-TREATMENT IS ASSOCIATED WITH FAST DOWNREGULATION OF THE SCAVENGER RECEPTOR CD163 AFTER SURGERY

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Background: Delta-9-Tetrahydrocannabinol (D9-THC), the active component of cannabis, may affect the expression of inflammatory markers in vivo. We hypothesized that D9-THC could modulate the extent of a perioperative SIRS (systemic inflammatory response syndrome)1.

Methods and patients: The clinical trial included 100 patients undergoing radical prostatectomy. For perioperative pain therapy, patients received randomly either the opioid piritramide alone or a partial substitution by 40 mg D9-THC over 3 days.

Blood samples were collected before (d-1) as well as on the first (d+1) and the second day (d+2) after surgery. Each specimen was analyzed by flow cytometry for 31 cell surface antigens. Inter- and intragroup differences of D9-THC related changes in cell surface antigen expression patterns of lymphocytes, monocytes, and granulocytes were evaluated by appropriate tests.

Results: Trauma induces SIRS and influences the following blood parameters: leukocyte count, CRP concentration, and lymphocyte subpopulations and concentrations of soluble inflammatory mediators. D9-THC treatment did not alter those variables which may be masked by a large variation of individual patients. Out of a panel of lymphocyte differentiation and activation antigens, neither the lymphocytes nor the natural killer-cell markers did change during the observation period. On day+1 after surgery, monocytic HLA-DR was decreased by D9-THC-treatment ($p< 0.078$). On day+2 after surgery, monocytic HLA-DR was decreased in the piritramid-only group ($p<0.058$). The downregulation of HLA-DR has been demonstrated to be associated with prolonged anergy2 and leads to a higher incidence of sepsis3. By contrast the D9-THC group had higher pronounced but not significant ($p> 0.720$) diminution of HLA-DR expression on monocytes. In addition, the SIRS-associated upregulation of the scavenger receptor CD163 was more rapidly downregulated on d+2 ($p<0.004$ in the D9-THC treated group, whereas significance for CD163 downmodulation was only $p< 0.025$ in the non-treated group). Thus, the attenuation of CD163 expression by antigen presenting cells the decrease was more pronounced in the D9-THC group.

Conclusion: Our results suggest that the substitution of perioperative pain therapy by D9-THC leads to a more rapid attenuation of HLA-DR and CD163 on APC post surgery. We therefore suggest that the immunomodulatory effect by D9-THC appears to target APC rather than lymphocyte subpopulations.

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HEMORRHAGIC SHOCK IN THE PIG: A NEW FIBRIN-DERIVED PEPTIDE REDUCES LUNG AND HEART INJURY

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Objective: The fibrin-derived peptide $B\beta_{15-42}$ has been shown to possess potent anti-inflammatory properties. It competes with fibrin E1-fragments for binding to endothelial VE-cadherin, thereby reducing leukocyte transmigration across endothelial junctions in vitro. Moreover, in rodent models for myocardial ischemia-reperfusion, this peptide reduces myocardial damage. We hypothesized that $B\beta_{15-42}$ reduces organ injury in a large animal model of hemorrhagic shock (HS), where also ischemia and reperfusion occur.

Materials and Methods: After ethical approval, general anesthesia was induced and male farm-bred landrace pigs (27-32 kg) were subjected to HS by bleeding from the left femoral artery to reach a mean arterial pressure of 40 mmHg. 60min later, animals were resuscitated with 60% of shed blood and twice the volume of shed blood as a crystalloid solution (for 60min). At the same time, $B\beta_{15-42}$ (2.4 mg/kg, n=8) or random peptide (control; 2.4 mg/kg, n=7) was administered as an iv bolus. Heart rate (HR), cardiac index (CI), stroke volume (SV), mean arterial pressure (MAP), and paO_2/FiO_2-ratio were measured at baseline, at the end of the shock phase as well as 1 and 5h after reperfusion. Blood gas analyses and serum-samples for troponin T (TnT) were taken. Data are presented as mean±SEM of n observations. Comparisons between groups were made using Prism 4.0 (GraphPad Software Inc., USA) using the t-test or ANOVA followed by Bonferroni's post-hoc test. Probability values of $*P<0.05$ were considered statistically significant.

Data: HS significantly compromised hemodynamic parameters in controls, reducing CI from 138 to 49 ml/kg/min and SV from 38 to 7 ml. During the early phase of reperfusion, hemodynamic parameters returned to baseline values but decreased over time (at 5h reperfusion; CI=106 ml/kg/min; SV=22 ml). TnT levels were 0.56 ng/ml at 5h reperfusion. In $B\beta_{15-42}$ treated animals, the following parameters were significantly different at 5h

reperfusion: *CI=151 ml/kg/min, *SV=37 ml and *TnT levels=0.14 ng/ml. Furthermore, we observed a significant impaired pulmonary function in control pigs. After 5h reperfusion, the paO_2/FiO_2-ratio was below 300 mmHg, fulfilling the criteria of acute lung injury. Sufficient oxygenation (paO_2>12<15 kPa) could only be established by increasing PEEP (max. 7 cmH_2O) and FiO_2 (max. 50%). We did not observe any pulmonary dysfunction in animals treated with $B\beta_{15\text{-}42}$.

Conclusion: $B\beta_{15\text{-}42}$ protects the heart and lung during HS in the pig. Further studies are required to investigate its potential to preserve organ function in a chronic model of HS.

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SIGNIFICANT MYOCARDIAL PROTECTION IN SWINE VIA THE FIBRIN-DERIVED PEPTIDE BB15-42

Kai Zacharowski, Jan Roesner, Peter Petzelbauer, Alex Koch, Michael Wolzt

Objective: The fibrin-derived peptide $B\beta_{15\text{-}42}$ mediates potent anti-inflammatory effects. It competes with fibrin E1-fragments for binding to endothelial VE-cadherin, thereby reducing leukocyte transmigration across endothelial junctions in vitro. Moreover, in rodent models for myocardial ischemia-reperfusion, this peptide reduces myocardial damage. To increase its potential for translation into the clinic, we studied the effects of $B\beta_{15\text{-}42}$ in pigs, where the coronary anatomy is similar to humans. In addition, we evaluated the pharmacokinetics and safety of $B\beta_{15\text{-}42}$ in several species including man.

Materials and Methods: After ethical approval, male farm-bred landrace pigs (25-30 kg) were subjected to 1h left anterior descending (LAD) coronary artery occlusion followed by 3h of reperfusion. At the time of reperfusion, $B\beta_{15\text{-}42}$ (2.4 mg/kg, n=6) or random peptide (control; 2.4 mg/kg, n=6) was administered as an intravenous bolus. As a positive control, pigs were subjected to ischemic preconditioning (IPC; 10min LAD-occlusion followed by 15min reperfusion, n=6). Markers of cardiac damage and inflammation were measured throughout the study. Biodistribution and pharmacokinetics of $B\beta_{15\text{-}42}$ were also determined. In a phase I trial studying 30 male healthy volunteers, pharmacokinetics and safety were tested in a randomized, double-blinded, placebo-controlled, parallel-group, single ascending dose study. Results are expressed as the mean ± SEM. Statistical analysis was performed with a One-Way-ANOVA followed by a Bonferroni's post-hoc test or a Two-Way-ANOVA if appropriate. Probability values of *P<0.05 were considered statistically significant.

Data: $B\beta_{15\text{-}42}$ and IPC significantly reduced myocardial ischemia-reperfusion injury, evident by a reduction in infarct size (% area at risk; control: 65±4; $B\beta_{15\text{-}42}$: *42±2; IPC: *36±7) and Troponin I levels (ng/ml/weight area at risk; control: 3.3±0.6; $B\beta_{15\text{-}42}$: *1.9±0.2; IPC: *1.7±0.4). $B\beta_{15\text{-}42}$ also reduces IL-6 levels underlining its anti-inflammatory properties. Furthermore, in man the pharmacokinetic of the peptide $B\beta_{15\text{-}42}$ was comparable to animals and no serious adverse effects were observed.

Conclusion: $B\beta_{15\text{-}42}$ elicits cardioprotection in pigs and is clinically safe in phase 1 testing of humans. This study confirms the new concept of a pathogenic role of fibrin derivatives in myocardial reperfusion injury, which can be inhibited by peptide $B\beta_{15\text{-}42}$. On the basis of this preclinical data package a phase II clinical trial in infarct patients undergoing PCI will be initiated.

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BACTERIAL DNA REDUCES THE INFARCT SIZE OF AN ISCHEMIA/REPERFUSION INJURY IN THE HEART

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Objective: Pretreatment with pathogenic ligands, e.g. lipopolysaccharide (LPS) is known to reduce the size of an ischemia-reperfusion (IR) injury in the heart. LPS binds to CD14/Toll-like receptor 4 thereby inducing inflammatory mediators. This pre-conditioning phenomenon seems to protect the tissue against ischemic injury. Bacterial DNA differs from eukaryotic DNA by the presence of CpG motives. CpG-DNA is specifically recognized by Toll-like receptor 9 (TLR9) leading to the activation of a signalling cascade followed by the induction of inflammatory mediators. Thus, we tested whether a specific CpG-oligonucleotide (1668-ODN) possesses pre-conditioning properties. 1668-ODN has been shown to induce sepsis in vivo earlier.

Material and Methods: In a first step, a thread was implanted around the LAD of C57BL/6 mice. Then, the animals were allowed to recover for 7 days. 16h before constriction of the LAD the animals were challenged intraperitoneally (i.p.) with CpG-ODN 1668 at different concentrations (1 nM, 5 nM, or 10 nM per animal). PBS and other synthetic oligonucleotides were used as control. After 1h of occlusion and 24h of reperfusion the hearts were double stained with 5% Phthaloblue and 1.5% triphenyltetrazolium chloride to calculate area at risk (AAR) and infarct area (IA). In addition, troponin T concentrations in the blood were measured using a Roche cardiac reader. Cytokine gene as well as TLR expression in the heart was detected at different time points using a RNase Protection Assay.

Data: In control hearts, IA was 28.3 % of AAR (SEM ± 3.4 n=15), in those pre-treated with 1 nM 1668-ODN IA did not significantly change (22 ± 7.0 %; n =7). However, pretreatment with 5 nM 1668-ODN significantly reduced IA by about 50% to 13% (SEM ± 3.0; n=12). Higher

CpG-DNA concentrations were not suitable as the survival rate of the animals was reduced. Plasma troponin T concentration were higher in control animals (6.7 ± 1.0 ng/ml) in comparison to 5 nM pretreated animals (2.2 ± 0.6 ng/ml). Control oligonucleotides did not seem to have preconditioning properties since IA did not significantly change compared to untreated WT mice. In contrast to control oligonucleotides, CpG-ODN 1668 challenge led to a strong increase in myocardial cytokine gene expression (TNF-α, IL-1β, IL-6) and to an upregulation of TLR2, whereas TLR4 levels remained unchanged suggesting that the initial inflammatory response contributes to the reduction of IA.

Conclusion: Pretreatment of bacterial DNA reduces the infarct size of an ischemia/reperfusion injury in the heart.

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EFFECT OF CALPAIN INHIBITION ON MYOCARDIAL INFARCTION FOLLOWING LOCAL ISCHEMIA AND REPERFUSION

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Introduction: This study was performed to test the protective effectiveness of a novel water-soluble and cell-permeable calpain inhibitor when administered before or after local ischemia.

Material and Methods: Isolated rabbit hearts were perfused in an open working Langendorff heart system at constant pressure (80 mmHg) and temperature (37°C) with Krebs-Henseleit buffer solution. After a steady state period of 20 min, the ramus interventricularis of the left coronary artery was blocked just below the origin of the first diagonal branch by means of a Tourniquet for 60 min, and subsequently reperfused for 120 min. The calpain inhibitor A-705253 (K_i = 27 ± 2.5 nM) from Abbott/ Ludwigshafen/Germany was added to the perfusion fluid in various final concentrations in one series (A) of experiments before the closure and in an other series (B) after the reopening of the coronary vessel.Hearts without inhibitor or with the administration of the Na+/H+-exchange inhibitor cariporide® served as controls. Hemodynamic monitoring and biochemical analysis of perfusion fluid from the coronary outflow were performed.Myocardial infarct size was determined by microscopic evaluation of left ventricular slices after a special staining procedure with Evans blue and 2,3,5 triphenyltetrazoliumchloride.

Results: The area of necrosis/infarction was 77.9 ± 2.3% of the area at risk in controls without calpain inhibition of series A (n=12), respectively 72.7 ± 4.0% of series B (n=8). Preischemic administration of A-705253 (n=8) reduced the area of infarction most effectively ($p<0.001$) to 49.3 ± 3.9% (n=8) with an inhibitor concentration of 10^{-8} mol/l. Even with postischemic inhibitor application (n=8) area of infarction could reduced significantly ($p<0.01$) to 48.3 ± 2.3% using an inhibitor concentration of 10^{-6} mol/l. Administration of cariporide® (n=8; 10^{-6} mol/l) resulted in a comparable reduction ($p<0.01$) to 50.2 ± 3.4% of the area at risk. The protective effect of calpain inhibition on myocardial infarction was dose dependant in relation to the time point of administration.

Conclusion: The experiments imply a major role of calpains in myocardial ischemia and reperfusion injury which can be attenuated as well by preischemic as by postischemic calpain inhibition. The potectiv effect of Na+/H+-exchange inhibition seems to be mediated by the inhibition of calpain activation.

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HMGB1 AND RAGE IN POST-ISCHEMIC BRAIN

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Severe cerebral ischemia in stroke triggers necrotic neuronal death. In the penumbra where ischemia is less severe tissue damage is due to apoptosis and inflammation. High mobility group box (HMGB) 1 has been shown to link necrotic cell death to inflammation in several other forms of tissue damage. HMGB1 is a DNA binding protein that is widely expressed in various tissues including the brain. Moreover, HMGB1 is a cytokine-like mediator of delayed endotoxin lethality and acute lung injury. HMGB1 binds to the receptor for advanced glycation end products (RAGE) and to TOLL-like receptors (TLR) 2 and TLR4. Here we show that HMGB1 is released by primary cortical neurons exposed to oxygen and glucose deprivation. In stroke patients we found an increase of serum HMGB1 concentrations 24 hours after admission to hospital. Animals lacking RAGE showed significantly reduced infarct volumes after permanent middle cerebral artery occlusion. However, opening of the blood-brain barrier in cerebral ischemia was not affected by RAGE deficiency nor did physiologic parameters such as blood pressure, cerebral blood flow, and blood gases differ between RAGE-/- and control animals. An intraperitoneal administration of soluble RAGE 15 minutes before and 90 min after middle cerebral artery occlusion significantly reduced the infarct volume. In summary, our data suggest that RAGE mediates ischemic brain damage at least in part due to binding of HMGB1 which is released from ischemic neurons.

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GLYCOGEN SYNTHASE KINASE-3B INHIBITION ATTENUATES THE DEVELOPMENT OF ISCHAEMIA/REPERFUSION INJURY OF THE GUT

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The aim of this study was to investigate the effects of TDZD-8, a potent and selective GSK-3b inhibitor, on the tissue injury caused by ischemia/reperfusion (I/R) of the gut. I/R injury of the intestine was caused by clamping both the superior mesenteric artery and the celiac trunk for 45 min followed by release of the clamp allowing reperfusion for 1 h or 6 h. This procedure results in splanchnic artery occlusion (SAO)-shock. Rats subjected to SAO developed a significant fall in mean arterial blood pressure, and only 10% of the animals survived for the entire 6 h reperfusion period. In a separate set of experiments, at 60 minutes after reperfusion animals were sacrificed for histological examination and biochemical studies. Administration of TDZD-8 (1 mg/kg i.v.) 5 min prior to the reperfusion significantly reduced the (i) fall in mean arterial blood pressure, (ii) mortality rate, (iii) infiltration of the reperfused intestine with polymorphonuclear neutrophils (MPO activity) (iv) production of pro-inflammatory cytokines (TNF-a and IL-1b) and (v) histological evidence of gut injury. Administration of TDZD-8 also markedly reduced the immunoreactivity of nitrotyrosine formation and the expression of ICAM-1 and P-selectin during reperfusion. Thus, based on these findings we propose that TDZD-8, may be useful in the treatment of various ischemia and reperfusion diseases.

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AN ANTI-INFLAMMATORY PROTEIN, FETUIN, ATTENUATES CEREBRAL ISCHEMIC INJURY

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Cerebral ischemia elicits inflammatory response, which precipitates progressive tissue injury in the surrounding penumbra. Anti-inflammatory agents capable of crossing the blood-brain barrier may attenuate the progression of cerebral ischemic injury. HMGB1 has recently been identified as a critical mediator of inflammatory diseases. A negative acute phase protein, fetuin (fetus protein in Greek), was recently characterized as a negative regulator of inflammation by opsonizing cationic immunosuppressive molecules (e.g., spermine).

Objectives: To further elucidate the potential roles of fetuin in the regulation of innate immune response, we examined its effects on active HMGB1 release in vitro, and progression of cerebral ischemia injury in vivo. **Methods:** Thioglycollate-elicited murine peritoneal macrophages or human peripheral blood mononuclear cells (HuPBMCs) were activated by bacterial endotoxin LPS (100 ng/ml) in the absence or presence of fetuin, and the levels of TNF and HMGB1 in the culture medium were determined 16 hours later. Male Lewis rats (270-300 g) were subjected to cerebral ischemia by occlusion of middle cerebral artery (MCAo), and peripherally (intravenously) administered with exogenous fetuin to determine its effect on the progression of cerebral ischemic injury.

Results: In vitro, fetuin dose-dependently inhibited LPS-induced release of TNF and HMGB1 in cultures of murine macrophages and HuPBMCs. Using an animal model of cerebral ischemia (middle cerebral artery occlusion, MCAO), we discovered that levels of fetuin and HMGB1 levels were low in normal brain tissue, but markedly increased in ischemic brain tissue at 24 h after the onset of cerebral ischemia. The dramatic increase in HMGB1 expression was predominantly noted in the surrounding penumbra. Similarly, the brain expression levels of proinflammatory cytokines (such as TNF) were also dramatically increased in the ischemic penumbra. Exogenous fetuin administered peripherally (intravenously) crossed the blood-brain barrier, and destined to the ischemic brain tissue. Furthermore, it dramatically attenuated expression of proinflammatory cytokines (such as TNF) in the ischemic brain tissue, and dose-dependently reduced brain infarct volume.

Conclusions: Fetuin occupies an important role in the regulation of the innate immune response in cerebral ischemia, and holds potential as an effective therapy for stroke. (*Supported by the NIH/NIGMS Grants R01GM063075 and R01GM070817 to HW).*

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ROLE OF ECTO-5′-NUCLEOTIDASE IN PROTECTION AGAINST GASTROINTESTINAL ISCHEMIA/REPERFUSION INJURY

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Objective: Gastrointestinal ischemia/reperfusion (GI/R) stems from interruption of blood flow within the mesenteric arteries, and leads to small intestine hypoperfusion. Injury from GI/R can result in local and systemic inflammatory responses that may lead to widespread organ dysfunction. Recent studies show that nucleotide phosphohydrolysis and nucleoside signaling via adenosine receptors (ARs) resemble innate protective signaling pathways that modulate acute inflammatory responses and organ function during hypoxia. However, their role in organ protection during GI/R is largely unknown. We hypothesized that ecto-5′-nucleotidase (CD73, conversion of AMP to adenosine) protects against the inflammatory response following GI/R.

Material and Methods: CD73 deficient ($CD73^{-/-}$) and littermate control mice (WT) were anesthetized with isoflurane. After a midline laparotomy, intestinal ischemia was produced by clamping the superior mesenteric artery for the indicated time periods, followed by unclamping for 3 hours of reperfusion. Sham-operated controls underwent the same surgical procedures but without GI/R.

Data: To clearly define the role of CD73 to PMN trafficking in vivo during GI/R, WT and $CD73^{-/-}$ mice were subjected to 5, 10 or 15 min ischemia, followed by 3 hours reperfusion. Increasing the ischemia time in $CD73^{-/-}$ and WT mice caused more infiltration of neutrophils into the intestinal and pulmonary tissue as measured by myeloperoxidase (MPO) activity. However, $CD73^{-/-}$ mice demonstrated significantly higher levels of MPO in the intestine and lung than WT mice following GI/R. Additionally, pharmacological inhibition of ecto-5′-nt with 5′-[αβ-methylene] diphospate (APCP) significantly increased pulmonary neutrophil infiltration in WT mice following GI/R. Collectively, these data indicate that CD73 protects against neutrophil infiltration induced by GI/R. We also assessed secondary hepatic injury by measuring serum ALT and AST following 15 minutes ischemia and 3 hours reperfusion. Biochemical analysis revealed that GI/R significantly increased serum ALT and AST concentrations in $CD73^{-/-}$ mice compared to WT mice after GI/R. $CD73^{-/-}$ mice also demonstrated significantly higher IL-1b and IL-6 proinflammatory cytokine concentrations in the serum than WT mice. **Conclusions**: These data suggest CD73 is protective during GI/R and that local and remote injuries associated with GI/R can be diminished by CD73. Taken together these data reveal an important role of extracellular adenosine generation via CD73 in organ protection during GI/R.

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GUT INJURY AND ASSOCIATED LUNG INJURY IS REDUCED IN FEMALE RATS FOLLOWING ISCHEMIA/REPERFUSION INJURY

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Objective: Our previous work has shown that female rats were more resistant to trauma-hemorrhagic shock (T/HS) induced gut injury and subsequent distant organ injury. The mechanism of why the female intestine is more resistant to gut ischemia/reperfusion (I/R) injury remains unclear. We tested the hypothesis that female intestine is more resistant to gut I/R injury than the male intestine.

Materials and Methods: In order to test this hypothesis, an isolated gut I/R injury model (superior mesenteric artery occlusion, SMAO) was employed. Adult Sprague-Dawley male and proestrus female rats were subjected to 45 minutes of SMAO. After 6 and 24 hours following SMAO, villous injury, lung permeability and lung neutrophil sequestration was measured. Lung permeability was quantified by calculating the percentage of Evan's Blue dye (EBD) and the total protein concentration in the bronchoalveolar lavage fluid (BALF) as compared to the plasma. Myeloperoxidase (MPO) levels were also measured in the lung tissue as a marker for lung neutrophil sequestration.

Results: Significant gut injury was found in male rats subjected to SMAO at either 6 (17±8% injured villi) and 24 hrs (14±2% injured villi) hours. In contrast, gut injury was not observed in the proestrus female rats subjected to SMAO at 6 hrs (2±1% injured villi) or 24 hrs (2±1% injured villi) which was not significantly different to male and female sham-SMAO rats. Evans blue lung permeability was significantly increased in male-SMAO rats at both 6hrs (10.4±0.7 %EBD in BALF) and 24hrs (10.3±2.2 %EBD in BALF). Additionally, BALF-to-plasma protein ratio was increased in the male-SMAO rats at 6 hrs (0.13±0.02) and 24hrs (0.11±0.02). However, EBD lung permeability was not significantly different in the female-SMAO as compared to sham at both 6hrs (2.4±0.8 vs 2.3±0.8 %EBD in BALF; $p>0.05$) and 24hrs (2.3±0.9 vs 2.7±0.6 %EBD in BALF; $p>0.05$). BALF-plasma protein ratio was also not significantly different when comparing female rats who underwent SMAO vs sham at both 6hrs (0.05±0.02 vs 0.04±0.01;$p>0.05$) and 24hrs.(0.04±0.01 vs. 0.05±0.01; $p>0.05$). Similarly the pulmonary MPO levels were increased in the male ($p<0.05$), but not the females tested.

Conclusion: These results suggest that female intestine is more resistant to gut I/R injury than the male intestine which subsequently may lead to decreased lung injury.

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TETRAHYDROBIOPTERIN ATTENUATES MICROVASCULAR REPERFUSION INJURY FOLLOWING MURINE PANCREAS TRANSPLANTATION

Manuel Maglione

Purpose: Tetrahydrobiopterin (BH_4) is an essential cofactor for nitric oxide synthases and thus a critical determinant of NO production. Recently we have shown that BH_4 depletion contributes to ischemia reperfusion injury (IRI) after pancreas transplantation. In this study we investigated the therapeutic potential of BH_4 supplementation during organ retrieval and the early post-transplant period.

Material and Methods: Male syngeneic C57BL6 (H-2b) mice, 10-12 weeks old were used as size-matched donor and recipient pairs. Murine cervical heterotopic vascularized pancreas transplantation was performed with a modified no-touch technique. Pancreatic grafts were subjected to 16 hours prolonged cold ischemia (CIT) time and different treatment regiments: untreated (I), BH_4 160μM to perfusion solution (II), BH_4 50mg/kg i.m. at reperfusion (III). Non transplanted animals served as

controls (IV). After 2h of reperfusion intravital fluorescence microscopy was used for analysis of graft microcirculation by means of functional capillary density (FCD) and capillary diameters (CD). Quantitative assessment of inflammatory responses (mononuclear infiltration) and endothelial disintegration (edema formation) was done by histology (H&E) and peroxynitrite formation was assessed by nitrotyrosine-immunostaining.

Results: After prolonged CIT FCD and CD was significantly reduced, paralleled by an increased peroxynitrite formation, when compared with controls (all $p<0.05$). Microcirculatory changes correlated significantly with intragraft peroxynitrite generation (Spearman: $r= -0.56$; $p<0.01$). Pancreatic grafts treated with BH_4 either during retrieval (II) or systemically (III) displayed markedly higher values of FCD ($p<0.01$) and abrogated nitrotyrosine staining ($p<0.05$). Systemically BH_4 treated recipients (III) and control animals (IV) displayed no differences in CD. Histological evaluation showed increased inflammation, interstitial edema, hemorrhage, acinar vacuolization and focal areas of necrosis after 16h CIT in group I, which could be diminished by both BH4 treatment regiments ($p<0.05$).

Conclusion: BH4 supplementation either during organ retrieval or in the early post-transplant period significantly reduces postischemic deterioration of microcirculation as well as histologic damage and might be a promising novel strategy in attenuating IRI in clinical pancreas transplantation.

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TNF-A PRECONDITIONING OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS DOES NOT IMPROVE STEM CELL ACUTE MYOCARDIAL PROTECTION AFTER ISCHEMIA/REPERFUSION

Troy Markel, Paul Crisostomo, Tim Lahm, Meijing Wang, Daniel Meldrum

Objective: Stem cell therapy is a promising treatment modality for injured cardiac tissue. Our previous work has shown that pretreatment with human bone marrow mesenchymal stem cells (BMSCs) acutely confers myocardial protection from ischemia and reperfusion (I/R) injury. Furthermore, a novel mechanism for this cardioprotection may include release of growth factors from BMSCs and subsequent protection of injured tissue via paracrine actions. We have previously shown that TNF-α stimulates human BMSCs to release paracrine growth factors. Recent studies have shown that hypoxic preconditioning of BMSCs decreases apoptosis and infarct size after myocardial infarction. It is not known however, if either early or late TNF-α preconditioning improves BMSC mediated acute protection after I/R. We therefore hypothesize that preconditioning BMSCs with TNF-α will confer cardioprotection to ischemic myocardium.

Material and Methods: Human BMSCs were plated (100,000 cells/well/ml) and cultured at 37C. Cells were preconditioned with TNF-α for 3 hours or 24 hours (50ng/ml) prior to cardiac infusion in order to simulate early or late preconditioning. Male Sprague-Dawley rat hearts were then isolated and perfused via Langendorff model. Hearts were subjected to 25 minutes of warm ischemia and 40 minutes of reperfusion. Two million BMSCs in 1ml of perfusate were infused immediately prior to ischemia in experimental hearts ($N=3$ per experimental group). Left ventricular developed pressure (LVDP) and cardiac contractility (+dp/dt, and –dp/dt) were continuously monitored. Results were considered significant if $p<0.05$.

Results: Ischemia / reperfusion resulted in markedly decreased LVDP in all groups. However, post-ischemic recovery of LVDP was not significantly different in early preconditioned hearts (37.1 ± 0.7 %) or late preconditioned hearts (25.4 ± 3.4 %) compared to controls (35.6 ± 3.5 %). Early or late preconditioning with TNF-α also did not confer protection to ischemic cardiac tissue as measured by end diastolic pressure, +dp/dt, or –dp/dt.

Conclusion: Although hypoxic preconditioning has been shown to confer protection to ischemic myocardium, it appears that preconditioning with TNF-α does not offer the same degree of protection. BMSCs are a potent source of growth factors that can offer protection to ischemic tissues. Understanding the mechanisms of growth factor production may allow for ex-vivo priming of stem cells both prior to and during therapeutic use.

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LYMPHO-LYMPHONODULAR ANASTOMOSES, PROOF OF PATENCY BY INJECTION OF PATENT BLUE DYE - A STUDY IN A RAT MODEL

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Objectives: Lymphedemas due to local posttraumatic lymphatic blocks can be treated by microsurgical transplantation of lymphatic vessels to bridge the blockage. In general end-to-end anastomoses are performed, but in some cases not enough appropriate draining recipient vessels can be found. Alternatively microvascular lymphatic grafts can be anastomosed to lymph nodes. This procedure enables the connection of the grafts without disruption of the recipient lymph stream. Additionally, grafts of any diameter can be sutured to incisions of adequate size in the capsule of the lymph node. This study was performed to proof the patency of lympho-lymphatic anastomoses in an experimental animal model.

Materials and Methods: Male Sprague-Dawley-rats were anaesthetized and the left truncus lumbaris was cut proximally and turned over to the right lumbal lymph nodes. It was anastomosed by a single stitch technique (Group A; n=12; 14 anastomoses). In the controls the left truncus lumbaris was either cut and ligated (Group B, n=6) or cut, turned over to the right side and fixed to the retroperitoneum close to the lymph node (Group C, n=6). After a healing period of 8 to 15.5 weeks (m=12.5 weeks) the situs was re-explored and the function of the truncus was examined by intranodular injection of patent blue dye into the left lumbar lymph node located upstream.

Data: In group A we found patent transposed lymph vessels stained with patent blue and a further draining off through the right retroperitoneal lymph vessels. In 10/12 animals the patency was proven directly by observation of blue dye transit through the anastomosis, in 11/12 animals indirectly by observation of blue staining of the right lumbal lymph node. In group B and C no direct lymphatic connection to the right lumbal lymph node was observed, but in group B in 6/6 animals a blue dye flow was oberserved from the left truncus lumbaris to the right truncus lumbaris downstream the right lumbal lymph node.

Conclusion: Patent blue staining demonstrated the passage of lymph through lympho-lymphonodular anastomoses. This microsurgical technique may be a useful alternative in lymphatic microsurgery.

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NITRIC OXIDE, HYDROGEN PEROXIDE AND PEROXYNITRITE IN INFLAMMATION

Guy Brown

Objective: To find out the relative roles of different oxidants derived from oxygen and NO (ROS and RNS), as well as mitochondria, in how inflammation is activated and how inflammation causes cell death and dysfunction.

Methods: Cultured macrophages or microglia (brain macrophages) and/or neurons were exposed to inflammatory stimuli or agents producing or removing (ROS or RNS), then proliferation, activation and death were quantified.

Results: A variety of inflammatory stimuli (LPS, LTA, beta amyloid, prion peptides, IL-1b, TNFa, ATP, PMA, arachidonate) caused an acute activation of hydrogen peroxide production from the NADPH oxidase of microglia. If the NADPH oxidase was inhibited (with apocynin or DPI) or if the hydrogen peroxide was removed (by adding catalase or catalase mimetics) the inflammatory stimuli no longer caused microglial proliferation or activation. Hydrogen peroxide alone (supplied by glucose oxidase or xanthine oxidase) was sufficient to cause microglial proliferation. Activation of the NADPH oxidase alone was not sufficient to cause death of co-cultured neurons. Similarly expression of iNOS was (normally) not sufficient to cause neuronal death. However, if the NADPH oxidase was activated when iNOS was also expressed, then neurons died, and this death was blocked by peroxynitrite scavengers. iNOS expression alone caused relatively little death, and can indeed protect cells and cause proliferation. However, if iNOS expression was combined with hypoxia, it caused death of neurons and aortic rings, via inhibition of mitochondrial cytochrome oxidase.

Conclusions: NO, superoxide, hydrogen peroxide and hypoxia are relatively non-toxic by themselves, and play a variety of signalling roles. However, NO combined with either superoxide, hydrogen peroxide or hypoxia can kill cells.

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NITROSATIVE STRESS OF CYTOCHROME OXIDASE IN ATAXIA TELANGIECTASIA

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Nitric oxide (NO) controls mitochondrial respiration via the rapid (milliseconds) reversible inhibition of cytochrome **c** oxidase (CcOX) [1, 2 and ref therein]. Ataxia Teleangiectasia patients are particularly responsive/exposed to oxidative and nitrosative stress. The mitochondrial response to NO of lymphoblastoid cells established from the blood of patients affected by Ataxia Teleangiectasia (AT) has been investigated and compared to that of normal lymphoblastoid cells collected from controls, having common genetic background. Stimulation of the constitutive NOS by N-methyl-D-aspartate led to a similar, minor, depression of mitochondrial membrane potential in controls and AT cells, respiring under standard cell culture conditions. In contrast, upon increasing the electron flux through the respiratory chain, the mitochondrial inhibition induced by NO was more severe in AT cells than in controls. The observation has been discussed on the bases of the previously proposed mechanisms of reaction of NO with CcOX [1]. Consistently, nitrosylation of CcOX was shown to be a function of concentration of cytochrome **c** [3], whose expression was found to be higher in AT cells. It is discussed how these findings might be relevant to ATmitochondrial pathophysiology.

Acknowledgements Supported by MIUR, Italy [PRIN "Meccanismi molecolari e aspetti fisiopatologici dei sistemi bioenergetici di membrana, to P.S., and PRIN "Basi genetiche molecolari e cellulari dell'Atassia con Aprassia Oculomotoria di tipo1, as well as FIRB - RBNE01RNN7-005, and Theleton E.0764, to LC].

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NITRITE AS A SOURCE OF NO IN MITOCHONDRIA: THE RELEVANCE FOR PERFUSION OF ISCHEMIC TISSUE

Andrey Kozlov

Objectives: Nitrite, considered previously as an end product of NO metabolism, has recently been rediscovered as biologically active substance, inducing vasodilatation. The aim of this study was to clarify the impact of nitrite for tissue perfusion.

Material and Methods: Infusions of nitrite and nitrosyl complexes of Hb (NO-Hb) were performed in control rats. Skeletal muscle ischemia was applied for 2 h using the rubber band tourniquet method; intestinal ischemia was made by superior mesenteric artery occlusion for 1 h.

Data: In our study the infusion of nitrite in blood resulted in vasodilatation and gave rise to the formation of Hb-NO complexes. Vasodilatation was observed at NO-Hb concentrations of 2 μM. Infusion of in vitro prepared RBC saturated with Hb-NO did not result in vasodilatation at Hb-NO concentrations reaching 15 - 20 μM. These data suggest that nitrite infused in the blood or taken alimentary is reduced to NO not only in RBC but also in other cells. To confirm this assumption erythrocytes were incubated in Petri dishes with or without cardiomyocytes (HL-1) and with or without nitrite. Nitrite incubated with erythrocytes alone gave rise to the formation of certain amounts of NO-Hb. The presence of cardiomyocytes resulted in the formation of additional NO-Hb amounts. These data suggest that an important mechanism of vasodilatation can be due to nitrite reduction inside the cells. This was confirmed by the fact that heart, liver, and intestine homogenates were able to reduce nitrite to NO. This process was catalyzed by mitochondrial fraction of homogenate and abolished by myxothiazol, a specific inhibitor of complex III of respiratory chain, suggesting mitochondrial complex III as the intracellular nitrite reductase. The reduction of nitrite was facilitated by anaerobic conditions, indicating that it is operating under ischemic/hypoxic conditions. In a model of skeletal muscle ischemia-reperfusion injury and intestinal ischemia reperfusion we observed an NOS-independent formation of NO in ischemic tissue.

Conclusion: Our data suggest that the reduction of nitrite by mitochondria as an important source of NO, which can ameliorate perfusion of ischemic tissue.

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HYPOXIA, NITRIC OXIDE AND MITOCHONDRIAL RESPIRATORY CONTROL BY CYTOCHROME C OXIDASE

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Mitochondrial respiratory control by oxygen is modified by nitric oxide which, in turn, exerts a direct influence on intracellular oxygen levels as a result of the balance between oxygen supply and demand. Although it is well established that NO is a membrane-permeant second messenger and competitive inhibitor of cytochrome c oxidase [1], little quantitative information is available on the kinetics of cellular respiration at low oxygen. We applied high-resolution respirometry with the OROBOROS Oxygraph-2k (Innsbruck, Austria) [2], incorporating a polarographic NO sensor for simultaneous recording of respiration and NO. NO production was stimulated by addition of arginine to HEK 293 cells stably expressing human iNOS. NO levels increased to 40 and up to 2000 nM as a function of iNOS activity, but invariably declined under hypoxia due to oxygen limitation of iNOS and predominance of NO degradation. Conventional models of linear competitive inhibition [3] or competitive and non-competitive inhibition [4] were inadequate to explain the complex interplay between oxygen and NO at pathophysiological concentrations. We developed, therefore, a new kinetic model of hyperbolic competitive inhibition to describe the inhibition of cell respiration at the level of cytochrome c oxidase by varying NO and oxygen concentrations at routine respiration rates and physiological substrates in the intact cell.

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THE ROLE OF CYTOCHROME C OXIDASE ISOFORMS IN MITOCHONDRIAL DYSFUNCTION AFTER HYPOXIA AND REOXYGENATION IN BRAIN AND HEART

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Brain and heart cells of mammalian organisms consume energy to a very large extent fulfilling their energetic requirements by mainly utilizing glucose and oxygen in mitochondrial oxidative energy metabolism. The terminal enzyme of the mitochondrial respiratory chain, cytochrome c oxidase (COX), directly consumes oxygen by catalyzing the electron transfer from ferrocytochrome c to oxygen. This process is coupled to the translocation of protons across the inner mitochondrial membrane and subsequently to production of ATP, an indirect product of COX, through the ATP synthase. Mammalian COX is composed of three catalytic, mitochondrially encoded and ten regulatory, nuclear encoded subunits. The regulatory COX subunit IV plays an important role in adjusting energy production to cellular energetic requirements by binding of ATP to the N-terminus of subunit IV thereby causing an allosteric inhibition of COX activity at high energy level, i.e. high ATP/ADP ratio [1]. COX subunit IV exists in different isoforms (IV-1 and IV-2) [2]. While the isoform IV-1 is ubiquitously transcribed in all adult mammalian tissues including brain and heart, isoform IV-2 showed high transcription levels only in the lung. Therefore, we examined the transcription pattern of subunit IV isoforms in brain and heart tissue and in different cell types thereof under normoxic and hypoxic conditions. Besides the expression of COX isoform IV-1 in brain and heart cells under normoxia, we detected also low levels of mRNA transcripts for the COX isoform IV-2 in excitable brain and heart cells. The expression of COX VI-2 in neurons and cardiomyocytes points at a cell type specific expression of COX subunit IV isoforms in these two organs. Under conditions of oxygen deprivation mRNA transcription of COX IV-2 is further up-regulated in excitable cells, while COX IV-2 is induced in non-excitable cells of brain (astrocytes) and heart (fibroblasts). Increased expression of COX isoform IV-2 caused an abolition of the allosteric inhibition of COX by ATP at high energy levels as determined by polarographic measurements of solubilized mitochondria [3]. We conclude that the expression of COX isoform IV-2 in excitable cells and under hypoxia in non-excitable cells suppresses the sensitivity of COX to its allosteric regulator ATP and overrules the regulation of COX by the cellular energy level. This is paralleled by a higher vulnerability of cell types expressing COX IV-2 isoform, i.e. excitable cells as neurons and cardiomyocytes, and may contribute to the development of several brain and heart diseases during energy deprivation at high cellular activity. Supported by DFG (Emmy Noether-Program to SA) References: [1] S. Arnold, B. Kadenbach (1997) Cell respiration is controlled by ATP, an allosteric inhibitor of cytochrome c oxidase. Eur. J. Biochem. 249, 350-354. [2] M. Hüttemann, B. Kadenbach, L.I. Grossman (2001) Mammalian subunit IV isoforms of cytcohrome c oxidase. Gene 267, 111-123. [3] S. Horvat, C. Beyer, S. Arnold (2006) Effect of hypoxia on the transcription pattern of subunit isoforms and the kinetics of cytochrome c oxidase in cortical astrocytes and cerebellar neurons. J. Neurochem. 99, 937-951.

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MITOCHONDRIAL INHIBITION AND PROTECTION OF THE ISCHEMIC HEART

Charles Hoppel, Qun Chen, Edward Lesnefsky

Ischemia (ISC) and reperfusion (REP) damages the electron transport chain (ETC) impairing oxidative phosphorylation (OXPHOS). Reversible blockade of the ETC before ISC with amobarbital (AMO) protects OXPHOS. We used AMO treatment as an experimental tool to test the hypothesis that damage to OXPHOS occurs mainly during ISC rather than REP, and if so, if protection of OXPHOS during ISC decreased cardiac injury during ISC-REP. Langendorff-perfused Fischer 344 rat hearts were treated with AMO (2.5 mM bolus for 1 min immediately before ISC) or vehicle and underwent 25 min. 37°C global ISC. Subsarcolemmal (SSM) and interfibrillar (IFM) populations of mitochondria were isolated at end of 25 min ISC or at 30 min. REP to measure respiration. Time control hearts were perfused for 40 min without ISC. For the infarct study, 120 min. REP was used. H2O2 generation from isolated mitochondria was detected by the amplex red technique and infarct size by triphenyltetrazolium chloride staining. ISC decreased 2 mM ADP stimulated respiration that did not recover during REP. AMO-treatment preserved OXPHOS in both SSM and IFM during ISC and that

Table:

OXPHOS (nAO/min/mg)	Time control N=6	ISC N=5	AMO+ISC N=5	ISC+REP N=12	AMO+ISC+REP N=11
SSM	199±6	114±17*	217±16 †	129±5*	183±12 ‡
IFM	313±17	173±22*	387±21 †	184±10*	299±22 ‡
	LVDP (mmHg)	Infarct size (%LV)	H2O2 (pmol/mg/30 min) SSM		IFM
ISC+REP	57±4 (n=12)	32±2 (n=7)	227±57 (n=7)		132±50 (n=7)
AMO+ISC+REP	79±5 ‡ (n=11)	13±2 ‡ (n=7)	58±28 ‡ (n=7)		33±16 ‡ (n=7)

Mean ± SEM. * $P < 0.01$ vs. Time control; † $P < 0.01$ vs. ISC alone; ‡ $P < 0.01$ vs. ISC+REP alone.
Supported by: NIH POI AG15885 and VA Medical Research Service

persisted during REP. Thus, OXPHOS damage occurred mainly during ISC. In AMO-treated hearts with intact OXPHOS, H2O2 release from SSM and IFM during REP was markedly decreased vs. untreated hearts. AMO-treated hearts had improved recovery of left ventricular developed pressure (LVDP) and strikingly decreased infarct size. Thus, protection of mitochondria during ISC attenuates mitochondrial ROS production and decreases myocardial injury during ISC-REP.

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HYPOXIA-INDUCED METHANE GENERATION - MECHANISM AND FUNCTION

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Aerobic life depends on redox homeostasis, an integrating network of intracellular oxidative and reductive processes. The absence of oxygen as an electron acceptor leads to a redox imbalance with a decreased level of ATP generation and progressive functional and structural cell damage. Although oxidative stress and generation of reactive oxygen intermediates (ROI) could be an important factor in the acute response to hypoxia, the potential of antioxidants to prevent or cure ROI-induced diseases is limited and the intracellular biochemical mechanisms that mediate this injury are not completely understood. We have demonstrated that electrophilic methyl groups attached to nitrogen or sulfur moieties may become substitute electron acceptors in the lack of oxygen. If the sources of the methyl groups are molecules such as phosphatidylcholine the substitution will lead to the generation of methane. In line with these, we hypothesized that temporary oxygen deprivation in aerobic cells induces methane generation as a response to anoxia. We have investigated this hypothesis at four experimental levels: (1) in model chemical reactions with choline and its metabolites in the presence of oxygen free radical generation with hydrogen peroxide, catalytic iron and ascorbate, (2) in rat liver mitochondria and mitochondrial subfractions under hypoxic conditions, (3) in endothelial cell cultures with or without oxygen and (4) in anesthetized dogs after experimental vascular occlusion and reperfusion. Significant methane generation was demonstrated in all four series of experiments. Oral pretreatment of the animals with phosphatidylcholine decreased the exhaled methane concentration and also diminished the local formation of oxygen free radicals after tissue ischemia and reoxygenation. In conclusion, these studies have revealed that hypoxia-induced methane emission can be normalized by exogenous means. Far from being irrelevant to the aerobic metabolism, methane generation occurs in aerobic systems and may have an essential function in promoting correction of an abnormal rise in electron-donor activity.

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NNOS GOES NUCLEAR: NNOS- AND NADPH OXIDASE-DERIVED REACTIVE OXYGEN/NITROGEN SPECIES PROMOTE OXIDATIVE NUCLEAR DAMAGE IN ALVEOLAR EPITHELIAL CELLS

Rhykka Connelly, Frank Schmalsteig, Daniel Traber

Rationale: Emerging evidence implicates a role for angiotensin II (Ang II)-stimulated reactive oxygen and nitrogen species (ROS/RNS) formation in acute lung injury (ALI). However, details of the mechanism are lacking. We hypothesized that compartmentalized generation of superoxide (O_2^-) and nitric oxide ($^\bullet$NO) may be key events in the Ang II-stimulated progression of ALI.

Methods: Human alveolar epithelial (A549) cells were treated with Ang II in the presence or absence of the specific nNOS inhibitor N-propyl-L- arginine, or the specific NADPH oxidase inhibitor, apocynin, and observed for ROS/RNS generation, as well as localization of nNOS and the NADPH oxidase homologue, Nox4. A549 cells were further analyzed via western blot, or confocal microscopy, for poly (ADP-ribose) (PAR) polymer formation, an indicator of oxidative nuclear damage.

Results: Compared to unstimulated controls, Ang II transiently increased ROS/RNS production 7.4 fold, an effect blocked by nNOS or NADPH oxidase inhibition. $^\bullet$NO fluorescence could only be observed in the presence of apocynin, suggesting that Ang II-generated $^\bullet$NO immediately reacts with O_2^- generated by NADPH oxidase. These data suggest that spatial and temporal sub-cellular co-localization may be critical for Ang II-stimulated NADPH oxidase and nNOS product reactions. Indeed, Nox4 and nNOS transiently co-immunoprecipitate, and co-localize at the peri-nuclear region 15 minutes post Ang II stimulation. Subsequently, nNOS translocates to the nucleus, suggesting that nNOS may regulate nuclear signaling directly. Furthermore, PAR polymers, which are undetectable in resting conditions, were generated following Ang II stimulation, an effect blocked with apocynin or n-propyl arginine.

Conclusions: These data suggest Ang II causes nNOS and Nox4 to co-localize at the peri-nuclear region of A549 cells, where superoxide produced by Nox4, and $^\bullet$NO produced by nNOS immediately react to form peroxynitrite, which leads to subsequent nuclear oxidative damage as evidenced by increased PAR polymer formation. Furthermore, these experiments demonstrate an inflammatory-stimulated nuclear trans-localization of nNOS, which has important implications for direct $^\bullet$NO-mediated nuclear activities.

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BLUE LIGHT FACILITATES RECOVERY OF MITOCHONDRIAL RESPIRATION INHIBITED BY NITRIC OXIDE: RELEVANCE FOR ENDOTOXIC SHOCK

Peter Dungel

Objectives: Endotoxic shock is known to induce iNOS, which results in increased NO production. In turn NO inhibits mitochondrial respiration by binding to cytochrome oxidase. Our recent study showed that the release of NO from NO complexes with heme proteins can be facilitated by blue light. The aim of this study was to clarify whether blue light can restore respiration of mitochondria inhibited by NO in concentrations similar to those observed in vivo under endotoxic shock.

Material and Methods: The experiments were performed in rats subjected to LPS challange and liver mitochondria prepared from control rats.

Data: The levels of NO in the liver of rats subjected to endotoxic shock determined by means of electron spin resonance spectroscopy were in a mmolar range[1]. Addition of 25 μM of NO either to mitochondria or to liver homogenate isolated from control rats induced total inhibition of mitochondrial respiration. Irradiation by blue light (470 nm, 10 lm) of mitochondria inhibited by NO resulted in an immediate and total recovery of respiratory function of mitochondria. Green (530 nm) and red (629 nm) light was less efficient although these light sources had higher luminous intensity (30 and 44 lm, respectively). Interestingly, nitroglycerin (NG) also causes an inhibition of mitochondrial respiration but this inhibition was not sensitive to blue light.

Conclusion: Our data shows that mitochondrial respiration is inhibited by NO levels approx. 40 times lower than those measured in liver from rats with endotoxic shock which can be reversed with intensive light of short wavelength. The fact that mitochondria inhibited by NG are not sensitive to light suggests that in case of NG other than NO species inhibit mitochondrial respiration.

[1] - Kozlov et al. Vascular Pharmacol. 2005 43:411-414

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UNDERSTANDING THE SIGNIFICANCE OF INFLAMMATION, MITOCHONDRIAL DYSFUNCTION, AND OXIDATIVE STRESS IN INFECTIOUS CHAGASIC CARDIOMYOPATHY

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Chagas disease, an infectious cardiomyopathy caused by Trypanosoma cruzi, is a major public health threat in Latin America, Mexico, and the USA. Studies with experimental models and the endomyocardial biopsies from human patients in different clinical stages of Chagas disease have shown persistent myocardial inflammation during progressive disease development. The cytotoxic inflammatory mediators (e.g. $O_2^{\cdot-}$, NO) may affect the host cellular and organelle components important in maintaining the cardiovascular function, and thus, contribute to chagasic pathogenesis.

Using a murine model, we have found that infection by Trypanosoma cruzi elicits antioxidant/oxidant imbalance and mitochondrial dysfunction in the heart. Our data showed a substantial decline in respiratory chain complex (CI and CIII) activities and ATP content in the myocardium of infected mice. These metabolic alterations were associated with increased mtROS release, substantial oxidative insult of mitochondrial membranes and respiratory complex subunits, and >60% inhibition of mtDNA-encoded transcripts for respiratory complex subunits in infected myocardium. Towards understanding the importance of free radicals in eliciting cardiac oxidative damage and mitochondrial dysfunction, we have tested the effect of phenyl-N-tert-butyl nitrone (PBN), an antioxidant, on T. cruzi-induced responses. PBN arrested the oxidative stress-mediated loss in mitochondrial membrane integrity, preserved redox potential-coupled mitochondrial gene expression, and improved the respiratory complex activities and cardiac ATP level in infected myocardium. Importantly, PBN resulted in a $\geq$ 2-fold decline in mtROS release rate in infected myocardium. Subsequently, PBN-treated mice arrested the cardiac oxidative stress evidenced by a decline in cellular level of malonyldialdehydes, protein carbonyls and GSSG and enhanced antioxidant (GSH) status compared to infected/untreated mice. Taken together, our data suggest that oxidative injuries affecting mitochondrial integrity-dependent expression and activity of the respiratory complexes initiate a feedback cycle of electron transport chain inefficiency, resulting in increased ROS production in chagasic hearts. We will discuss the role of mtROS in sustenance of inflammatory reactions and oxidative stress-mediated responses in chagasic cardiomyopathy.

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FREE IRON DERIVED FROM HO-ACTIVITY IS A POSSIBLE SOURCE OF MITOCHONDRIAL DYSFUNCTION IN CRITICAL CARE DISEASES

Catharina Duvigneau

Objectives:. Activation of the heat shock protein HSP32 (heme oxygenase (HO-1)) accompanies a number of serious diseases including sepsis and hemorrhagic shock. One of the products of HO is Fe^{2+}, which multiplies damaging potential of reactive oxygen species catalyzing Fenton reaction. The aim of this study was to clarify pathophysiological potential of Fe^{2+}.

Material and Methods: Rats were challenged with lipopolysaccaride or subjected to hemorrhagic shock and sacrificed during first 12 hours after challenge.

Data: We observed that the expression of HO-1 was associated with increased levels of free iron. In endotoxic shock in rats "free" iron levels in liver (EPR-spectroscopy) were increased at 4h after endotoxin challenge. Expression of transferrin receptor (real-time reverse transcription polymerase chain reaction) was decreased, suggesting that the observed increase in "free" iron levels is not a consequence of increased iron uptake. Additionally the expression of ferritin and ferroportin, the iron exporter, was decreased, indicating that liberated ferrous iron from HO-1 activity is not sufficiently sequestered or removed from the cell and can therefore reach elevated levels within the cells. We have found a positive correlation between HO-1 expression and "free" iron levels and a negative correlation between HO-1 expression and respiratory function of mitochondria. In-vitro we have shown that within all products of HO-1 only free iron reduced the performance of mitochondria.

Conclusion: Our data suggest that up-regulation of HO-1, leads to a subsequent increase in intracellular free iron levels. The latter can be the source of mitochondrial dysfunction and consequently organ failure in a number of critical care diseases, like sepsis and hemorrhagic shock.

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EXOGENOUS EPIDERMAL GROWTH FACTOR IMPROVES INTESTINAL INTEGRITY IN SEPSIS

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Introduction: The intestine plays a central role in the pathophysiology of sepsis. Epidermal growth factor (EGF) is a potent cytoprotective peptide that exhibits trophic, maturational and healing effects on the intestinal mucosa. Exogenous administration of EGF has been shown to attenuate intestinal tissue damage and decrease mortality in animal models of ischemia/reperfusion and colitis. However, the role of EGF in sepsis is unknown.

Hypothesis: Exogenous administration of EGF preserves intestinal architecture and improves survival in sepsis.

Methods: FVB/N mice were subjected to 2x25-gauge cecal ligation and puncture (CLP) and treated post-operatively with or without EGF (twice daily intraperitoneal (i.p.) injections, 75 μg/kg per dose). Control mice underwent a sham laparotomy. Antibiotic therapy (12.5 mg/kg metronidazole + 25 mg/kg ceftriaxone, given i.p.) was initiated 3 hrs after CLP and a second dose was given 12 hrs later. Mice were sacrificed 24 hours after surgery. EGF receptor (EGF-R) localization and expression were assessed by immunohistochemistry and Western blot, respectively. Intestinal morphology was quantitatively evaluated in H&E stained sections by measuring villus length and crypt depth in 12 well-oriented crypt-villus units per animal (n=5-11 per group). Survival studies were performed in a separate cohort of mice (n=12 per group) and subjected to a more severe injury (2x23-gauge CLP) to detect differences in mortality. Mice were treated post-operatively with or without EGF (75 μg/kg i.p., twice daily for 7 days). Antibiotics were given as described above but were continued for a total of 48 hrs. Mice were followed for survival for 7 days.

Results: At 24 hrs, EGF-R was markedly increased by both immunohistochemistry and Western blot in the intestine of septic mice compared to sham mice. Exogenous administration of EGF resulted in normalization of EGF-R expression to similar levels seen in sham mice. Septic mice had significantly shorter villi compared to sham mice (215.2 μm ± 8.2 vs. 365.7 μm ± 20.4; $p<0.001$) and exogenous EGF resulted in normalization to sham villi lengths (352.5 μm ± 23.4 vs. 365.7 μm ± 20.4; p=ns). While crypt depths were significantly shorter in EGF treated mice compared to septic mice (64.5 μm ± 3.6 vs. 88.7 μm ± 3.7; $p<0.001$), the biological relevance of these results remains unclear. At 7 days, there was a trend towards decreased mortality, with 67% survival in mice treated with exogenous EGF compared to 33% survival in septic mice (p=0.06).

Discussion: The EGF/EGF-R axis is perturbed in the intestine of septic mice. Administration of exogenous EGF after the onset of sepsis preserves intestinal architecture to levels seen in sham animals. In addition, exogenous EGF is associated with a trend towards decreased mortality in sepsis. Thus, EGF may be a novel therapeutic agent for the treatment of sepsis, potentially by directly modulating the intestinal epithelium.

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RECONSTITUTION OF IMMUNOCOMPETENCE BY GM-CSF IN PATIENTS WITH SEVERE SEPSIS AND IMMUNOPARALYSIS

Christian Meisel, Jörg Schefold, Heidrun Zuckermann-Becker, Nina Polze, Tycho Baumann, et al

Objective: Despite modern intensive care medicine severe sepsis and septic shock remain a leading cause of mortality and morbidity in ICU units. While the initial stages of sepsis may be characterised by an overwhelming release of inflammatory mediators, many sepsis patients show signs of severe systemic immunodepression, also termed as immunoparalysis, during the later stages of disease. Immunoparalysis is characterised by impaired cell-mediated immune responses including diminished T-cell proliferation and reduced monocytic HLA-DR expression and cytokine secretion capacity. Patients with prolonged immunoparalysis have an increased suscepti-

bility to secondary infections and worse outcome, suggesting that these patients may benefit from immunostimulatory therapies. Restoration of monocyte function from sepsis patients with immunoparalysis by interferon-gamma or granulocyte-macrophages colony stimulating factor (GM-CSF) was demonstrated previously both in in vitro experiments and in small clinical pilot trials. We performed a prospective randomized, double-blind, placebo controlled clinical trial to further evaluate the effects of recombinant human GM-CSF (rhGM-CSF) on reversal of immunoparalysis and clinical outcome in a larger cohort of patients with severe sepsis or septic shock and immunoparalysis.

Data: Design: Prospective randomized, double-blind, placebo controlled study. Setting: Three adult intensive care units of a university hospital. Patients: 38 surgical and medical patients with severe sepsis or septic shock and immunoparalysis as defined by monocytic HLA-DR expression of less than 8.000 antibodies bound per cell (mAb/cell) for at least 48h prior to intervention. Intervention: Patients (19 per treatment arm) were randomly allocated to subcutaneous administration of a daily dose of either 4 ug/kg rhGM-CSF or placebo over a period of 8 days in addition to standard therapy. Drug dose was increased to 8 ug/kg on days 6 to 8 if monocytic HLA-DR expression on day 5 did not show normalization to values above 15.000 mAb/cell.

Primary Outcome: Sustained normalisation of monocytic HLA-DR expression as a measure of monocytic immunocompetence. Secondary outcomes: monocytic and lymphocytic cytokine secretion capacity, plasma pro- and anti-inflammatory markers (IL-6, TNFalpha, PCT, IL-10), disease severity and degree of organ failure determined by APACHEII and SOFA scores, 28-day mortality.

Data and Conclusion: Patient recruitment was started in September 2005 and ended in November 2006. The 28-day observation period for the remaining patients will be completed in December 2006. Data on primary and secondary outcome measures will be presented.

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EFFECT OF THE PEROXYNITRITE CATALYST WW-85 ON HEMODYNAMICS AND CEREBRAL MICROVASCULAR BLOOD FLOW IN OVINE SEPTIC SHOCK

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Objective: The production of nitric oxide (NO) and peroxynitrite ($ONOO^-$) typically contributes to the pathophysiology seen in septic shock. We hypothesized that administration of the novel $ONOO^-$ catalyst WW-85 may prevent these disturbances.

Material and Methods: Eighteen sheep (33-37 kg) were operatively prepared for chronic study (1) and randomly allocated (n=6 each) to 1) an uninjured and untreated sham group, 2) an injured control group, and 3) an injured and treated intervention group that received WW-85 intravenously administered 1 hr post injury, with an initial bolus of 0.1 mg/kg followed by a continuous infusion of 0.02 $mg \bullet kg^{-1} \bullet h^{-1}$ until the end of the 24-h experimental period. Control and WW85 animals were subjected to smoke inhalation and sepsis as previously reported (1). Sheep were resuscitated with Ringer's lactate solution and ventilated with 100% O_2 in awake state for 24hrs. Blood flow was measured with fluorescent microspheres.

Data: In the sham group, the cardiac index (CI, $L \bullet min^{-1} \bullet m^{-2}$), systemic vascular resistance index (SVRI, $dynes \bullet sec \bullet cm^{-5} \bullet m^{-2}$) and regional microvascular blood flow (mL/min/g tissue) in cerebral cortex, cerebellum, and medulla oblongata remained near baseline (BL) levels during the 24hr study period. In septic sheep, CI increased significantly (BL 5.6±0.4 vs. 24h 7.5±0.4) over time, and was associated with a significant drop in SVRI (BL 1412±90 vs. 24h 729±96) compared to sham and WW-85 treated animals ($p<0.05$). In the WW-85 group, the CI (BL 5.9±0.2 vs. 24h 6.2 ± 0.3) remained stable and SVRI did not fall to the same extent as in controls (BL 1241±50 vs. 24h 984±71). Regional blood flow in septic sheep significantly increased in cerebral cortex (175±20% of BL), cerebellum (190±30% of BL) and medulla oblongata (145±19% of BL, each $p<0.05$). Regional blood flow in WW85 treated septic animals remained at BL levels (cerebral cortex: 87±20%, cerebellum: 99±15%, and medulla oblongata: 87±14% of BL, $p<0.05$ each, compared with controls).

Conclusion: These results suggest that the peroxynitrite catalyst WW-85 may be useful to prevent cerebral microcirculatory disturbances triggered by NO and $ONOO^-$ in septic shock.

Reference: Murakami et al., Crit Care Med 2002;30(9):2083-90.

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THE ROLE OF ACTIVATED PROTEIN C AND ANTITHROMBIN III AS MODULATORS OF THE LPS-INDUCED INFLAMMATORY RESPONSE IN PBMCS OF HEALTHY VOLUNTEERS AND PATIENTS UNDERGOING MAJOR SURGERY

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Activated Protein C (APC) and Antithrombin III (ATIII) are not just physiologic inhibitors of the coagulatory cascade but furthermore influence inflammation. Objective of this experimental ex-vivo study was to assess the capacity of APC and ATIII to modulate the LPS-induced inflammatory response in human immune cells from

healthy volunteers and moreover, to observe if APC and ATIII would affect the LPS-induced inflammatory response in immune cells primed by tissue trauma following major surgery.

Mononuclear peripheral blood cells (PBMCs) were obtained from healthy volunteers (n=8) and patients undergoing abdominal surgery (n=8). Blood was drawn before surgery (-1) and day 1, 3, and 7 after surgery. After Ficoll separation, cells were cultured in serum-free medium AIM V and were pre-incubated with either physiologic (APC 4µg/ml, ATIII 1IU/ml) or supraphysiologic (APC 100µg/ml, ATIII 20IU/ml) doses of APC and ATIII, respectively. After 60 minutes of incubation, bacterial lipopolysaccharide (LPS, 1µg/ml) was added and stimulated for 20h. We measured LPS-induced Tumor Necrosis Factor alpha (TNF) and Interleukin 10 (IL10) in culture supernatants by use of Bioplex Suspensions Assay System. Data was analyzed by Analysis of Variance (ANOVA) or non-parametric test (T-test, Mann-Whitney-Test) as appropriate. Statistical significance was accepted at $p<0.05$.

Without LPS-stimulus, there were no levels of cytokine detectable. In healthy volunteers, LPS-induced TNF and IL10 were significantly depressed in presence of ATIII (20IU/ml). Surgical patients showed similar effect in LPS-induced TNF-levels on day 1 and 7 after surgery. LPS-induced IL10 levels in surgical patients showed a trend towards reduction, however without reaching statistical significance. ATIII at 1IU/ml had no effect. There was no effect of APC on the inflammatory response of PBMCs under serum-free conditions.

Our data indicates that ATIII in supraphysiologic conditions is capable to significantly reduce proinflammatory (TNF) and antiinflammatory (IL10) cytokine production in PBMCs from healthy volunteers. This effect is not altered by surgery. However, APC has no effect on the inflammatory response as far as investigated in our model. It is possible that APC requires a co-factor no available under serum-free conditions. In conclusion we suggest, that anti-inflammatory effects of APC as observed in clinical studies is not likely to be result of immunomodulatory effects of this substance.

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THERAPEUTIC EFFECTS OF NEUROPEPTIDES IN EXPERIMENTAL SEPSIS BY INHIBITING HMGB1 RELEASE

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Objective: Several data suggest that HMGB1 is a necessary and sufficient late mediator of sepsis. Ghrelin (GHR), urocortin (UCN), and vasoactive intestinal peptide (VIP) are three neuropeptides with endogenous anti-inflammatory activities and therapeutic effects on autoimmune and inflammatory diseases. The aim of this study was to investigate the effect of GHR, UCN, and VIP in experimental sepsis and the involvement of HMGB1 secretion in their potential therapeutic action.

Material and Methods: Endotoxemia and sepsis were induced in BALB/c mice by intraperitoneal (i.p.) injection of LPS (100 µg/mouse), and by cecal ligation and puncture (CLP), respectively. Vehicle (controls), GHR (5 nmol), UCN (5 nmol) or VIP (5 nmol) were administered i.p. at time of LPS injection or beginning 12 or 24 h after CLP, survival was monitored and serum collected. Murine peritoneal and RAW 264.7 macrophages were cultured with LPS and different concentrations of the neuropeptides in serum free medium (Opti-MEM). HMGB1 content in serum from septic mice and macrophage culture supernatants were assayed by Western blotting.

Data: The administration of GHR, UCN or VIP 12h after CLP attenuates serum HMGB1 levels and increases survival in experimental sepsis (control = 35.3%; GHR = 100%; UCN = 88,2%; VIP = 67%). GHR, UCN, and VIP specifically down-regulate HMGB1 secretion by endotoxin activated macrophages. In addition, treatment with GHR reduced the number of peritoneal bacteria in vivo. In vitro, GHR exhibits bactericidal activity. Finally, GHR, UCN, and VIP protect from sepsis lethality even if they are administered 24h after CLP.

Conclusion: The present report describes GHR, UCN and VIP as new physiological inhibitors of HMGB1 secretion, and GHR as a neuropeptide with antimicrobial properties. The therapeutic window observed in our study suggests that GHR, UCN, and VIP have a significant therapeutic potential for the treatment of sepsis.

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PREVENTION OF SIRS-RELATED LOSS OF CARDIAC FUNCTION BY MEANS OF THE LEUKOCYTE INHIBITION MODULE (LIM), AN EXTRACORPOREAL IMMUNE THERAPY DEVICE

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Objective: The leukocyte inhibition module (LIM) has been shown to limit overshooting neutrophil activity after trauma and large surgical interventions. In a porcine model for aseptic SIRS, we now studied whether early inhibition of neutrophil hyperactivity by LIM may prevent the loss of posttraumatic cardiac function.

Material and Methods: German landrace pigs (40-45 kg) underwent immune stimulating extracorporeal circulation (ECC) analogous to the heart-lung machine without (group I; n=5) or with LIM (group II; n=5) connected to

the arterial line of the ECC. The cardiac indices (CI) and cardiac function (endsystolic pressure volume relationship; ESPVR) were analyzed pre and post ECC with a Swan-Ganz catheter and the cardiac function analyzer. The left ventricular outflow tract systolic acceleration (LVOTacc) was measured by echocardiography.

Data: Pigs in group I developed neutrophil hyperactivity and SIRS-like syndrome. The CI were significantly reduced post ECC (3.26 ± 0.31 l/min/m^2) versus pre ECC (4.05 ± 0.45 l/min/m^2; $p<0.01$). In contrast, in group II, the CI was only slightly reduced post ECC versus pre ECC (3.86 ± 0.49 versus 4.21 ± 1.32 l/min/m^2; $p=0.23$). Between groups, CI significantly differed post ECC ($p<0.05$). Pre-load independent ESPVR values in group I were significantly lower post ECC (1.57 ± 0.18) versus pre ECC (2.32 ± 0.41; $p<0.001$). In group II, posttrauma decrease of ESPVR was not significant (post ECC: 1.93 ± 0.16; pre ECC: 2.19 ± 0.24; $p=0.08$). The analysis of the LVOTacc confirmed the beneficial effects of LIM on the pre-load independent left ventricular (LV) contractility ($p<0.01$). The intergroup analyses showed a significantly better cardiac function in group II versus group I ($p<0.05$). In conjunction with the cardiac function data, the neutrophil activity (cytokine secretion and chemotactic activity) was significantly reduced in group II versus group I ($p<001$).

Conclusion: The inhibition of neutrophil activity by LIM has been shown to prevent ECC-related SIRS and impairment of cardiac contractility and function. LIM ought to be regarded as an important tool to improve the outcome of patients with risk to develop SIRS.

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ETANERCEPT ATTENUATES THE DEVELOPMENT OF CERULEIN-INDUCED ACUTE PANCREATITIS IN MICE. A COMPARISON WITH TNF-A GENETIC DELETION

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TNF-α plays a pivotal role in the pathogenesis of acute pancreatitis. Recent studies have shown that TNF-α inhibition significantly ameliorates the course of experimental acute pancreatitis, but in this context the effects of Etanercept, a novel anti TNF-α agent, have not been investigated so far. The aim of the present study is i) to assess the effects of pharmacological inhibition of Tumor Necrosis Factor (TNF)-α by means of Etanercept on the inflammatory response and apoptosis in a murine model of necrotizing acute pancreatitis; ii) to compare the results to those observed in TNF-α receptor 1 knockout (TNFR1-KO) mice . Necrotizing acute pancreatitis was induced in TNF-α wild type for TNFR1 (WT) and TNFR1-KO mice by intraperitoneal injection of cerulein (hourly x5, 50 μg/kg). In another group of WT mice Etanercept was administered (5 or 10 mg/kg subcutaneously) at 1 hour after first cerulein injection. Control groups received saline treatment. After 24 hours, biochemical, histological and immunohistochemical evidences of acute pancreatitis developed in all cerulein-treated mice; apoptosis was also present in the pancreas. Contrarily, pancreatitis histological features, amylase and lipase levels, pancreas water content and MPO activity were reduced in a similar degree in Etanercept treated and TNFR1-KO mice. Likewise, in these two groups immunohistochemical stainings and TUNEL assay were found negative. TNF-α receptor 1 gene deletion and Etanercept administration ameliorate the course of experimental acute pancreatitis in a similar degree. Future studies on clinical applications of Etanercept in pancreatitis seem promising.

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MECHANISM OF THE SALUTARY EFFECTS OF FINASTERIDE ON IMMUNE RESPONSE FOLLOWING TRAUMA-HEMORRHAGE: UPREGULATION OF ESTRADIOL SYNTHESIS

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In the last decade, a number of studies have provided evidence for a gender dimorphism in host defence following trauma. Under stress conditions such as trauma-hemorrhage, androgenic hormones have immunosuppressive effects leading to increased susceptibility to sepsis, morbidity and eventual mortality. Testosterone is converted by 5α-reductase to 5α-dihydrotestosterone (DHT), a more potent androgen for depressing immune responses. We hypothesized that administration of Finasteride, a 5α-reductase inhibitor; will improve immune function following trauma-hemorrhage. To determine this, male C3H/HeN mice (8-10 weeks) were randomly assigned and either treated with Finasteride or vehicle, and then subjected to trauma-hemorrhage or sham operation. Trauma-hemorrhage was induced by a midline laparotomy and approximately 90 min of hemorrhagic shock (blood pressure 35 mmHg), followed by fluid resuscitation (four times the shed blood volume in the form of Ringer's lactate). Animals were sacrificed two hrs after resuscitation or sham operation and Kupffer cells were isolated. Plasma levels and Kupffer cell production of cytokines (TNF-α, IL-6, IL-10, MCP-1, KC and MIP-1α), lung neutrophil infiltration and edema were evaluated. Finasteride administration prevented the increase in plasma cytokine levels and Kupffer cell cytokine production following trauma-hemorrhage. In addition, neutrophil infiltration and edema formation in the lung were reduced by finasteride. Furthermore, Finasteride administration increased plasma 17β-estradiol concentrations. No adverse effect of finasteride was observed in sham animals. These results suggest that inhibition of DHT synthesis has salutary effects on the posttraumatic immune response which are associated with increased

Data/Results:

	C5aR Flow	C5aR++ Flow	C5aR Micro	C5aR++ Micro
Resting	100	100+/-1	100	100+/-2
1 min C5a	65+/-4	*95+/-8	-	-
5 min C5a	45+/-2	*108+/-20	72+/-20	#121+/-24
10 min C5a	36+/-3	*85+/-2	44+/-16	#126+/-15

plasma estradiol levels. The increased estradiol levels most likely upregulates estrogen receptors (as observed following the administration of estradiol) and thus produces salutary effects on immune responses following trauma-hemorrhage (supported by USPHS grant R01 GM37127).

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PHYSIOLOGIC HYPEROSMOLARITY INHIBITS C5A ENDOCYTOTIC SIGNALING POST-RECEPTOR IN NEUTROPHILS

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Objective: C5a is an integral part of the complement system and causes activation and sequestration of granulocytes and is an important early effector in trauma. Hypertonicity has been shown to inhibit macrophage responsiveness to endotoxin and diminish reactive oxygen species generation in neutrophils, yet this mechanism has yet to be elucidated. Many chemotactic messengers transduce their message via G-protein-coupled receptors (GPCRs), like C5a, where internalization of the receptor is a critical step of the signaling process. We hypothesize that physiologic hyperosmolarity attenuates C5a-activation of PMNs via inhibition of C5a receptor endocytosis.

Materials and Methods: PMN were isolated (>95% purity) by dextran sedimenatation, Ficoll-Hypaque gradient centrifugation, and hypotonic lysis. In vitro experiments were performed in isotonic Krebs Ringer dextrose phosphate buffer, pH 7.35 (KRDP) or hyperosmolar KRDP (180 mmol Na+) at 37°C and stimulated by human recombinant C5a [$1x10^{-7}$M]. Surface expression of the C5a receptor (C5aR) was measured by flow cytometry (5000 events/sample). Digital microscopy was performed on fixed PMNs, and the statistics from these images are representative of 25 cells per image/treatment group. Western blots were completed on whole cell lysates and cytosolic Ca^{2+} flux was measured in indo-1 loaded PMNs using a spectrofluorimeter.

Sodium hyperosmolarity preserved C5aR expression on human PMNs (++) following C5a stimulation (1, 5 10 min) compared to C5a stimulation of buffer-treated PMNs (*$p<0.05$). Microscopy confirmed the inhibitory effect of hypertonicity on C5a-induced receptor endocytosis and shape change as compared to normotonic-treated PMNs (#$p<0.05$). Superoxide anion production was inhibited 95± 5% in ++ cells post C5a stimulation. Western blot analysis of whole cell lysates from ++ cells post C5a displayed significantly less phospho-p38 MAPK activation ($p<0.05$). However, hyperosmolarity did not alter the C5a-mediated Ca^{2+} flux.

Conclusion: Hyperosmolar-treated PMNs evidenced marked decreases in C5a induced 1) p38 MAPK activation, 2) C5aR endocytosis 3) shape change, and 4) activation of the NADPH oxidase. However the C5a mediated cytosolic Ca^{2+} was unaffected indicating that the ligand-receptor binding was similar in both groups and suggesting that hyperosmolarity interferes with post-receptor, downstream, signal transduction rather than an alteration of the C5a:C5aR interaction.

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SELECTIVE INHIBITION OF PLATELETS BY THE GPIIB/IIIA RECEPTOR ANTAGONIST TIROFIBAN REDUCES LEUKOCYTE-ENDOTHELIAL-CELL-INTERACTION IN MURINE ANTIGEN-INDUCED ARTHRITIS

Oliver Gottschalk, Oliver Gottschalk, Philip Metz, Volkmar Jansson, Marcus Schmitt-Sody

Introduction: Inflammation is associated with the invasion of leukocytes into affected tissues and with the upregulation of platelet activation and adhesion. Assuming that leukocyte accumulation is linked to platelet aggregation in affected endothelium, the aim of our study was to examine the effects of selective platelet inhibition by the glycoprotein (GP) IIb/IIIa receptor antagonist Tirofiban on the leukocyte-endothelial cell interaction.

Materials and Methods: We used an established model of antigen-induced arthritis (AiA) to induce inflammatory changes in the synovial microcirculation. Ex vivo labelled platelets and in vivo fluorescence-labelled leukocytes were visualized by means of intravital microscopy (IVM). C 57/Bl6 mice were allocated to 4 groups; 2 control groups with saline or Tirofiban and 2 groups with AiA that also received either saline or Tirofiban (0,5 µg/g BW) intravenously. The severity of arthritis was assessed by changes in the transverse knee joint diameter.

Results: There was no significant change in platelet- or leukocyte-endothelial cell interaction in the endothelium

in healthy control animals, regardless of whether saline (platelets: rolling: 0.066±0.01; adherence: 189.7±40.1 mm^{-2}; leukocytes: rolling: 0.098±0.13; adherence: 521.3±148 mm^{-2}) or Tirofiban (platelets: rolling: 0.048±0.01; adherence: 148.9±24.7 mm^{-2}; leukocytes: Rolling: 0.088±0.02; adherence: 412.1±74.3 mm^{-2}) was injected. In contrast, after selective inhibition of platelets by the use of Tirofiban, the platelet (rolling: 0.152±0.03; adherence: 700.6±140.9 mm^{-2})-and leukocyte (Rolling: 0.161±0.04; 915.1±168 mm^{-2})-endothelial cell interaction was significantly reduced in arthritic mice compared to AiA animals treated with saline (platelets: rolling: 0.277±0.02; adherence: 1809.1±239.3 mm^{-2}; leukocytes: rolling: 0.288±0.02; adherence: 1491.8±283.5 mm^{-2}) and reached the level of the healthy control groups.

Conclusion: Selective platelet inhibition by Tirofiban resulted in reduced leukocyte-endothelial cell interactions in AiA. Consequently, platelets contribute to leukocyte adhesion in AiA via GP IIb/IIIa and therefore platelet inhibition could potentially become an additional option in the therapy of chronic arthritic disease.

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STANDARD VS. HIGH VOLUME ADMINISTRATION IN EXPERIMENTAL SEPSIS: EFFECTS ON REGIONAL BLOOD FLOWS AND JEJUNAL IN-VITRO MOTILITY

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Introduction: Gut dysfunction during sepsis promotes multiorgan failure (MOF). Goal-oriented therapy has resulted in both improved and worsened outcome in different patient groups. The aim of this study was assess the effects of sepsis in combination with standard vs. high fluid administration on regional abdominal blood flow and in-vitro jejunal motility.

Materials and Methods: After abdominal exploration and regional flow probe fitting, 30 anesthetized and mechanically ventilated pigs were randomized to 24 hours of peritonitis (P) and standard (10 ml/kg/hour, n=6) or high fluid administration (15 ml/kg/hour, n=5), endotoxin infusion (E) and standard (n=5) or high fluid administration (n=5) or placebo infusion (P) and standard (n=5) or high fluid administration (n=4). Cardiac output was measured by thermodilution and superior mesenteric (SMA) and celiac trunk (CT) blood flow by Doppler ultrasound. After 24 hours, jejunal tissue was removed and effects of acetylcholine (ACH) and sodium nitroprusside (SNP) on jejunal strips was assessed in organ chambers. ACH and SNP effects were quantified as integral under the curve during 2 minutes after drug exposure and expressed as percentage from baseline.

Data: Cardiac output increased in all septic groups and in controls during high volume administration (all $p<0.05$). Regional blood flows did not change in the control groups while fractional SMA- and CT-flow decreased in all septic groups. ACH- induced jejunum contractility was similar in all groups (but NO-induced relaxation was attenuated in both E and P groups and similarly with standard and high volume administration (C:84±10, P:92±11, E:93±7; p= 0.023)

Conclusions: In this experimental model sepsis did not decrease acetylcholine-induced jejunum motility but impaired NO-induced jejunum relaxation. The amount of fluid administration did not alter the responses.

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INHIBITION OF ENDOGENOUS HYDROGEN SULPHIDE FORMATION PROTECTS THE LIVER FROM SCALD BURN INJURY IN THE RAT

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Hydrogen sulphide (H_2S) is a naturally occurring gas with important roles both in physiology and disease. Cystathionine-g-lyase (CSE) and cystathionine-b-synthase use L-cysteine as substrate to produce H_2S. The role of H_2S in inflammation is beginning to emerge. Recent evidence has suggested that endogeous H_2S plays a known role in the pathophysiology of cerulean-induced pancreatitis, hemorrhagic shock, endotoxemia, and in local inflammation. The liver and kidney are particularly susceptible to remote organ injury following scald burn injury, wich is associated with multiple organ failure. Here we investigate the effects of a CSE inhibitor, dl-propargylglycine (PAG 100 mg/kg, i.p., administered 5 min before burn injury and 3 hours after injury), on the liver and kidney injury caused by skin burn. Male Wistar rats were divided in 3 groups (n=11 each): (i) one receiving a 60% total body surface area scald burn (99°C for 10 seconds) under general anesthesia; (ii) a second group pre-treated with PAG 5 min before receiving a 60% total body surface area scald burn and 3 hours after, also under general anesthesia; and (iii) a sham group not subjected to burn injury. Anesthesia was maintained throughout the experiment and rats were sacrificed 6 hours after initial injury and blood samples were collected for the determination of biochemical markers of hepatic and renal injury. Target organ samples for wet/dry weight ratio determinations and ligh microscopy were also collected. Our results show that burn injury induced a statistically significant increase (vs. Sham) in the serum levels of AST (771±131 vs. 283±31 IU/l), ALT (234±83 vs. 70±11 IU/l), creatinine (1.5±0.2 vs. 0.48±0.028 mg/dl)), and urea (94±4 vs. 37±2.1 mg/dl). Serum levels of AST (294±39 IU/l) and ALT (294±39 IU/l) were significantly reduced in animals treated with PAG. The same was not observed for the kidney (creatinine 2.01±0.14 mg/dl, urea 103±4 mg/dl). In conclusion, H_2S might play a significant role in the pathology of liver injury associated to burn scald injury, but this mediator might have an endogenous protective role in the kidney, which seems to be abolished by PAG treatment.

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TARGETED DRUG DELIVERY TO INFLAMMATION SITES

Sandra Reichstetter, Zdravka Medarova, Anna Moore, Geraldo Castillo, Alexei Bogdanov, Elijah Bolotin

Protected Graft Copolymers (PGC) is a new drug delivery technology developed by PharmaIn, that allows reversible binding of therapeutic molecules such as small molecules, peptides, proteins, and others to our unique nanocarriers for use in vivo. Each PGC carrier is able to bind several therapeutic molecules in a single carrier via a metal bridge or hydrophobic core interaction. This technology provides new drug characteristics including protection from degradation in the serum and due to the size of the carrier from excretion through the kidney, thus extending the in-vivo half-life of a drug significantly. Most importantly, the PGC-carriers accumulate in areas of increased vascular permeability and therefore allow to concentrate drugs in sites of inflammation. This makes PGC formulations a useful tool for the delivery of a multitude of molecules. We show the extension of the half-life using the incretin hormone GLP-1 as an example. GLP-1 in its unprotected native form is subject to degradation by a serum protease called DPPIV and excretion through the kidney which leads to a half-life of under 5 min. The PGC-bound protected GLP-1 has a half-life, depending on the species, of 10 to over 20 h. Due to the size of the carrier, PGC-nanocarriers accumulate in the tissue in sites of increased vascular permeability, leading to exciting possibilities of delivering immunomodulating and other drugs directly into the area of inflammation. Here, we demonstrate the targeting of the PGC-drug formulations to sites of inflammation with MRI imaging agents loaded to the PGC in two different models of inflammation. In a rat model of experimental infection, the imaging agent accumulates in the side of infection over a 24 h period. In another model of inflammation, streptozotozin-induced diabetes in mice, the imaging agent accumulates in the inflamed pancreas over a 17 h period after injection.

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HUMORAL AND CELLULAR MUCOSAL IMMUNE RESPONSES IN CRITICAL ILLNESS

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Although advances have been made in our knowledge of the pathology of sepsis and the co-morbid development of multiple organ dysfunctions, much remains to be understood about the septic process if we are to develop better therapies. In this respect, mucosal surfaces represent not only important sites of nutrient exchange with the outside environment, but also one of the primary barriers (innate defense) to the microbial world (varying from indigenous to pathogenic populations). Thus, maintenance of these processes (barrier function and competent capacity to respond to a diversity of antigenic challenges) requires a competent humoral/cellular system. However, following the onset of sepsis mucosal immune responsiveness, as defined by changes in the ability to produce IgA (humoral) as well as a decline in cell mediated responsiveness (decreased Th1 cytokine release and/or CD3-induced proliferation). This is associated with the onset of marked apoptosis in both immune and non-immune cells and increase in gut permeability. The pathological significance of the apoptotic changes is evidenced by the capacity of agents, which block Fas-FasL (death receptor) signaling as well as, by the gut epithelial cell restricted over expression of Bcl-2, to not only limit mucosal cell death but also protect the animal from septic morbidity. The significance of the loss of T-cell immune responsive arm is indirectly demonstrated by the observation that in the genetic absence (knockout) of γδ-T-cells (a subpopulation of T-cells found in the highest frequency within the murine mucosa) mice more readily succumb to lethal effects of septic challenge (Chung CS, et al [2006] Amer J. Physiol. 291:R1338). Because this T-cell subpopulation is thought be uniquely positioned to contribute to both early innate as well as late adaptive/cell mediated immunity and can express antigens like FasL as it is activated; we speculate that it may not only serve as a sentinel of change (pathogenic challenge), but also contribute to maintenance of mucosal barrier homeostasis, via its communication/interaction with antigen presenting cells and non-professional immune cells, like the epithelia. (Supported by NIH GM-46354 & GM-53209).

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EFFECTS OF FEVER AND HEAT SHOCK ON EPITHELIAL CELL FUNCTIONS AND SURVIVAL IN ACUTE LUNG INJURY

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We have previously demonstrated that whole body febrile-range hyperthermia (FRH) activates the heat shock (HS) response, accelerates pathogen clearance, and increases collateral tissue injury. The ultimate effect of FRH on survival depends on the nature and site of infection or injury. Our previous work has identified the lung as one site that is particularly susceptible to FRH-augmented collateral tissue injury. In diffuse lung injury models, FRH increases expression of neutrophil-attracting CXC chemokines, augments neutrophil recruitment, and worsens lethal lung injury. We have identified two important effects of FRH on pulmonary epithelium that contribute to augmented lung injury. First, immuno-

histochemistry analysis demonstrated that the respiratory/bronchiolar epithelium is the predominant site of CXC chemokine expression in mice co-exposed to intratracheal bacterial lipopolysaccharide (LPS) and FRH. In the mouse respiratory epithelial cell line, MLE15, TNF?-induceded secretion of the CXC chemokines, KC, LIX, and MIP-2, was increased two- to four-fold by co-exposure to HS (42°C for 2h). In the human respiratory epithelial-like cell line, A549, and in primary cultured human small airway epithelial cells, TNF?-induced expression of the human CXC chemokine, IL-8, was also amplified by co-exposure to HS. Using the A549 model, we showed that HS increased IL-8 transcription through a novel mechanism in which HS-activated transcription factor-1 (HSF-1) co-activates IL-8 transcription by binding to at least two HSF-1 binding sequences in the IL-8 promoter. In the LPS-challenged mouse model, augmentation of CXC chemokine expression by FRH was abrogated in HSF-1 knockout mice, indicating that HSF-1 plays a similar role in FRH augmented CXC chemokine expression in the mouse lung. In addition to its effects on chemokine expression, co-exposure to FRH and intratracheal LPS also caused extensive bronchiolar epithelial death that was out of proportion to the increased neutrophil burden. Co-exposing MLE15 cells to 39.5°C or HS (42°C for 2h) greatly augmented TNFα-induced caspase-3 activation and apoptosis. This effect is profoundly different than the cytoprotection conferred when exposure to HS precedes exposure to cytotoxic agents by several hours. The mechanisms of these effects are under investigation. In summary, we have identified two important effects of FRH and HS on pulmonary epithelium that contribute to the susceptibility of the lung to FRH-augmented collateral injury. These results demonstrate that the HS response, generally believed to be cytoprotective, exerts effects that may contribute to lung injury.

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THE ROLE OF NF-KAPPAB IN CRITICAL ILLNESS: WHAT WE HAVE LEARNED FROM ANIMAL MODELS

Timothy Blackwell

The NF-κB pathway impacts a number of key biological processes, including innate immunity, through transcriptional regulation of target genes. Innate immunity is critical for host defense against bacteria and other pathogens, but dysregulated or exaggerated immune responses can result in tissue injury. Investigations using transgenic and knockout mice have helped to shed light on aspects of the NF-κB dependent inflammatory response that regulate host defense and tissue injury. Studies have shown that the timing, intensity, and duration of NF-κB signaling are critical determinants of tissue injury. Inhibition of NF-κB activation has differential effects on host defense and tissue injury depending on the particular cells and tissues that are targeted. Despite much progress, further pre-clinical investigations are needed to identify treatment strategies that limit tissue injury while preserving key host defense functions.

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INHIBITION OF NUCLEAR FACTOR KAPPA B DURING INFLAMMATION: WHEN AND HOW?

John W. Christman

The basic molecular biology of the NF-kB activation pathway is well described, and approaches to modify this axis have involved inhibition of various components of the classical activation pathway, including ubiquitination and proteosomal degradation of IkB. Recently, there have been detailed characterizations of molecular mechanisms that involve reversible post-translational modification of RelA, including phosphorylation and acetylation that might be amenable to therapeutic interdiction. Alternately DNA decoy, antisense and siRNA technologies that interfere with NF-kB binding and inhibition of gene expression, respectively, of NF-kB proteins have been employed in experimental settings, but this has not been practically or effectively applied in human disease. A very promising approach is inhibition of inhibitory kappa B kinases (IKK) since these appear to be highly specific for the NF-kB activation pathway and amenable to conventional small molecule pharmaceutical approaches. The timing and magnitude of these interventions is of great concern because of the necessity of the NF-kB pathway for normal host defense against nosocomial pathogens.

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IS NF-KAPPAB ALWAYS A THERAPEUTIC TARGET DURING CRITICAL DISEASES?

Uwe Senftleben

There is a plethora of evidence that activation of the NF-kappaB pathway is a critical proximal step in the development of stress answers and, moreover, fatal complications during critical diseases such as sepsis. A pathophysiological concept describes increased NF-kappaB-induced transcription of pro-inflammatory target genes as key mechanism of systemic hyperinflammation. Thus, specific inhibition of NF-kappaB signaling is a promising therapeutic approach to suppress hyperinflammation and, presumably, the development of septic shock and multiple organ dysfunction syndrome (MODS). In this context it has to be noted that signal transduction via the NF-kappaB pathway is not only crucial for the control of pro-inflammatory immune responses. It also plays a precisely defined role during programmed cell death. Mainly considered as pro-survival transcription factor it targets a number of anti-apoptotic genes. Moreover,

prevention of TNFalpha-induced programmed cell death is a hallmark of classical NF-kappaB signalling. It occurs in different cell types such as murine embryonic fibroblasts, hepatocytes and Jurkat cells. Activation of the canonical NF-kappaB pathway, however, also prevents TNFalpha - induced lymphocyte apoptosis in vivo. It is well known that sepsis induces extensive lymphocyte apoptosis that contributes to immunosuppression and mortality. Sepsis is characterized by systemic hyperinflammation due to cytokine overproduction. Thus, TNFalpha-induced anti-apoptotic NF-kappaB signalling may play a decisive role in the context of lymphocyte apoptosis during sepsis. Thus, therapeutic inhibition of NF-kappaB is a double edged sword. On the one hand, NF-kappaB inhibition might be associated with increased apoptotic rates of immune cells such as lymphocytes. As a result, immunosuppression might occur. On the other hand, it was demonstrated that regular activation of NF-kappaB in leukocytes is necessary to resolve the inflammatory process in vivo. Hence, inhibition of NF-kappaB activation might result in prolonged inflammation. It was further shown in a murine ischemia-reperfusion model that complete inactivation of canonical NF-kappaB signaling in enterocytes prevents the typical systemic inflammatory response. However, in the same model local NF-kappaB inactivation resulted in severe apoptotic damage to the reperfused intestinal mucosa. Nevertheless, NF-kappaB is an attractive target for anti-inflammatory therapeutic interventions in the field of critical care medicine. Especially during the initial phase of the inflammatory response it might be appropriate to block excessive NF-kappaB activation. In contrast, during phases of anergy or anti-inflammatory responses it most likely is not reasonable to inhibit NF-kappaB signaling. However, it should be emphasized that adequate inhibition of NF-kappaB signaling probably requires routine monitoring of NF-kappaB activity to prevent adverse effects of complete inactivation. The development of such routinely applicable methods as well as the evaluation of specific NF-kappaB inhibitors during certain diseases is an exciting field of future research.

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TREATMENT WITH ANTI-GITR AGONISTIC ANTIBODY CORRECTS T CELL DYSFUNCTION AND IMPROVES SURVIVAL IN MURINE SEPSIS

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Objectives: Reversal or prevention of apoptosis in cells of the adaptive immune system can improve outcome in sepsis. In this study, we wished to characterize the defects in the adaptive immune system and examine whether improving adaptive immune system function via costimulation of T cells can improve outcome to sepsis, similarly to blocking apoptosis.

Materials and Methods: Female C57Bl/6 or DO11.10 T cell receptor transgenic mice underwent cecal ligation and puncture (CLP) or sham laparotomy. One hour before surgery, some mice were given 500 μg of anti-glucocorticoid inducedTNF receptor (GITR) antibody At the time of surgery, some B6 mice were pre-bled and immunized with nitrophenyl keyhole limpet hemocyanin (NP-KLH) in alum or NP-Ficoll to measure NP-specific T cell dependent (NP-KLH, 10 days later) or T cell independent (NP-Ficoll, 7 days later) antibody production. DO11.10 mice were immunized with Ovalbumin peptide 323-337 (Ova) in incomplete freud's adjuvant to measure antigen specific T cell proliferation (7 days later). In another experiment, sham or CLP B6 mice were euthanised 24 hours after sepsis to determine the presence of antigen non-specific T cell dysfunction. CD4+ T cells were isolated using Miltenyi microbeads and were restimulated with irradiated APCs and anti-CD3/CD28 (1 day after sepsis) or Ova (7 days after sepsis). Media was analyzed for cytokines using Luminex 10-plex assay and proliferation was measured using 3H-thymidine incorporation.

Data: We found that immunization with the T cell dependent antigen NP-KLH and alum at the time of sepsis causes a decrease in NP-specific immunoglobulin (IgM and IgG2a) production when compared to sham mice, whereas immunization with the T cell independent antigen NP-Ficoll causes increased NP-specific antibody production (IgM and IgG3). These data indicate that CD4+ T cells are most likely dysfunctional in sepsis. Next, we found that CD4+ T effector cells from septic mice display decreased nonspecific proliferation to anti-CD3/CD28 stimulation one day after sepsis as well as decreased specific proliferatiion to Ova 7 days after sepsis. Furthermore, septic DO11.10 T cells produced dramatically less IL-2 and interferon-γ when restimulated with ovalbumin ex vivo, but showed no increase in IL-4, IL-5, or IL-10 production. Treatment of mice with anti-GITR antibody one hour before sepsis restored T cell proliferation, improved NP-KLH specific class switching to both IgG1 and IgG2a isotypes, and improved sepsis survival by ~25-30% in normal mice, but not in mice depleted of CD4+ T cells.

Conclusions: These data indicate that sepsis induces dramatic CD4+ T cell dependent adaptive immune system dysfunction and correction of this dysfunction can improve sepsis survival. Costimulation through GITR may be a useful strategy to prevent adaptive immune dysfunction in sepsis.

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DAMAGE TO ENDOPLASMIC RETICULUM BUT NOT TO MITOCHONDRIA IS A POSSIBLE MECHANISM FOR ENDOTOXIN INDUCED LIVER FAILURE

Andrey Kozlov

Objectives: This study aimed at determination of subcellular compartment(s) responsible for liver dysfunction in a model of endotoxic shock in rats.

Material and Methods: Rats were challenged with lipopolysaccaride (at approx. LD_{50} dose) and sacrificed after 16 hours; blood, liver, microsomal and mitochondrial fractions of liver were analyzed.

Data: We observed damage to liver cells resulted in focal necroses and elevated alanine-aminotransferase levels in blood. Simultaneously, we observed a drastic decrease in monoxygenase activity of P450 indicating damage to endoplasmic reticulum (ER), while respiratory activity of mitochondria was even better than in controls. Using 2D electrophoresis we found dramatic changes in the ER proteome. In contrast, a previous study had shown very modest changes in mitochondrial protein patterns. Main changes had been a significant up-regulation of two proteins, mitochondrial SOD and ATP-synthase [1]. Transmission electron microscopy examination of liver showed no remarkable changes in the quantity and morphology of mitochondria, but significant decrease in quantity and damage to ER. The latter was particularly pronounced in ER located in close vicinity to mitochondria. Using electron spin resonance spectroscopy we have shown that mitochondria isolated from LPS treated animals had significantly increased reactive oxygen species (ROS) generation, preferentially in complex 1. In spite of excessive mitochondrial ROS generation mitochondria were not damaged probably due to protection by up-regulation of mitochondrial SOD. In contrast, significant morphological and biochemical injury was found in endoplasmic reticulum, which was not protected.

Conclusion: Our data suggest that damage to ER may be an important mechanism causing liver dysfunction induced by endotoxic shock.

[1] - Miller et al. FEBS Lett. 2006 580:1257-62.

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THERMAL INJURY AND SEPSIS CAUSE AN INCREASE IN MONOCYTES THAT ARE THE MAJOR PRODUCERS OF TNF

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Hematopoiesis is responsible for the development of myeloid progenitor cells that differentiate into monocytes and macrophages. The objective of our studies was to determine if burn or sepsis leads to a shift or different phenotype of monocyte or macrophage. For these studies, an 18% scald burn was used for the thermal injury model and cecal ligation and puncture was used for the sepsis model. Animals from both models were euthanized 8 days after treatment and spleen cells isolated for FACS analysis.

Thermal injury increased some, but not all of the macrophage subpopulations in the spleen. $CD11b^{-}F4/80^{+}$ macrophages were not altered by the burn injury. In contrast, a four-fold increase in $F4/80^{-}$ $CD11b^{+}$, cells was determined (5.5% vs 22.5%). Further analysis of the $CD11b^{+}$ population indicated that a significant increase in $LY6C^{+}$ PMN (2% vs 8%) and $LY6C^{++}$ monocytes (5% vs 17%) occurred after thermal injury. Additional experiments examining TNF production by different monocyte/macrophage subpopulations clearly showed that inflammatory $CD11b^{+}$ $LY6C^{++}$ monocytes were the cells most capable of expressing TNFα in the post burn spleen. Over 90% of these cells stained for TNFα, and these cells had the highest TNFα MCF of any cell type. A similar pattern was displayed in the sepsis model. $CD11b^{-}F4/80^{+}$ macrophages were not altered by sepsis. However, a significant 2.8% vs. 37.7 % increase was found for $F4/80^{-}CD11b^{+}$ cells. Sepsis significantly increased $LY\text{-}6G^{-}$ LY-6C $^{+}$ (monocytes) from 2.8% to 29.7%. Sepsis significantly increased $LY\text{-}6G^{+}$ LY-6C $^{+}$ (neutrophils) 4.9% to 13.3%. Sepsis significantly increased GR-1dim $CD11b^{+}$ (monocytes) from 5.6% to 16.1%. A significant total TNF production 2.3% vs 13.5% correlated with the increase in $CD11b^{+}$ cells. These monocytes were the cells most capable of expressing TNFα in the post CLP. In conclusion, burn and sepsis significantly increased monocytes but not macrophages. The increased monocytes are responsible for increased TNF production after thermal injury or sepsis.

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GENETIC POLYMORPHISMS OF NOD1 AND IL-8 BUT NOT POLYMORPHISMS OF TLR4 GENES ARE ASSOCIATED WITH HELICOBACTER PYLORI-INDUCED DUODENAL ULCER AND GASTRITIS

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Objective: Intracellular pathogen receptor Nod1 is involved in the epithelial cell sensing Helicobacter pylori, (HP) which results in a considerable IL-8 production. The aim was to evaluate the relationship between Nod1 and IL-8 genetic polymorphisms and the development of H. pylori – induced gastritis and duodenal ulcer (DU), as compared with the TLR4 polymorphisms.

Material and Methods 85 patients with DU and 135 patients with gastritis were enrolled in the study. Seventy five HP-positive subjects without gastric or duodenal disease served as controls. The G796A (E266K) Nod1 polymorphism was determined by RFLP, and the – 251 IL-8 polymorphism by ARMS method. The TLR4 (ASP/299/Gly and Thr/399/Ile) gene polymorphisms were examined by melting point analysis.

Data: AA homozygote mutant variants of NOD1 were detected in 20 % of the HP-positive patients with DU vs 7 % of HP-positive patients with gastritis and vs 6 % of the HP positive healthy controls. The IL-8 heterozygote mutant variant was detected with a significantly higher frequency among the DU patients and those with gastritis than among the HP - positive healthy subjects. However,

no significant correlation concerning the frequency of the TLR4 gene polymorphism could be revealed between any group of patients and the controls.

Conclusion: E266K CARD4/NOD1, but not the TLR4 gene polymorphism increases the risk of peptic ulceration in HP-positive patients. The -251 IL-8 polymorphism was significantly associated with either gastritis or DU in HP infected subjects. Host factors including intracellular pathogen receptors and IL-8 production play an important role in HP-induced gastric mucosal damage.

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POST-TRANSCRIPTIONAL REGULATION OF ICAM-1 EXPRESSION BY P38 MAPK PATHWAY IN HUNAM MICROVASCULAR ENDOTHELIAL CELLS: THE ROLE OF HSP27

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Heat shock protein 27 (HSP27) phosphorylation is involved in p38 MAPK-mediated cellular responses, such as cytoskeleton remodeling and cell migration. Previous studies suggest that the p38 MAPK/HSP27 pathway modulates endothelial inflammatory response. The expression of adhesion molecules in lung microvascular endothelial cells is critical in neutrophil recruitment and lung injury. However, the role of the p38 MAPK/HSP27 pathway in lung microvascular endothelial cell expression of adhesion molecules remains to be determined. The objective of this study was to determine the effect of the p38 MAPK/HSP27 pathway on TNF-induced ICAM-1 expression in human lung microvascular endothelial cells (HMVECs). Methods and results: HMVECs in culture were stimulated with TNF (10 ng/ml) for a varied period of time for the analysis of ICAM-1 mRNA, total cellular ICAM-1 and cell surface ICAM-1. TNF stimulation induced rapid activation of p38 MAPK and phosphorylation of HSP27. Inhibition of p38 MAPK abolished HSP27 phosphorylation, and reduced total and cell surface ICAM-1 protein levels. However, inhibition of p38 MAPK did not affect ICAM-1 mRNA levels following TNF stimulation. Treatment with HSP27 siRNA reduced cellular HSP27 levels and resulted in attenuated HSP27 phosphorylation following TNF stimulation. However, knockdown of HSP27 had a minimal influence on TNF-induced ICAM-1 expression.

Conclusions: The results suggest that the p38 MAPK pathway modulates ICAM-1 expression in HMVEC through a post-transcriptional mechanism. While HSP27 phosphorylation is a result of p38 MAPK activation, the amount of total and phosphorylated HSP27 is not critical in the post-transcriptional regulation of ICAM-1 expression by the p38 MAPK pathway.

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SITE-SPECIFIC IS MODIFIED BY INJURY DNA-BINDING ABILITY OF HNF-4

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Objective: The injury response is a complex and regulated process. Hepatocyte nuclear factor 4a (HNF-4a), a Liver Enriched Transcription Factor, is involved in the transcriptional regulation of many liver-specific genes which are altered during the liver's acute phase response. Previous studies have shown that injury reduced HNF-4a binding. To define whether injury induced changes in HNF-4a binding ability are binding site specific, we studied the effect of injury on HNF-4a binding to known HNF-4a binding sites in genes whose transcription is changed after injury.

Material and Methods: Utilizing a cytokine stimulated acute phase response in HepG2 cells, we characterized and compared the effect of injury on the binding activity of HNF-4a to the HNF-4a sensitive genes, a1-antitrypsin (a1-AT), transthyretin (TTR), classic positive and negative acute phase proteins, and apolipoprotein B (ApoB). HepG2 cells were treated with a cytokine mixture of IL-1, IL-6 and TNF-a over time. Electrophoretic mobility-shift (EMSA) and chromatin immunoprecipitation (ChIP) assays were used to detect injury-induced changes in HNF-4a binding activities. The specific HNF-4a binding sites found in the promoter regions of a1-AT, TTR and ApoB were utilized and analyzed.

Data: The HNF-4a binding sites from the a1-AT, TTR and ApoB genes were all capable of binding HNF-4a in untreated cells. Cytokine treatment revealed a different pattern of response that was specific for each site. The binding capacity to the TTR binding site was rapidly and significantly reduced in a time-dependent manner, while the ApoB site only showed a slight decline at later time points (18 hours), and the a1-AT site exhibited a small increase at early time points followed by a minor drop from baseline at 18 hours. To further confirm our EMSA results in vivo, ChIP assays were performed. HepG2 cell extracts (cytokine untreated and treated) were immunoprecipitated with HNF-4a antibody, and the amount of the immunoprecipitated DNA fragments containing bound HNF-4a to their promoters was amplified by PCR for these three genes. Cytokine treatment substantially reduced the amount of HNF4a bound to TTR chromatin and slightly reduced the HNF-4a bound to the a1-AT and ApoB chromatin. These results were consistent with our EMSA findings.

Conclusion: HNF-4a is capable of binding to sequence specific sites present in the a1-AT, TTR and ApoB gene promoter regions. Cytokine treatment causes the modification of HNF-4a binding ability. The degree of modification appears to be specific to the HNF-4a binding site in a given gene promoter. This represents a novel mechanism by which the injury response could specifically regulate the transcriptional output of the liver through injury-induced alteration in HNF-4a specific binding abilities that are site specific.

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JAM-A MEDIATES LEUKOCYTE TRANSMIGRATION DYNAMICALLY AND IN A STIMULUS-SPECIFIC MANNER

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JAM-A is a transmembrane protein expressed on endothelial and epithelial cells as well as on platelets and leukocytes. Several in vitro and in vivo studies have implicated JAM-A in the transmigration of leukocytes, though not all published data have been consistent.

In the present study, leukocyte rolling, adherence, and transendothelial migration were analyzed in the cremaster muscle of anaesthetized C57BL/6 mice in response to different inflammatory stimuli including LTB4, IL-1β, and ischemia-reperfusion (I/R). Migration parameters were obtained in wild-type (WT) and JAM-A-/- mice (n=6 each group), respectively, using near-infrared-reflected light oblique transillumination microscopy.

Whereas no differences were detected in numbers of rolling and adherent leukocytes among groups, I/R- as well as IL-1β-elicited leukocyte transmigration was significantly attenuated in JAM-A-/- mice as compared to WT mice (70.3±0.2% and 48.5±0.3% inhibition, $p<0.05$). In contrast, JAM-A-deletion did not affect leukocyte transmigration after stimulation with LTB4. Additionally, migration parameters were assessed in mice lacking endothelial JAM-A (eJAM-A). In response to I/R and IL-1β, leukocyte transmigration in eJAM-A-/- mice was significantly reduced to the level of JAM-A-/- mice (59.7±0.2% and 51.1±0.2% inhibition). Finally the role of endothelial JAM-A for transmigration of leukocytes was evaluated in chronological sequence. Whereas leukocyte transmigration was significantly decreased in eJAM-A-/- mice after 4h of stimulation with IL-1β, these effects were abolished after 24h of stimulation.

In conclusion, these in vivo data demonstrate that i) JAM-A mediates transendothelial migration of leukocytes in a stimulus specific manner, ii) within the model employed, endothelial JAM-A dynamically regulates the leukocyte transmigration process.

Supported by EU NoE MAIN (LSHG-CT-2003-502935) and DFG (GRK 438)

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VASCULAR SMOOTH MUSCLE CELL CALCIUM SENSITIVITY IS DECREASED DURING LIPOPOLYSACCHARIDE-MEDIATED INFLAMMATION

Yves Ouellette

Sepsis is associated with microvascular hyporeactivity to agonists. Vasoconstriction in resistance arteries involves both elevation of smooth muscle intracellular Ca^{2+} levels and sensitization of the contractile myofilaments to the intracellular Ca^{2+} increase. Whether the hyporesponsiveness to vasoconstrictors seen in sepsis is reflective of reduced Ca^{2+}-sensitivity within smooth muscle cells remains unclear. Our hypothesis is that impaired vasoreactivity during LPS-mediated inflammation is associated with decreased Ca^{2+} sensitivity in small mesenteric resistance arteries (SMRA). SMRAs (190-220 μm) isolated from LPS-treated (15 mg/kg ip, 18 hours) and saline-treated control mice were mounted on a pressure myograph, superfused, and loaded with fura-2. Arteriolar diameter and global intracellular Ca^{2+} were simultaneously measured. Smooth muscle Ca^{2+}-sensitivity of SMRAs was assessed by stepwise increases in extracellular Ca^{2+} (0 to 2 mM) under depolarizing conditions (120 mM K^+). SMRA isolated from mice treated with LPS demonstrated hyporesponsiveness to Ca^{2+}. Extracellular mediated Ca^{2+} vasoconstriction in intact vessels resulted in an increase in EC_{50} (0.23 ± 0.01 mM vs 0.39 ± 0.03 mM*) and a reduction in E_{max} (42.8± 1.1% vs 26.6 ± 3.4%*). Removal of the endothelium resulted in near normal response to Ca^{2+}. In intact vessels, LPS treatment decreases vascular smooth muscle Ca^{2+} sensitivity as compared to controls. NOS inhibition (L-NNA, 1 μM) restores responsiveness to Ca^{2+} without restoring Ca^{2+} sensitivity. LPS-induced inflammation results in NO-independent decrease in Ca^{2+}-sensitivity within vascular smooth muscle cells.

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THROMBIN-ACTIVATABLE FIBRINOLYSIS INHIBITOR (TAFI) IMPAIRS HOST DEFENSE IN GRAM-NEGATIVE SEPSIS CAUSED BY BURKHOLDERIA PSEUDOMALLEI

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Objective: Thrombin-activatable fibrinolysis inhibitor (TAFI) has been implicated as an important negative regulator of the fibrinolytic system. Melioidosis is a severe infection caused by the Gram-negative bacterium Burkholderia pseudomallei that is endemic in SE-Asia. We aimed to characterize the expression and function of TAFI in sepsis caused by B. pseudomallei.

Material and Methods: Subjects: Patients with culture proven sepsis caused by B. pseudomallei, and male wild-type (WT and TAFI gene-deficient mice (TAFI KO) C57BL/6 mice intranasally infected with a lethal dose of B. pseudomallei.

Data: (1) 34 patients with septic melioidosis demonstrated strongly decreased TAFI antigen plasma levels when compared to healthy controls. Patients who survived from this debilitating disease showed normalization of their TAFI levels after successful treatment. However, no correlation with outcome was seen. (2) To determine the role of TAFI in the host defense against B. pseudomallei, we intranasally inoculated TAFI KO and WT mice with a lethal dose of B. pseudomallei. TAFI KO mice displayed significantly less bacterial outgrowth in their lungs, liver and blood compared to WT mice. This corresponded with a distinctly decreased inflammation (cytokine profiles, histopathology) and a markedly increased survival of TAFI KO mice. Additional experiments with mice infected with lethal doses of the Gram-negative bacterium Klebsiella pneumoniae or the Gram-positive bacterium Streptococcus pneumoniae did not show any differences in the inflammatory response between TAFI KO and WT mice, highlighting the unique role of TAFI in sepsis caused by B. pseudomallei.

Conclusion: Together these data indicate that TAFI is (1) downregulated in sepsis caused by B. pseudomallei and (2) plays a detrimental role in the inflammatory response to this severe infection.

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EXTRACELLULAR HYDROLYSIS OF SPHINGOMYELIN IN PATIENTS WITH SYSTEMIC INFLAMMATION AND ORGAN FAILURE

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Background and Objectives: Recent data indicate that bioactive lipids such as ceramide (Cer) have significant effects on cells relevant for inflammation, which may therefore be regarded as candidate mediators in systemic inflammatory response syndrome (SIRS). In pts with sepsis, Cer concentration in mononuclear cells has been identified as a possible marker to predict multiple organ failure. In order to elucidate the role of ceramide formation in the development and progression of systemic sequelae of SIRS, we addressed the question whether there is a difference in the sphingolytic activity of the secreted isoform of sphingomyelinases (pSMPD1) in plasma of pts with various degrees of SIRS or sepsis of different origin.

Methods: Plasma samples were obtained from pts with SIRS after off pump coronary bypass surgery through a mini-left anterior thoracotomy (MIDCAB), after CABG using an extracorporeal circuit (cardiopulmonary bypass - CPB) as well as from pts with sepsis. Activity was determined by the hydrolysis of fluorescently labelled sphingomyelin, plasma presence of the enzyme was identified by immuno blotting. In endothelial cells exposed to patient plasma, we determined the rate of sphingomyelin hydrolysis and the resulting Cer pattern by chromatographical separation as well as the formation of Cer enriched macro domains by fluorescence microscopy.

Results: Plasma activity of sphingomyelinase (pSMPD1) in samples of critically ill patients (median 262.3 pmol/(ml*h)) were significantly higher than those of age matched controls (median 123.6 pmol/(ml*h); $p < 0.005$). In pts with fatal outcome (n=7) sphingolytic activity increased during the study period (+ 77.4 pmol/(ml*h)), while a decrease in the subgroup of sepsis survivors was observed (- 252.1 $p < 0.02$). Low and high pSMPD1 activity levels were paralleled by equally low or high values of established clinical severity markers such as SOFA score or proCT value. Comparing the absolute increase of pSMPD1 activity 24 hours after either MIDCAB surgery or CABG with CPB we found the increase of enzyme activity to be significantly lower in both the MIDCAB group as well as in patients without SIRS at the first day post op. Beyond immunological detection of increased pSMPD1 in septic pts, we found an increase in breakdown of sphingomyelin in endothelial cells after stimulation with pts plasma as well as endotoxin or prototypic pro-inflammatory cytokines such as TNF-alpha. Also we found formation of ceramide enriched macro domains by immuno-staining using specific antibodies directed against Cer, CD14, Fas-receptor and TNF-Receptor1.

Conclusion: Here we demonstrate an increased pSMPD1 activity in pts with SIRS and sepsis. Together with data from in vitro experiments and animal models, the results provide the first demonstration of a bio-functional relevant activity of pSMPD1 resulting in an altered signal transduction in systemic inflammation. The association with sepsis related mortality suggests that increased pSMPD1 activity may be involved in the complex network of processes finally resulting in an unfavourable outcome.

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FOCAL ADHESION KINASE EXPRESSION AND ACTIVATION IN INTESTINAL INFLAMMATION

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Objective: Focal adhesion kinase (FAK) is a nonreceptor protein tyrosine kinase that plays an important role in a number of biologic functions, including cellular proliferation, motility, and survival. Recent data suggest a role for FAK in the intestinal epithelial cell's response to inflammatory mediators. Induced FAK expression has

been linked to suppression of programmed cell death (apoptosis) via NF-kB expression in an in vitro model of intestinal epithelial cells; additionally, FAK has been identified as an important component of adhesion disassembly through relationships with Src and myosin light chain kinase (MLCK). Given these findings, we hypothesize that FAK plays a central role in intestinal epithelial cell function in response to sepsis and inflammation, and aim to further characterize this role.

Material and Methods: Human intestinal epithelial cells (CaCo-2, ATCC, Rockville, Maryland, USA) were grown three to five days past confluence under standard conditions. To demonstrate the response of FAK to various inflammatory mediators, time-response curves were generated: at time 0, cells were stimulated with 100 ng/mL human tumor necrosis factor-alpha (hTNF-a), E. coli 0111:B4 lipopolysaccharide (LPS), or purified P. aeruginosa flagellin (flagellin). At 2, 6, 12, 24, 36, 48, and 72 hours following stimulation, cells were harvested and lysates prepared for immunoblotting. To demonstrate the response of FAK to varying doses of inflammatory mediators, dose-response curves were generated: at time 0, cells were stimulated with hTNF-a, LPS, and flagellin in increasing concentrations from 0 to100 ng/mL. Eight hours after stimulation, cells were harvested and lysates prepared for immunoblotting.

Data: Following stimulation with high dose (100 ng/mL) hTNF-a, LPS, and flagellin, a dramatic and sustained increase in FAK phosphorylated at tyrosine 397 was seen, indicating activation of FAK. This activation persisted for 72 hours. Concomitantly, total FAK levels demonstrated only slight changes. When stimulated with increasing doses of hTNF-a, LPS, and flagellin, a dose-dependent increase in FAK phosphorylated at tyrosine 397 was also seen. Again, total FAK levels demonstrated only minimal changes.

Conclusion: Focal adhesion kinase is activated in intestinal epithelial cells by known inflammatory agents, including hTNF-α, LPS, and flagellin. This response is rapid (within two hours) and sustained (present at 72 hours following stimulation). Importantly, the response generated is also dose-dependent. Further elucidation of the specific pathways of FAK activation and their ultimate outcomes should provide insight into the function and behavior of the intestinal epithelium in infection and inflammation.

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EFFECT OF TEMPERATURE ON PROTEIN BINDING OF CORTISOL

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Biological effects of cortisol are substantially determined by protein binding of the hormone. Some small previous studies suggest a decreased protein binding of cortisol in serum associated with temperatures above 37°C. The aim of our study was to further characterize temperature effects on cortisol protein binding by use of an equilibrium dialysis method. Serum samples obtained from ten healthy volunteers were submitted to equilibrium dialysis. Each one sample from each individual was incubated for 16 hours at 37°C, 38°C, 39°C, 40°C and 41°C, respectively. In the dialysate samples obtained, cortisol concentrations were measured by immunoassay in order to characterize unbound serum cortisol concentrations present in the incubated samples. For samples incubated at 37°C, a mean dialysate cortisol concentration of 0.41 ug/dL (SD 0.14) was found. Gradual increase of dialysate cortisol concentration was observed with increasing incubation temperatures. For samples incubated at 41°C, a mean dialysate cortisol of 0.75 ug/dL (SD 0.24) was found. Thus, the mean percentage of free-to-total cortisol increased by about 80% from 3.7% (SD 1.1) at 37°C to 6.7% (SD 1.8) at 41°C. The results of our in vitro experiments suggest that during fever the free-to-total ratio of cortisol is increased substantially compared to normal conditions, and that administration of antipyretic drugs is probably associated with a considerable decline in the bioavailability of cortisol.

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THE PROBLEM: COAGULOPATHY OF POSTTRAUMATIC MASSIVE TRANSFUSION (PMT)

Frederick Moore, Bruce McKinley, Zsolt Balogh

Advances in trauma care in the 1980's [e.g. trauma center triage, Advance Trauma Life Support (ATLS), damage control surgery & goal directed intensive care unit (ICU) resuscitation] allowed patients to survive exsanguinating hemorrhage. In the mid 1990's as a result of aggressive application of these strategies, an epidemic of abdominal compartment syndrome (ACS) emerged. In the late 1990's we conducted a series of prospective studies aimed at defining the epidemiology of postinjury ACS. We showed that ACS was an early modifiable link in the multiple organ failure (MOF). Its clinical trajectory is declared soon after trauma center (TC) arrival in patients with ongoing severe bleeding who require hemorrhage control interventions. ACS can be accurately predicted upon ICU admission (roughly 6 hours after TC arrival). Despite conventional wisdom patients with impending ACS (i.e. severe intra-abdominal hypertension without secondary organ dysfunction) do not respond well to boluses of isotonic crystalloid to increase pre-load. In fact, modest volume loading can precipitate full blown ACS. To minimize the occurrence of what we believe is largely an iatrogenic complication; we implemented fundamental changes in the early care of patients who present with severe bleeding. The primary emphasis is early identification and control of ongoing bleeding. As part of this effort, we developed a PMT protocol in which fresh frozen plasma (FFP) was started after patients had received six units of packed red blood cells (PRBC's) and thereafter FFP was administered at a ratio of 1 unit

per 1 unit of PRBC. This was based on the traditional teachings that posttraumatic coagulopathy develops over time because of worsening acidosis, hypothermia, hemodilution and consumption of factors. We recently analyzed a prospective database which included 97 patients who had entered into our PMT protocol and survived long enough to be entered into our ICU shock resuscitation protocol. All patients (age = 39+/- 2 years, ISS=29+/-1, 73% blunt mechanism) required hemorrhage control interventions. Mean emergency department INR was 1.8 +/- 0.2. Despite appropriate application of our PMT protocol (pre ICU PRBC's = 12 +/- 1 unit and FFP = 5 +/- 0.4 units), initial ICU INR was 1.6 +/- 0.1. Prolonged INR upon ICU admission was shown to correlate well with subsequent mortality. With standardized ICU care associated hypothermia and acidosis quickly normalized (by 4 and 8 hours respectively) but INR did not (despite patients receiving FFP and PRBC's at a ratio of 1 to 1). These PMT patients required substantial ongoing blood transfusion (10+/- 1 units of PRBC's in ICU) and 29 (30%) subsequently died. Causes of death included 5 exsanguinations, 5 early refractory hypoxemia from ARDS, 18 late MOF and 1pulmonary embolus. In conclusion, these data indicate that coagulopathy associated with PMT was present upon TC admission and did not correct despite adherence to the pre ICU PMT and ICU protocols. We believe that more aggressive pre ICU FFP to correct coagulopathy will decrease PRBC requirements and improve outcome. By our current protocol, we start FFP as soon as we recognize the need for PMT and are currently working on developing prediction models and/or bedside testing to assist with early decision making concerning when to implement "damage control resuscitation" (see Dr. Holcomb's abstract).

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VALUE OF FIBRINOGEN IN TRAUMA BLEEDING

Herbert Schoechl

The final step of the coagulation process is the formation of fibrin from its precursor fibrinogen. The resulting fibrin monomers undergo polymerisation to form an insoluble fibrin clot. Activated platelets express glycoprotein receptors IIb/IIIa on their surfaces which have a high affinity for fibrinogen. So it plays an important role in platelet aggregation. Fibrinogen concentrations of less than 1g/l are generally insufficient to curtail blood loss in massive bleeding. Low fibrinogen concentrations in trauma are associated with:

Blood loss: Hippalla et al observed that fibrinogen deficiency develops earlier than any other haemostatic abnormality. After a blood loss of 142% of the total blood volume, plasma fibrinogen decreased to the critical level of 100mg/dl. (2) In severe trauma fibrinogen dropped from 1,6g/l in the field to less than 0,9g/l on arrival in the emergency room. (4)

Dilution: As hypovolemic shock is primarily treated with crystalloids and colloids a dilution of the remaining coagulation factors is common. Colloidal volume replacement may also impair fibrin polymerisation. (1)

Consumption: In severe tissue trauma huge amounts of fibrinogen are consumed. Martini et al measured simultaneously the synthesis and breakdown of fibrinogen in a pig model. They found that after moderate hemorrhagic shock, fibrinogen breakdown was accelerated, but synthesis remained unchanged. (5)

Hyperfibrinolysis: tPA binds to clot-bound fibrin resulting in local generation of plasmin from plasminogen. In severe trauma this localisation can be lost resulting in systemic fibrinolysis. The consequence is a degradation of both fibrin polymers and circulation fibrinogen.

Acidosis: In a pig model, Martini et al observed a decrease of approximately 20% in fibrinogen concentration shortly after induction of acidosis. The underlying mechanism can not be explained by a reduced synthesis rate. Altered sequestration or increased degradation of fibrinogen is more likely.(6)

Fibrinogen replacement options

Fresh Frozen Plasma (FFP): The concentration of fibrinogen in FFP is only around 2,5 g/l and it requires 20 to 30 minutes of thawing prior to use. Large quantities are necessary to achieve significant increases in the plasma levels of coagulation factors. Only high volumes of FFP could increase the concentrations of coagulation factor, in particular fibrinogen.(2).

Cryoprecipitate: Cryoprecipitate is recommended when fibrinogen falls below the critical level of 1g/l. The dose is 2ml/kg and one unit should increase the fibrinogen level by 0,1g/l. It does not contain a consistent standardized quantity of fibrinogen and can not be virally inactivated.

Fibrinogen Concentrates:

Fibrinogen concentrates are immediately available and contain a well-defined concentration of fibrinogen. It has a good safety profile with respect to transmission of infections diseases. To increase the plasma fibrinogen concentration by 1g approximately 3g of fibrinogen concentrate is necessary.

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MASSIVE TRANSFUSION PROTOCOL-CIVILIAN EXPERIENCE

David Hoyt

The definition of the patient at risk for coagulopathy varies from institution to institution and a protocol for massive transfusion of red cells and repletion of coagulation factors also is highly variable. The recognition that coagulopathy of trauma is present at the time of admission is increasingly being identified, but historically coagulation factor repletion has been incremental based on laboratory testing. This is further augmented by the fact that historically blood banking has separated coagulation components and restoration of the equivalent of "fresh whole blood" has been challenging.

Traditionally, the focus in civilian trauma has been to restore coagulation factors in an incremental fashion in the patient with generalized micro vascular oozing. The first focus is reversal of hypothermia as this is known to be a common problem following massive Bleeding and transfusion. In the patient who has received greater than ten units of packed red cells or whole blood and has a platelet count less than hundred thousand treatment of four to six units of platelets or restoration of the platelet count to a greater than a hundred thousand is generally the second step. Measurement of the pro time and if greater than 1.5 should be accompanied by restoration of FFP and if the fibrinogen level is less than 100 milligrams percent treatment with cryoprecipitate should be considered.

Recent data has suggested that early treatment with FFP maybe be accompanied by reduced coagulopathy and as such many civilian trauma center protocols have moved toward earlier use of FFP based on the number of units transfused. There is clear evidence that early characterization of a high risk patient and earlier repletion of coagulation of factors are accompanied by a reduction of complications and mortality, but further research in this area is warranted.

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AGE AND RAGE IN MACROVASCULAR DISEASE

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Diabetes is associated with the accumulation of advanced glycation end products and increased expression of RAGE. At sites of diabetes-associated vascular injury, there is a high level of expression of AGE receptors that co-localize with AGEs and other atherogenic mediators that have been implicated in the development and progression of atherosclerosis.

It has been postulated that the interactions of AGEs with the receptor RAGE trigger the expression of extracellular proteins such as fibronectin, type I and type IV collagen via induction of profibrotic cytokines and growth factors including TGF-b1 and CTGF. In addition, the AGE-RAGE interaction promotes inflammatory pathways. In previous studies we have demonstrated increased macrophage infiltration, chemokine expression and phenotypic transdifferentiation of cells in plaques of diabetic apoE KO mice.

Administration of soluble RAGE, inhibition of AGE accumulation with an inhibitor of AGE accumulation or the cross link breaker alagebrium significantly reduce plaque area in association with reduced expression of RAGE and markers of inflammation and fibrosis in a model of diabetes accelerated atherosclerosis, the diabetic apoE KO mouse. It has also been shown that interventions that reduce vascular AGE accumulation are able to reduce established atherosclerosis, a setting more relevant to the clinical situation.

More recently, we studied the effects of the deletion of RAGE on the development of macrovascular disease in a murine model of type 1 diabetes, in diabetic RAGE (-/-)-apoE (-/-) null mice. The mice were rendered diabetic with streptozotozin and followed for 10 and 20 weeks of diabetes. The diabetic apoE (-/-) mice developed complex atherosclerotic lesions after 20 weeks of diabetes. Diabetic RAGE (-/-)-apoE (-/-) mice had significantly reduced plaque area when compared to the diabetic apoE (-/-) mice. There was also a consistent reduction in the extent of atherosclerosis in the non-diabetic RAGE (-/-)-apoE (-/-) mice when compared to non-diabetic apoE (-/-) mice. In the diabetic mice, the deletion of the RAGE also appeared to attenuate diabetic kidney injury, as assessed by a reduction in mesangial matrix and reduced glomerulosclerosis. This study demonstrates attenuated macrovascular disease in diabetic RAGE (-/-)-apoE (-/-) mice. These studies extend the rationale for RAGE antagonism as an effective therapeutic approach in combating diabetic micro- and macrovascular complications, presumably as a result of interruption of key inflammatory and pro-fibrotic pathways.

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AGE AND RAGE IN DIABETIC NEUROPATHY

Angelika Bierhaus

The molecular mechanisms underlying pain and loss of pain perception in diabetic neuropathy are poorly understood. Experimental animal models recently provided evidence, that reduced detoxification of advanced glycation end products (AGEs) by the glyoxalase system, engagement of the receptor RAGE and RAGE dependent sustained activation of the proinflammatory transcription factor NF-κB might significantly contribute to functional deficits in diabetic neuropathy. Glyoxalase-I controlled reactions are important in determining pain in mouse models of diabetes, and in controlling apoptosis in dorsal root ganglia. Consistently, RAGE-mediated suppression of glyoxalase-I expression and activity contributes to increased pain in early phases of diabetic neuropathy. In long-standing diabetes, however, AGE-accumulation, RAGE-ligation and RAGE mediated sustained NF-κB-activation reduces nociception and impaires neuronal blood-flow. Consistently, the diabetes-induced RAGE-mediated impairment of glyoxalase-1-expression and -activity and the reduction in neuronal function is largely prevented in $RAGE^{-/-}$-mice. Identification of the AGE-RAGE-NF-κB pathway as one pathomechanism mediating neuronal dysfunction in diabetic neuropathy may therefore provide new targets for a pathogenetically-oriented treatment.

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USE OF SINGLE-POINT MUTATED HMGB1 BOX A VARIANTS AS THERAPEUTIC AGENTS IN HMGB1 AND RAGE RELATED PATHOLOGIES

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One of the approaches we are developing to antagonize/inhibit circulating HMGB1 foresees to exploit Box A ability to antagonize circulating HMGB1 on its receptors. It has been demonstrated that Box A (84 aas N-term domain of HMGB1) is an extremely weak agonist of the inflammatory cytokine release triggered by HMGB1 and competitively inhibits the proinflammatory activities of Box B and of the whole protein. It acts blocking HMGB1 interactions with its receptors.

One of the identified receptors for HMGB1 is the receptor for advanced glycation end products (RAGE). HMGB1 binds to RAGE in a concentration-dependent manner (Hori et al., 1995) and the engagement of RAGE with its ligands (not only HMGB1, but also AGEs, transthyretin) activates the NF-kB signalling pathway (Thornalley, 1988; Souse et al., 2000; Sappington at al., 2002) and the MAPK pathways (Degryse at al., 2001). RAGE activation and consequent increased expression is associated with several pathological states, such as diabetic vasculopathy, neuropathy, retinopathy and other disorders, including Alzheimer's disease and immune/inflammatory reactions of the vessel walls.

From a pharmacological point of view, Box A behaves as an antagonist in the pathological conditions induced and/or sustained by HMGB1 (and Box B) and as inhibitor of RAGE in RAGE-associated diseases.

However, as expected HMG Box A being a protein has a very short half life in plasma, resulting in low bioavailability. Consequently the related pharmacological action requires high dosages and frequent administrations.

On this basis, we applied the proprietary approach of Nautilus biotech (Paris) that foresees the in silico identification of "proteolysis hot spots" and their substitution with appropriate replacing aminoacids in order to obtain a single-point mutated Box A with consistent increased resistance to proteases and consequently improved PK/PD performance. This technology has already been successfully applied to six different proteins, among which IFN-α (Belerofon®) and GH (VitatropinN™).A list of positions along the HMGB1 Box A (84 amino acids) representing potential targets for proteolysis has been established (53 positions) and one or more replacing amino acids identified. A total of 115 mutants of Box A with one single mutation have been generated and each mutant has been compared to wild type Box A by in vitro testing its capability to inhibit HMGB1-induced migration of NIH/3T3 cells. About 67 mutants out of 115 have shown equivalent or improved efficacy compared to wild type. These mutants have been further tested for resistance to proteases in comparison to wild type by time-points incubation with a proteases mixture followed gel electrophoresis and image analysis. About 21 have shown improved resistance and of these 7 displayed a resistance to proteases dramatically increased. 5 best performing variants have been selected for further development, a scale-up of production method set up together with an ELISA assay for plasma quantification of wild type and variants. PK/PD studies as well as efficacy studies in different in vivo models are being performed and on the basis of the results obtained the single-point mutated Box A variant with the best performance is going to be selected for further development.

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RAGE-DEFICIENCY BOOSTS BACTERIAL KILLING BY NEUTROPHILS

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Background: Engagement of advanced glycation end products (AGEs) to their cellular receptor RAGE (receptor for advanced glycation end products) results in perpetuated cellular activation. In a mouse model for sepsis after cecal ligation and puncture, however, mice

deficient for RAGE (RAGE-/- mice) are protected from the lethal effects of sepsis observed in wildtype mice. As one underlying mechanism explaining the improved survival of RAGE-/-mice we hypothesized an increased neutrophil activity and a more effective bacterial kill mediated by the neutrophils of RAGE-/- compared to wildtype mice.

Objektive: An Escherichia coli-killing assay was developed to compare the properties of neutrophils from RAGE-/- mice and wildtype mice in killing bacteria.

Study Design: Neutrophils from bone marrow of RAGE-/- mice and wildtype mice were isolated and the cell suspensions were incubated for 20, 40 and 60 minutes together with E. coli.. Samples of these suspensions were plated on Columbia agar and grown overnight before the survival of E. coli was assessed. Supernatants were collected.

Results: Compared to wildtype cells, neutrophils of RAGE-/- mice showed an enhanced bacterial killing suggesting a RAGE-mediated impairment of neutrophil capacity to attack bacteria. First analysis of the supernatants have shown an increased lactoferrin activity in RAGE-/- mice.

Conclusion: These data demonatrate for the first time that RAGE modulates cellular defense mechanism mediated by neutrophils.

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A BETTER SURVIVAL OF RAGE-/-MICE COMPARED TO WT-MICE IN AN ANIMAL MODEL OF SEPTICEMIA COULD BE LINKED TO A DIFFERENT LEUKOCYTE DISTRIBUTION

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Objective: Advanced glycation end products (AGEs), S100-proteins and HMGB1 bind to their cellular receptor RAGE, a signal transduction receptor, involved in processes linked to chronic inflammation. Ligand/RAGE-Interaction induces a perpetuated NF-kappaB-activation, followed by inflammatory gene expression. Consistently RAGE-deficiency limits ligand induced inflammation and improves survival in an animal model of septicemia.

Material and Methods: RAGE knockout-mice (RAGE-/- mice) and Wildtype-mice (WT-mice) on the C57Bl/6 background were immunized three times at day 1, 14 and 21 with intraperitoneal injections of Tetanus Toxoid (TT) or PBS, for control purpose. At day 0, leucocytes were determined with a coulter-counter (automated hematology system) and at day 28, the TT-specific, cellular and humoral immune response in TT immunized versus PBS immunized mice was analyzed in the proliferation assay, ELISA and FACS. For statistical analyses the two-tailed T-test was used.

Data: Immunization to TT induced a similar anti-TT IgG immune response in RAGE-/-mice as well as in WT-mice and the total IgG did not differ in RAGE-/- compared to WT-mice. In contrast to the humoral immune response, the absolute leukocytes, the relative number of T-helper cells and of DN T-cells (double negative; CD4-/CD8-) are higher in nonimmunized, but comparable high in immunized RAGE-/-mice and WT-mice. The relation between B- and T-cells and cytotoxic T-cells doesn't differ in naïve and immunized RAGE-/-mice compared to WT-mice as analyzed by FACS and no difference between RAGE-/-mice and WT-mice was found in the TT stimulated cellular proliferation.

Conclusion: A similar humoral immune response and cellular proliferation of RAGE-/- compared to WT-mice is indicative for a comparable adaptive immune response. However, the increased number of leukocytes in naive RAGE-/-mice might augment the innate immune response and contribute to the improved survival of RAGE-/-mice in septicemia.

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ESRAGE IS ELEVATED IN SEPTIC PATIENTS AND ASSOCIATED WITH PATIENTS OUTCOME

Christian Bopp, Christian Bopp, Stefan Hofer, Angelika Bierhaus, Markus Weigand

Objectives: a) To evaluate in septic patients the plasma levels of esRAGE, a soluble splice variant of the full length receptor RAGE, which is involved in acute inflammation b) to determine whether esRAGE could be used as a diagnostic and prognostic marker in sepsis in the surgical intensive care unit.

Design: Observational clinical study. Setting: Surgical intensive care unit of the University Hospital of Heidelberg, Germany.

Patients: Patients admitted to the intensive care unit over a 6-month period with clinical evidence of severe sepsis or septic shock and eight healthy controls.

Interventions: None.

Measurements and Main Results: Twenty-nine intensive care patients were enrolled in the study within the first 24 h after onset of severe sepsis or septic shock. Eight healthy controls served as controls. Plasma esRAGE concentrations were elevated in septic patients compared with healthy volunteers (1.764 ± 138 vs. 1.026 ± 177, $p < 0.05$). Additionally, nonsurvivors after 28 days have had higher plasma esRAGE concentrations than survivors (2.302 ± 189 vs. 1.326 ± 112, $p < 0.001$). ROC curve analysis of plasma esRAGE concentrations of septic patients showed a specificity of 75 % and a sensitivity of 84.6 % with 1596 pg/ml as cutoff.

Conclusion: This is the first study showing elevated plasma esRAGE concentrations in septic patients. Noteworthy, nonsurvivors had higher plasma esRAGE concentrations than survivors suggesting that esRAGE is related to severity and outcome of septic patients and may serve as a new sepsis marker.

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A 274

ROLE OF STAT3 IN THE LUNG INFLAMMATORY RESPONSE

Peter Ward

Objective: To assess activation of STAT3 in the IgG immune complex (IgGIC) model of acute lung injury (ALI) in rodents and determine the mechanisms involved.

Materials and Methods: The model of IgGIC ALI has been extensively described in our recent publications. Endpoints related to STAT3 were RT-PCR and electrophoretic mobility shift assays (EMSA) as well as phosphorylation of Tyr^{705} and Ser^{727} in STAT3. Lung IL-6 or IL-10 was neutralized by intratracheal administration of anti-IL-6 or anti-IL-10 (50 μg). Similarly, blockade of anti-C5a was also achieved in lung. Endpoints were STAT3 activation as detected by EMSA as well as albumin leak into lung (a measure of the intensity of lung injury). Requirements for phagocytic cells for STAT3 activation were determined by PMN depletion (using intravenous rabbit anti-rat PMN IgG) or airway instillation of phosphonate liposomes to deplete lung macrophages.

Data: STAT3 is a cytoplasmic transcription factor that is activated (phosphorylated) by Janus kinases (JAKS). STAT3 modulates gene expression. Depending on the cell type, STAT3 may promote or inhibit inflammatory responses. Acute lung injury (ALI) induced in rodent lungs by deposition of IgG immune complexes (IgGIC) resulted in STAT3 activation (as measured by EMSA), which peaked at 4 hr in whole lung extracts, whereas in BAL macrophages activation peaked within 30 min. In this model of ALI, mRNA and protein for STAT3 also increased, peaking between 2-4 hr. Proof of activation of STAT3 was found by phosphorylation of Tyr^{705} and Ser^{727}, peaking at 4 hr in whole lung extracts. Since IL-6 and IL-10 are known to be involved in STAT3 activation, antibodies to these two cytokines were employed in the ALI model and were found to suppress activation of STAT3. In vivo blockade of C5a also suppressed activation of STAT3 in the ALI model, indicating that the STAT3 activation requires the complement activation product, C5a. Depletion of either lung macrophages or blood PMNs also reduced lung activation of STAT3, indicating that products of both cell types are involved in STAT3 activation.

Conclusion: STAT 3 activation robustly occurs in the model of ALI employed. The data indicate that C5a, IL-6 and IL-10 enhance STAT3 activation in lung, resulting in regulation (modulation) of the lung inflammatory response. STAT3 activation also requires products of both PMNs and lung macrophages. Pathways leading to STAT3 activation likely represent a counter balance to the strong pro-inflammatory responses that are also linked to C5a in this ALI model of lung injury.

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ROLE OF MACROPHAGES IN LUNG INFLAMMATION

Loems Ziegler-Heitbrock

Lung macrophages (alveolar, bronchial, interstitial) can orchestrate inflammation by production of chemokines and of pro- and anti-inflammatory cytokines. During inflammatory processes as seen in adult respiratory distress syndrome (ARDS), cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD) new macrophages are recruited. These newly recruited monocyte-like cells show high CD14 and DR expression and low levels of CD68 and they are smaller in size as compared to the lung macrophages that are present under non-inflammatory conditions. Bronchial macrophages from induced sputum of COPD patients show high level expression of TNF protein in flow cytometry and isolated cells exhibit high mRNA levels for the pro-inflammatory TNF and the chemotactic IL-8. Similarly, alveolar macrophages from lavage of patients with ARDS and with interstitial lung disease like sarcoidosis show increased CD14 levels, increased TNF and many of these cells are of lower size. These finding suggest that newly recruited monocytes may be a driving force in lung inflammation.

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LUNG INFLAMMATION CAUSED BY DISTANT ISCHEMIA

Alex Lentsch

Organ injury resulting from ischemia/reperfusion often cause secondary inflammatory injury to the lung. The mechanisms responsible for this are complex and often dependent upon the site and duration of ischemia. Experimental animal models have been developed to examine this phenomenon. Of these, models of limb and liver ischemia/reperfusion have been among the most widely studied. In each of these models, there is abundant evidence to suggest that elaboration of proinflammatory mediators from the injured organ during reperfusion is responsible for induction of an acute inflammatory

response in the lung. The most prominent of these include TNFα, IL-1β and activated complement components. All of these mediators are potent priming stimuli for circulating neutrophils. Complement activation has been noted in circulating blood during organ ischemia and this is linked to intrapulmonary expression of thromboxanes, leukotrienes, and substance P. Substance P activates the transcription factor, NF-κB, leading to the expression of chemokines. In a similar fashion, TNFα and IL-1β activate NF-κB and induces the expression of vascular cell adhesion molecules in pulmonary endothelial cells and chemokines in pulmonary macrophages and epithelial cells. Collectively, the pulmonary expression of chemotactic agents along with vascular expression of adhesion molecules promotes the adhesion and diapedesis of activated neutrophils into lung causing tissue injury.

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REGULATION OF LUNG INFLAMMATION BY NUCLEAR FACTOR KAPPA B

John W. Christman

Activation of NF-kB is the "hallmark" of acute inflammation and plays a critical role in regulating transcription of a wide array of pro-inflammatory gene products that include COX-2, and are involved in the pathobiology of inflammatory lung disease. Both airway epithelial cells and pulmonary macrophages have been shown to be involved in the generation of lung inflammation through signaling mechanisms that are dependent on activation of the NF-kB pathway. Our data show that intentional activation of NF-kB in airway epithelial cells results in ARDS-like lung inflammation in a murine model. Although activation of NF-kB occurs surprisingly early in airway epithelial cells, elimination of macrophages attenuates lung inflammation whereas increasing macrophage numbers increases the intensity and duration of the neutrophilic inflammatory response. In order to address this paradox, we have used in vitro methodology to show a unidirectional ICAM-1 dependent cell-to-cell signaling mechanism that is related epithelial cell stimulated macrophage COX-2 gene expression. Although activation of NF-kB in macrophages is sufficient to result in COX-2 gene expression, two other transcription factors, PU.1 and YY-1, play a combinatorial and synergistic role in COX-2 gene expression, possibly through epigenetic effects on chromatin structure that is mediated by phosphorylation of histones. Unraveling the details on this complicated mechanism is gradually indentifying critical points that result in activation or modification of the NF-kB activation pahway that are susceptible to therapeutic interventions.

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ADRENERGIC REGULATION OF LUNG INFLAMMATION

Michael Flierl

Recent studies indicate that stimulation of the cholinergic parasympathetic nervous system suppresses the inflammatory response, but much less is known about the sympathetic (adrenergic) nervous system. Acute lung injury (ALI) was induced in pathogen-free male Sprague-Dawley rats (275-300 grams) through intra-tracheal administration of LPS and α- and β- adrenoceptors were blocked with selective compounds in vivo and alveolar macrophages and neutrophils were obtained and evaluated for TNF-α production after LPS exposure in vitro in presence of adrenergic antagonists. When acute lung injury was triggered in rodent lungs, injury was accentuated by the presence of α_2-adrenoceptor agonists and depressed by the use of α_2-adrenergic antagonists. When phagocytes were exposed to LPS, the co-presence of adrenergic agonists or antagonists enhanced or depressed TNF-α production, respectively. We further showed that LPS upregulated mRNA of noradrenaline producing enzymes in phagocytes. When specific antagonists for α_{2A}- and $\alpha_{2B/C}$-adrenoceptors were used, the α_{2A}-antagonist powerfully reduced LPS-induced generation of TNF-α. These data indicate that the adrenergic pathway regulates the lung inflammatory reponse.

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INFLAMMATION AND IMMUNITY IN STROKE

Ulrich Dirnagl

Stroke poses a massive clinical, social and economic burden, yet we have very limited effective therapies. This inadequacy is in spite of intensive research efforts and numerous failed clinical trials. The latest of these (SAINT II, using a free radical scavenger) showed no efficacy in spite of extensive and convincing pre-clinial data. These failures demand a re-examination of the pathophysiology of ischemic brain injury and subsequent repair processes. Animal models as well as clinical observations have demonstrated that after a very dynamic phase of early damage the lesion may continue to grow even many hours or days after the onset of ischemia. We have also learnt that, paradoxically, at the same time the brain mounts a potent but only partially successful defensive response against many of the deleterious secondary mechanisms which are active during the process of lesion maturation. In addition, it has been known for a long time that the brain can at least partially compensate the loss of function by plasticity, and recent evidence shows that the brain even attempts to repair itself. I propose that understanding the role of inflammation and immunity after stroke, which appear to be of equal importance for secondary damage as well as for repair, may hold the key to novel and effective therapeutic strategies for stroke

patients.
Inflammation in stroke is Janus-faced: Destruction of brain tissue and the containment of damage or even regeneration may be governed by the same inflammatory molecules and mechanisms, with time, cellular context and stimulus intensity as important switches between destruction and repair. This important role for inflammation in stroke should not come as a surprise: Inflammation is the basic mechanism by which tissues of multicellular organisms respond to injury: Inflammation first eliminates pathogens or noxious agents and clears debris, then it controls the restoration of tissue integrity and function. In higher vertebrates inflammation is a highly complex process, not the least because they not only have an innate, but also an adaptive immune system. Moreover, in the adult mammalian brain lesion repair and restoration of function are inherently limited. Not surprisingly then, the role of inflammation after stroke is a multifaceted process, which is also strongly affected by the immune status of the stroke victim at the time of stroke (atherosclerosis, infection). Reciprocally, stroke itself affects peripheral immunity (stroke induced immunodepression).. I propose that a deeper understanding of inflammation after stroke, beyond the simple dichotomy of good vs. bad, will result in the identification of targets for innovative and promising therapies in human stroke. In particular, it will widen the time window for therapeutic interventions, and may lead to improved regeneration of lost function after stroke.

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ROLE OF INFLAMMATION FOR NEURODEGENERATION AND NEUROREGENERATION - CNS TRAUMA

Philip Stahel

Traumatic brain injury remains the leading cause of death and persistent neurological impairment in mainly young patients. Research efforts in the past years have been aimed at elucidating the role of the inflammatory response in the injured brain in mediating neuropathological sequelae which are, in large part, responsible for the adverse outcome after central nervous system (CNS) trauma. In contrast, recent data support the notion that the posttraumatic neuroinflammation may mediate neuroprotective effects as well. This so-termed "dual role" of the inflammatory response represents the focus of the present overview and provides the basis for the current understanding of the role of inflammation for neurodegeneration and neuroregeneration after CNS trauma. New potential pharmacological approaches aimed at modulating the neuroinflammatory response after head injury will be discussed.

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THE CLINICAL SPECTRUM OF CARDIOGENIC SHOCK

Joseph Parrillo

This lecture will provide a brief review of the history, diagnostic approach, etiology and pathogenesis, clinical assessment, and management of cardiogenic shock. Mechanical causes, (right ventricular infarction, ventricular septal rupture, severe mitral regurgitation, and a ruptured left ventricle) of cardiogenic shock will also be considered. Important links between pathogenetic mechanisms and management principles will be highlighted. Management of cardiogenic shock will emphasize pharmacologic support of blood pressure and ventricular dysfunction; mechanical (intraaortic balloon pumping and ventricular assist device) support of the failing ventricle; and revascularization therapy with percutaneous coronary intervention and/or coronary bypass surgery. Prognosis from cardiogenic shock has improved during the past four decades, however, mortality still remains high (about 40%) despite our best present management. There is a critical need for additional effective treatment for this serious disease.

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THE PEPTIDE BBETA15-42: A NEW TREATMENT OPTION FOR THE HEART IN SHOCK?

Kai Zacharowski, Peter Petzelbauer

We have recently identified novel anti-inflammatory properties of the fibrin-derived peptide Bβ15-42. It consists of 28 amino acids corresponding to the N-terminal sequence of the β-chain of fibrin. It competes with fibrin E1-fragments for binding to endothelial VE-cadherin, thereby reducing leukocyte transmigration across endothelial junctions in vitro and in vivo (1-2).

In 2004 2.3 million people were involved in accidents on German roads, 5.842 died, and 81.000 people were seriously injured. The incidence of haemorrhagic shock in polytraumatized patients is high and associated with organ-dysfunction and failure, thus increasing morbidity and mortality. Often hypotension, hypoxia and hypoperfusion lead to cardiac ischemia and reduced cardiac output worsening the outcome. Volume (e.g. crystalloid and/or colloid and/or blood products) and oxygen delivery are mandatory to treat hemorrhagic shock. These interventions can improve the clinical situation, however, contribute indirectly to reperfusion injury. Currently no drug is available to treat this injury.

We tested Bb15-42 in several models focussing on the heart during conditions of local or global ischemia. We can show that in rodent models (mice and rats) for

myocardial ischemia followed by reperfusion, the peptide Bb15-42 reduces myocardial inflammation and infarct size (1, 3). Similar results were obtained in a pig model of left coronary artery occlusion and reperfusion.

In a pig model of hemorrhagic shock we can demonstrate that myocardial damage occurs during reperfusion (cardiac troponin T release). This was significantly reduced in animals which were treated with Bb15-42 during reperfusion.

We can conclude that Bβ15-42 protects the heart during various conditions of ischemia and reperfusion including shock and is clinically safe in phase 1 testing of humans.

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THE SPECTRUM OF CLINICAL SYNDROMES DUE TO DYSREGULATION OF IL-1BETA RELEASE

Charles Dinarello

One can consider chronic inflammatory diseases as being "autoimmune" or "autoinflammatory". Although both TNFα and IL-1β play a role in these disease TNFα and IFNγ appear to dominate in autoimmune diseases whereas IL-1β is the sole mediator in autoinflammatory diseases. Also, in auto-inflammatory diseases, the monocytes/macrophage rather than the T-cell plays the dominant pathological role. In fact, dysregulation of processing and secretion of the inactive IL-1β precursor accounts for the basis of these diseases.

This dysregulation is at the level of increased secretion of IL-1β as a mature active cytokine. In general, the secretion of processed, mature IL-1β is tightly controlled. But in auto-inflammatory diseases, secretion of IL-1β from cultured monocytes is clearly greater than in healthy controls. Although the amount of IL-1β secreted in vitro is greater than in controls, circulating IL-1β is not particularly high. In fact, since IL-6 is IL-1β-dependent, IL-6 is more likely to be measured that IL-1β itself. The auto-inflammatory diseases are characterized by recurrent fevers, elevated white blood cell counts with a prominent neutrophilia, rashes, serositis, and generalized fatigue. The proof of he concept is the rapid and near complete remission of the signs and symptoms of the disease upon blocking IL-1 receptors or administration of anti-IL-1β monoclonal antibodies or the IL-1Trap.

The most marked responses to IL-1 blockade have been observed in patients with adult onset Still's disease, macrophage activation syndrome, familial Mediterranean fever, and mutations in the NALP-3 gene , for example, Muckle-Wells syndrome, neonatal onset multisystem inflammatory disease and familial cold autoimmune-inflammatory syndrome. Although it is often difficult to demonstrate elevated circulating levels of IL-1β in these patients, the rapid reduction in fever, neutrophilia and acute phase reactants by IL-1 receptor antagonist demonstrates that these are IL-1-mediated diseases and particularly IL-1β dependent. There is no question that hereditary auto-inflammatory diseases are rare. The lesson learned from treating these rare diseases with specific blockade of IL-1, however, is that the clinical symptoms of the patients, the intensity of the inflammation, the hematological upheaval and biochemical manifestations are hardly rare; in fact, they are the hallmarks of systemic inflammation. These clinical findings also place IL-1 in a unique position in the cascade of cytokines during several inflammatory diseases. They raise the question whether there are "unique IL-1 diseases" or whether IL-1 mediates the inflammation induced by more proximal cytokines such as TNF or IL-18. It should be noted that many patients with or without the NALP-3 mutation as well as patients with adult onset Still's disease were initially treated with infliximab or etanercept with partial responses, suggesting that neutralization of TNF results in decreased IL-1 activity in those patients. These studies supports the concept that IL-1 contributes to the inflammatory component of most diseases and that efficacy of antibodies to TNF is due, in part, to a reduction in IL1 activities. However, uniquely IL-1-mediated diseases do exist due to dysfunction in IL-1 gene expression, processing and release as well as receptor expression.

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GENETIC BASIS OF IL-1 MEDIATED FEVER SYNDROMES

Hal Hoffman

The inherited periodic fever syndromes are autoinflammatory diseases that are characterized by recurrent episodes of systemic inflammation resulting in fever and inflammatory symptoms involving various tissues including joints, skin, and the central nervous system. These diseases include Familial Mediterranean fever, Hyper IgD syndrome, TNF receptor associated periodic syndrome, familial cold autoinflammatory syndrome, Muckle-Wells syndrome, and neonatal onset multisystem

inflammatory disease. Genetic mapping of families with these inherited syndromes over the last decade has successfully localized associated disease genes including MEFV, MVK, TNFR, and CIAS1. The identification of the responsible genes has resulted in increased understanding of the pathogenesis and improved diagnosis and treatment of these disorders. Many of the inflammatory mechanisms involved in these diseases intersect with the IL-1 pathway and this is supported by the remarkable efficacy of IL-1 targeted therapies in some of these diseases.

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PATTERNS OF IL-1BETA SYNTHESIS AND SECRETION AND RESPONSE TO ANTI-IL-1 TREATMENT IN PATIENTS AFFECTED BY SYSTEMIC ONSET JIA (SOJIA)

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Objective: To analyze the pattern of synthesis and secretion of IL-1β and the clinical and biological effects of the IL-1 blockade in SoJIA patients. Patients and Methods: 15 patients with SoJIA were selected for treatment with recombinant IL-1 receptor antagonist (rIL-1Ra, Anakinra) and followed longitudinally. Monocytes from SoJIA active patients and from healthy donors were isolated from peripheral blood and activated with LPS for 3 h. Intracellular and secreted pro-IL-1β and IL-1β were determined by western blot and ELISA with or without ATP stimulation for 15 minutes.

Data: The production of pro-IL-1β by monocytes from SoJIA patients was highly variable and overall low. One group synthesized IL-1 spontaneously and scarcely increased production after LPS stimulation. The second group produced no or low pro-IL-1β that increased after LPS stimulation, like in healthy individuals. The third group produced minute quantities of pro-IL-1β both before and after LPS stimulation. In all groups the amount of IL-1β secreted during 3 h with LPS was variable and lower than in healthy controls. Also ATP stimulation was much less efficient than in healthy controls in inducing IL-1β secretion, with some cases not responding at all as observed in CIAS1 mutated patients. After treatment with rIL-1Ra, 7 patients displayed a prompt control of systemic and articular manifestations and a normalization of acute phase reactants (complete responders), whereas 8 patients displayed a variable clinical response, with a general improvement soon after the beginning of the treatment, but with a tendency to a persistence of active disease activity during follow-up (incomplete responders). Unlike in CINCA patients, one week of treatment did not change significantly the amount of pro-IL-1β synthesis and secretion. However in the complete responders analyzed after 6 month of treatment, monocytes, that produced very low IL-1β before treatment, increased IL-1β synthesis and secretion thus acquiring a normal phenotype.

Conclusion: Monocytes from SoJIA patients display a variable response to IL-1 blockade, and low but variable levels of IL-1β synthesis and secretion. Furthermore, a subgroup of complete responder displays some functional similarities with CIAS-1 mutated individuals. Interestingly, unlike in CINCA patients, the efficacy of IL-1Ra does not correlate with inhibition of IL-1β synthesis and secretion. Together these data confirms the heterogeneity of the syndrome and suggest the existence of different pathways converging on IL-1β mediated clinical signs.

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PATTERN OF INTERLEUKIN-1BETA SECRETION IN RESPONSE TO LPS AND ATP IN PATIENTS WITH CIAS-1 MUTATIONS BEFORE AND AFTER INTERLEUKIN-1 BLOCKADE

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Objective: The synthesis, processing and secretion of interleukin (IL)-1β, as well as the clinical and biological effects of the IL-1 blockade, were analyzed in patients affected by Chronic Infantile Neurologic Cutaneous Articular (CINCA) and Muckle-Wells (MW) syndromes, with the aim to understand the molecular mechanisms linking mutations in the cryopyrin gene and IL-1β hypersecretion, and underlying the response to IL-1 receptor antagonist (IL-1Ra).

Patients and Methods: 6 patients with CINCA/MW syndrome have been treated with recombinant IL-1Ra and followed longitudinally. Monocytes from CINCA/MW and 25 healthy donors were activated with Lipopolysaccharide (LPS) for 3 h and intracellular and secreted IL-1β was determined by western blot and ELISA before and after exposure to exogenous ATP. Data: LPS-induced IL-1β secretion was markedly increased in monocytes from cryopyrin-mutated patients. However, unlike in healthy subjects, secretion of IL-1β was not induced by exogenous ATP. Treatment with IL-1Ra resulted in a dramatic clinical improvement paralleled by an early and strong down-regulation of LPS-induced IL-1β secretion by the patients cells in vitro. **Conclusion**: 1.The requirements of ATP stimulation for IL-1β release observed in healthy individuals are by-passed in patients bearing cryopyrin mutations. This indicates that crypyrin is the direct target of ATP and that mutations release the protein from the need of ATP for activation. 2. The dramatic amelioration induced by IL-1Ra treatment is due at least in part to the strong decrease in IL-1β secretion that follows the first injections of the antagonist. These findings may have implications for other chronic inflammatory conditions characterized by increased IL-1β.

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CD14 EXPRESSION IS INCREASED AFTER TREATMENT WITH STATINS, ENHANCING THE RESPONSE TO LPS

Antonio De Maio, Tiffany Fre

Although it is well accepted that septic shock is the product of a poorly controlled inflammatory response, anti-inflammatory therapy has not been totally successful in the treatment of this condition. Statins, which are widely used for the treatment of hypercholesterolemia, have been found to be anti-inflammatory. Thus, we investigated the effect of statins on the response of macrophages (Mϕ) to bacterial lipopolysaccharide (LPS). Treatment of RAW 264.7 Mϕ with lovastatin resulted in an increase of tumor necrosis factor (TNF)-a levels following stimulation with LPS. This increase in TNF-a was inhibited by co-incubation of lovastatin with mevalonate, indicating that the effect is specific for inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. The elevation in LPS-induced TNF-a levels was correlated with increased levels of CD14, the major LPS binding site on the macrophage surface. The elevated expression of CD14 was apparently due to a small decrease in the cellular cholesterol pool as well as reduced levels of geranylgeranyl pyrophosphate. The presence of soluble CD14, which is an important mediator in septic shock, was significantly reduced after lovastatin treatment. This decrease in soluble CD14 may play a role in the anti-inflammatory effect attributed to statins and is possibly related to inhibition of CD14 processing or trafficking.

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STEM CELL THERAPHY: STATE OF THE ART - FORMATION OF LARGE CORONARY ARTERIES BY CARDIAC STEM CELLS

Piero Anversa, Jochen Tillmans, Marcello Rota, Annarosa Leri, Jan Kajstura,

Coronary artery disease is the major cause of cardiac failure in the Western world and accounts for 500,000 cases of bypass-surgery per year in the United-States alone. To date, there is no alternative to bypass-surgery for severe coronary atherosclerosis, which increases with age and dramatically affects the elderly population. Therefore, we tested whether c-kit-positive cardiac stem cells (CSCs), which have the ability to differentiate into smooth muscle and endothelial cells, are capable of creating large, functionally competent coronary vessels, which reestablish blood flow to the distal myocardium. For this purpose, CSCs were injected in proximity of the site of occlusion of the left coronary artery in rats. A relevant fraction of implanted cells survived within the myocardium, and divided and differentiated. CSCs formed rapidly conductive and intermediate-sized coronary arteries together with small resistance arterioles and capillary profiles. The new vessels more than doubled myocardial blood flow to the infarcted myocardium. This beneficial effect attenuated the development of the post-infarction dilated myopathy and improved cardiac function. Our studies indicate that locally delivered activated-CSCs generate de novo coronary vasculature, and might be implemented clinically for restoration of blood supply to the ischemic myocardium.

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NHLBI/NIH RESOURCES FOR GENE AND CELL-BASED THERAPIES

Sonia Skarlatos

This presentation will focus on several programs of interest to investigators that are involved in cell and gene-based therapies for cardiovascular diseases. Programs such as the NHLBI Gene Therapy Resource Program and the NHLBI Production Assistance for Cellular Therapies will be briefly discussed. These two new NHLBI Programs offer resources to NIH-supported investigators for preclinical and clinical grade vector productions, toxicology studies and clinical grade cell products. In addition, an overview of several initiatives in vector development and stem cell biology will be presented.

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PRECONDITIONING ENHANCES SURVIVAL AND TRANSDIFFERENTION POTENTIAL OF STEM CALLS AFTER TRANSPLANTATION IN INFARCTED MYOCARDIUM

Muhammad Ashraf

Recent studies show that cardiac bone marrow stem cells can colonize the infarcted myocardium and participate in de novo regeneration of the infarcted myocardium. The survival of donor cells after transplantation is limited by lack of oxygen and nutrients in the infarcted heart. The phenomenon of ischemic preconditioning is known to impart cytoprotective influence against subsequent lethal ischemic injury. We hypothesize that preconditioning of stem cells with preconditioning mimetics or cytokines evokes multiple signaling pathways leading to their enhanced survival and engraftment in the ischemic myocardium.The stem cells can be preconditioned by several methods, i.e. ischemic preconditioning mimetics, cytokines/ growth factors, heat shock, etc. In this study, Sca_1^+ mesenchymal stem cells were treated with 100nM insulin like growth factor (IGF) for 20 minutes and then exposed to 8 hours of anoxia. Preconditioning with IGF

significantly improved their survival via MAPK and PI-3k/Akt signaling pathways. After 7 days treatment with IGF, Sca_1+ cells showed significant upregulation of connexin-43 and Gata-4. The implantation of preconditioned cells into infarcted myocardium significantly reduced infarct size and induced both angio- and myogenesis. Similarly preconditioning of skeletal myoblasts with preconditioning mimetic, diazoxide enhanced cell survival upon exposure to 100uM H2O2 as shown by reduced enzyme release, cytochrome c translocation and apoptosis. These cells expressed elevated levels of Akt, bFGF, HGF compared to non preconditioned cells. Upon transplantation into rat hearts after LAD ligation, these hearts after 4 weeks revealed marked improvement in cardiac function as measured by echocardiography. Capillary density was the highest in the hearts treated with preconditioned cells compared to hearts treated with non PC cells. These results suggest that activation of signaling pathways of preconditioning promotes the survival of stem cells by release of paracrine factors and upregulation of cell survival genes. Increased upregulation of these cells with cardiac transcription factors promotes their cardiomyogenic potential. Transplantation of preconditioned stem cells is an innovative approach and will have solid impact on the success of cell based therapies to treat cardiovascular diseases.

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GENDER DIFFERENCE IN BONE MARROW FUNCTION FOLLOWING TRAUMA-HEMORRHAGE

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It is well known that sepsis and subsequent multiple organ dysfunction syndrome (MODS) are the most common causes for death in the SICU. In this regard, injury-induced anergy of the immune system has been postulated to be the major factor in the high susceptibility of trauma patient to sepsis and subsequent MODS. Bone marrow failure is one facet of the MODS and is commonly seen in patients recovering from severe trauma and hemorrhagic shock. Earlier experimental studies have indicated that various adverse circulatory conditions such as hemorrhagic shock, acute hypoxia and endotoxin impair bone marrow proliferative capacity, in particular the growth of granulocyte-macrophage progenitor cells. Other studies have reported that the bone marrow is driven to monocytopoiesis following thermal injury and infection in mice. However, studies have also demonstrated a decreased ability of peripheral mononuclear cells to support bone marrow growth in patients following severe torso trauma. Although traumatic injury appears to be associated with bone marrow alterations, the mechanisms responsible for producing this remain unclear. While gender is a crucial determinant under such conditions, the role of gender on bone marrow function has not been extensively examined. The available information indicates that gender specific effects take place in bone marrow differentiation and immune responses following trauma-hemorrhage. Females in the proestrus state of the estrus cycle, with high levels of estrogen, show maintained bone marrow immune response as compared to males following trauma-hemorrhage. Trauma-hemorrhage also increases the development of granulocyte/macrophage progenitor cells. The proliferative responses to GM-CSF were maintained in proestrus females, but decreased in males under those conditions. In addition, augmented differentiation into monocyte/macrophage lineage in proestrus females was observed and associated with the maintained release of TNF-α and IL-6. Alternatively, increased IL-10 and PGE_2 production was observed in the male trauma-hemorrhage groups. Thus, gender specific effects in bone marrow differentiation and immune responses occur following trauma-hemorrhage which likely contributes to the gender related differences in the systemic immune responses under those conditions (supported by USPHS grant R01 GM37127).

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STEM CELLS AND CARDIAC REGENERATION

Loren Field

Cardiomyocytes in the adult mammal exhibit little capacity to undergo cell division. It is generally thought that increasing the number of function cardiomyocytes in a diseased heart would result in improved cardiac function. A variety of approaches have been used in an attempt to accomplish this, including (1) transplantation of donor cardiomyocytes or cardiomyogenic stem cells, (2) delivery of genes or cytokines to reactivated proliferation of host cardiomyocytes, (3) delivery of cytokines to mobilize endogenous stem cell pools, and (4) reactivation of cell cycle activity in surviving cardiomyocytes. While all four approaches offer promise, all are also subject to technical limitations. For example, while it is clear that transplanted fetal cardiomyocytes can functionally couple with the host myocardium, the number of cells that can be successfully transplanted is low. While myogenic stem cell transplantation appears to have a positive impact on cardiac function post MI, the degree of bona fide cardiac differentiation (as opposed to donor cell-induced angiogenesis, inhibition of apoptosis, or other "indirect" mechanisms) remains controversial. Data will be presented updating our current progress in the areas of cell transplantation. Particular emphasis will be placed on recent studies examining the differentiation and function of transplanted donor cells.

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MRI FOR THE ASSESSMENT OF THERAPEUTIC EFFECTS AFTER AUTOLOGOUS STEM CELL THERAPY

Bernd Wintersperger

Background: Experimental studies and early phase clinical trials suggest that g-CSF based stimulation of bone marrow-derived stem cells may have an impact on improvement of cardiac function and potentially nonviable myocardium after myocardial infarction. Published studies have either been based on mobilization of peripheral autologous bone marrow stem cells (1) or direct coronary infusion of harvested bone marrow stem cells (2,3).
Monitored parameters during initial phase (baseline) and follow-up (up to 18 month) included assessment of global cardiac function, regional wall motion, myocardial viability and myocardial perfusion.
Magnetic resonance techniques have been the predominately applied imaging techniques for parameter assessment.

Material and Methods: Modern magnetic resonance imaging (MRI) techniques allow for functional analysis with high spatial and temporal resolution with high reproducibility and volumetric accuracy. The Cine MR protocol used by Engelmann et al. provided an in-plane resolution of 1.5x1.5mm. The additional application of Gd-based contrast media also allow for assessment of myocardial perfusion and myocardial viability. In early phase after MI delayed enhancement imaging enables delineation of microvascular obstruction within the core of the infarcted area. In addition the technique shows the exact transmural extent of the infarction.

Data: Recent published animal and patient studies did not show a beneficial outcome in regard to global functional performance after bone marrow stem mobilization or coronary bone marrow stem cell infusion. The therapy however may lead to an earlier improvement after AMI with accelerated LV EF recovery (4). Beside changes in global parameters also regional wall motion did not show significant differences within infracted areas in patients treated with either verum or placebo (1). The volume of infarction as determined by delayed enhancement for both groups showed a comparable reduction following a natural shrinking and scarring process (1).

Conclusions: The effect of autologous stem cell therapy on functional outcome after acute myocardial infarction may be controversially discussed. The use of state-of-the-art MR imaging techniques in monitoring of these studies though, allow for relatively small patient groups based on the MRI accuracy.New techniques and higher field strength may even allow to reliably tracking the stem cells themselves in patients (4).

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STEM CELL PARACRINE EFFECTS: POTENTIAL IN CARDIAC SURGERY

Daniel Meldrum

Heart disease remains the leading cause of death in the industrialized world. Stem cell therapy is a promising treatment modality for injured cardiac tissue. A novel mechanism for this cardioprotection may include paracrine actions. Cardiac surgery represents the unique situation where pre- and post-ischemia treatment modalities exist which may allow the optimal use of stem cell paracrine mediated protection. This presentation will: (1) recall the history of stem cells in cardiac disease and the unraveling of its mechanistic basis for protection; (2) outline the pathways for stem cell mediated paracrine protection; (3) highlight the signaling factors expressed; (4) explore the potential of using stem cells clinically in cardiac surgery.

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IMMUNOMODULATION BY HUMAN AMNION AND ADIPOSE TISSUE DERIVED STEM CELLS

Susanne Wolbank, Anja Peterbauer, Simone Hennerbichler, Martijn van Griensven, Heinz Redl, Christian Gabriel

Objective: Human adult stem cells (SC) isolated from sources like amnion and adipose tissue can be expanded in an undifferentiated state or differentiated along multiple lineages and are regarded to be promising candidates for regenerative medicine and tissue engineering.

Material and Method: We have evaluated the in vitro immunomodulatory potential of human amniotic mesenchymal and human amniotic epithelial cells in comparison to human adipose-derived stem cells under identical experimental conditions. Peripheral blood mononuclear cell (PBMC) stimulated in mixed lymphocyte reactions (MLR) or in phytohemagglutinin activation (PHA) assays were cocultured with SC at different cell ratios and PBMC proliferation was evaluated. Additionally, the applied SC populations were pre-exposed to cytokines like interferon-g and tumor necrosis factor a.

Data: All investigated SC inhibited activated PBMC proliferation in a cell dose-dependent manner in MLR (66 - 93% inhibition at equal amounts of SC and PBMC) and PHA assays (67 - 96% inhibition at equal amounts of SC and PBMC). The lowest effective SC to PBMC ratio

was 1 to 8. The immunoinhibitory properties were independent of passage number (passage 2-6) but were significantly reduced by prior cryopreservation. Furthermore immunosuppression was not limited by the presence of proinflammatory cytokines.

Conclusion: Concluding the presented in vitro data, all three stem cell types may be considered for future allogeneic transplantation in cell therapy and regenerative medicine.

This work was partially supported by the European STREP Project HIPPOCRATES (NMP3-CT-2003-505758) and the Lorenz Boehler Fonds and was carried out under the scope of the European NoE EXPERTISSUES (NMP3-CT-2004-500283).

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THE USE OF INTEGRA™ IN PRIMARY BURN RECONSTRUCTION

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Background: Early excision with autograft-allograft closure is standard in severe burn management. Cadaver skin is associated with risks such as antigenicity, infection, limited availability and shelf life. Previous studies have shown that Integra™ is safe to use in <20% total body surface area (TBSA) burns. However, the suitability of its use in large burns over 50% TBSA, its effects on post-burn hypermetabolism, and long-term cosmetic and functional results have not yet been evaluated.

Materials and Methods: Twenty children with an average burn size of 73±15% TBSA (71±15% 3rd full thickness burn) were randomized to be treated with either Integra™ or with autograft-allograft technique. Outcome measures such as length of hospital stay (LOS), mortality, incidence of infection and sepsis, and acute phase protein levels, were compared between and within groups during the acute stay (admit to discharge). Outcome measures such as resting energy expenditure (REE), body composition data (measured by Dual-Energy X-Ray Absorptometry, DEXA), cardiac function indices, and number of reconstructive procedures, were compared during acute hospital stay and at long-term follow-up (up to two years post injury). Scar evaluation was performed at long-term follow-up.

Results: There were no significant differences between Integra™ and controls in burn size (70±5% vs. 74±4% TBSA), mortality (40% vs. 30%), and LOS (41±4 vs. 39±4 days). In the short term, REE significantly decreased ($p<0.01$), and serum levels of constitutive proteins significantly increased ($p<0.03$) in the Integra™ group compared to controls. Long-term follow-up revealed a significant increase in bone mineral content and density (24 months post burn, $p<0.05$), as well as improved scar in terms of height, thickness, vascularity and pigmentation (12 months and 18-24 months, $p<0.01$) in the Integra™ group.

Conclusion: In summary, Integra™ can be used for immediate wound coverage in children with severe burns without the associated risks of cadaver skin. Its use was not associated with an increased incidence of infection or sepsis in our patient cohort. Integra™ was associated with an attenuation of post-burn hepatic dysfunction and improved resting energy expenditure in the short-term, and an improved aesthetic outcome in the long-term.

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PROTECTIVE ROLE OF I-NOS INHIBITOR IN PARTIAL THICKNESS BURN WOUND

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Introduction: In 2005, we presented a clinical report establishing the role of apoptosis in thermally injured tissue in the zone of stasis. This zone is exposed to oxidative stress resulting from reperfusion injury, particularly after sustaining major partial thickness burns. Reperfusion injury patterns result in predominantly apoptotic cellular death. Nitric oxide (NO) plays a significant role in the initiation of the inflammatory cascade, notably from increased expression of macrophage inducible nitric oxide synthase (i-NOS) and is pertinent to burn pathophysiology. An experimental study was designed to develop an animal model of apoptotic cell injury in the zone of stasis in a mouse deep partial thickness burn injury. We also investigated the possible protective effect of a specific i-NOS inhibitor against apoptosis in partial thickness burn wound.

Methods: 40 mice (C57BL/6) were anesthetized and received a 30 % total body surface area dorsal scald burn. Control group (n=20) received no interventional medicines besides standard resuscitation with normal saline and analgesic agents. Study group (n=20) received 3 mg/kg i.p. S-methylisothiourea (SMT), a specific i-NOS inhibitor every 12 hours. 10 animals in each group were sacrificed at 24 h and 48 h. Visible burn wound and adjacent tissues in the zone of stasis were biopsied for histological review. TUNEL assay, M30 Cytodeath assay, PARP assay and measurement of apoptotic index were carried out on these specimens.

Results: Mean apoptotic index (AI) for control group were 0.248 (± 0.04 SE) and 0.181 (± 0.02 SE) respectively at 24 and 48 hrs. These AI's are comparable to that seen in our clinical observation obtained from human burn samples. The AI for the i-NOS inhibitor group was 0.147 and 0.141 at 24 and 48 hrs respectively. The AI for control and i-NOS inhibitor groups were compared using ANOVA test. The difference between the groups is

statistically significant (p=0.004). The difference was more pronounced at 24 hr time point.

Conclusions: This murine scald model provides a standardized and reproducible methodology for studying tissue injury, apoptosis as well as possible therapeutic interventions. Our results suggest a protective role for i-NOS inhibition in partial thickness burn wounds. This protective effect may be due to modification in NO induced vascular permeability as well as free oxygen radical inhibition. This therapeutic intervention may have clinical application for tissue preservation in the thermally injured as well as IR wound states.

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RAPAMYCIN IMPAIRS WOUND HEALING: POSSIBLE ROLE OF VEGF AND NITRIC OXIDE

Michael Schaeffer, Robert Schier, Stefan Michalski, Markus Napirei, Peter Schenker, Richard Viebahn

Objective: Rapamycin (Ra), used in solid organ transplantation, inhibits cellular immune function. Clinically, rapamycin appears to impair wound healing. Little is known, however, about the mechanisms of action leading to diminished repair. Rapamycin decreases the expression of vascular endothelial growth factor (VEGF) and nitric oxide (NO). VEGF and NO, both, have been shown to be critical to wound repair. We, therefore, investigated the effect of systemic treatment with rapamaycin on wound healing and wound VEGF and NO expression in rats.

Material and Methods: Groups of ten rats underwent dorsal skin incision and polyvinyl alcohol sponges were implanted subcutaneously. Beginning at the day of wounding, rats were treated by gavage with 0.5, 2.0 or 5.0 mg Ra/kg/day. Animals were sacrificed ten days later to determine wound breaking strength (WBS) and reparative collagen deposition (sponge hydroxyproline content - OHP). Expression of VEGF and inducible NO synthase (iNOS) was studied in wounds by immunohistochemistry. Nitrite + nitrate levels, an index of wound NO synthesis, were measured in wound fluid (WF). Splenic lymphocytes were tested for proliferative activity. Rapamycin levels in blood and wound fluid were measured by MEIA..

Data: Systemic rapamycin treatment was well tolerated by all rats, as reflected by equal weigth gain. Splenic lymphocyte proliferative activity was significantly decreased by rapamycin ($p < 0.05$), indicating that the rapamycin doses used were immunosuppressive. Rapamycin accumulated in wound fluid. Rapamycin levels in wound fluid were found to be approximately two to fivefold higher than blood levels ($p < 0.01$). Rapamycin (2.0 and 5.0 mg/kg/day) reduced wound breaking strength ($p < 0.01$, Table) and wound collagen deposition ($p < 0.05$). This was paralleled by decreased nitrite + nitrate levels in WF, indicating diminished wound NO synthesis. In addition, immunohistochemically, expression of VEGF and iNOS in the wound was decreased.

Conclusion: Experimentally, our data show for the first time that rapamycin impairs wound healing in a dose dependent manner and this is reflected by diminished expression of VEGF and NO in the wound.

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AN APPRAISAL OF ICODEXTRINA SOLUTION IN THE PREVENTION AND DEVELOPMENT OF INTRA-ABDOMINAL ADHESIONS. AN EXPERIMENTAL STUDY

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Background: The development of intra-abdominal adhesions accompanied by both an increased morbility and even mortality, if a reintervention is necessary, is one of the most recurrent and important complications in abdominal surgery. Due to their capacity to completely cover the peritoneum surface at both the visceral and the parietal levels, interest has recently focused on the value of the intra-abdominal administration of anti-adhesion fluids on completion of a surgical intervention.

Aim: The aim of this study was to assess the efficacy of icodextrina solution in the reduction of the formation of intra-abdominal adhesions during a thirty day period subsequent to doing a cholecystectomy using a laparotomy in pigs.

Material and Methods: 28 pigs with a weight of between 23 kg and 27 kg were used. The animals were divided into two groups.

Table

	WBS (N)	OHP (mg/mg sponge)	Nitrite + Nitrate in WF (mM)
Control	13.4 ± 0.6	23.4 ± 1.4	167 ± 8
Ra 0.5 mg/kg	11.8 ± 0.5	20.0 ± 0.9	149 ± 7
Ra 2.0 mg/kg	8.6 ± 0.6*	13.2 ± 1.1*	118 ± 8*
Ra 5.0 mg/kg	6.5 ± 0.7*	9.1 ± 1.0*	110 ± 8*

n = 10, means ± SEM, *$p < 0.01$ vs. control, ANOVA, Scheffe-test

Group I. (Control group of 10 animals.) Under general anaesthesia and skin disinfected with iodine povidone, a laparotomy of 10-12 cm in length was performed on the right subcostal. A cholecystectomy using the standard technique was carried out through it. Next, the abdominal wall was closed at two levels with Vycril 0 while skin closure was made with staples.

Group II. (Study group of 18 animals.) The same technical procedure as in group I was followed. On termination of the exeris of the gallbladder, 500cc of icodextrian solution at 4% (Adept Ò) at 37°C was introduced into the abdominal cavity.

So that an analysis of the development of intra-abdominal adhesions could be made, a medium laparotomy under general anaesthesia was performed after 30 days. The number of adhesions, their extension, consistency and vascularization were taken into account. Samples of the adhesions were taken for a histological study. The animals were sacrificed by the anaesthetist. The statistical analysis was made by using the c2 test ($p \leq 0.05$).

Results: Group I. 100% of the ten animals studied developed intra-abdominal adhesions, which were multiple in all cases.

Group II. 17 (94.4%) of the 18 animals studied developed post-operative intra-abdominal adhesions (p=NS), which were multiple in 14 pigs (82.4%). Most of them were located either between the liver and the parietal peritoneum, between the omentum and the gallbladder bed, or between the omentum and the abdominal wall. Additionally, adhesions were also found between the omentum or the small intestine and the metallic clips placed in the cystic duct, as well as between the spleen and the abdominal wall.

Conclusion: This experimental study has demonstrated the ineffectiveness of icodextrina solution at 4% in the prevention of the development of intra-abdominal adhesions after performing a cholecystectomy in pigs.

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A NEW VISCOUS ADHESION BARRIER

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Purpose: Function and biocompatibility of a new adhesion barrier (polyvinyl alcohol + carboxymethylated cellulose) were evaluated.

Material and Method: In a prospective randomized controlled study 80 female albino rabbits underwent trauma of the abdominal side-wall. PVA-gel was placed at the side-wall defect in 50 cases. In two further groups 18 animals had no treatment (control group) and 12 animals were treated with Icodextrin 4%. Biocompatibility, mechanical properties, adhesion development, device handling was observed.

Result: PVA-gel (polyvinyl alcohol + carboxymethylated cellulose) showed good biocompatibility, no side-effects and excellent adhesion prevention. While the untreated control group as well as the animals treated with Icodextrin 4% developed adhesion formation in 100%, those rabbits which were treated with PVA-gel showed adhesions in around 25 %.

Conclusion: PVA-gel functions as an excellent adhesion barrier

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MEDIATORS FROM ACTIVATED MYELOID LEUKOCYTES INDUCE OSTEOGENIC DIFFERENTIATION OF MESENCHYMAL STROMAL CELLS

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Autologous transplantation of expanded mesenchymal stromal cells (MSC) from bone marrow may offer a new approach for the therapy of critical bone defects. It is known that polymorphonuclear neutrophil granulocytes (PMN) and peripheral blood mononuclear cells (PBMC) were activated after tissue trauma or at biomaterial surfaces and release inflammatory mediators. Thus, it was the purpose of this study to analyze the influence cell culture supernatants of activated leukocytes (conditioned media) on the differentiation of human mesenchymal stromal cells. Isolated PMN and PBMC were stimulated with lipopolysaccharide (LPS) or toxic shock syndrome toxin-1 (TSST-1) and these conditioned media were transferred to human mesenchymal stromal cells. After an additional cell culture period up to 28 days it was observed that conditioned media obtained from LPS-stimulations induced osteogenic cell differentiation indicated by Alizarin-positive mineralization areas and bone nodule formation. Control experiments revealed that LPS alone did not lead to osteogenic cell differentiation. Conditioned media obtained by stimulation of leukocytes with TSST-1 did not induce osteogenic differentiation of mesenchymal stromal cells. In contrast, the isolation of monocytes from lymphocytes within the PBMC by counterflow centrifugation elutriation revealed myeloid cells as producers of the osteogenic activity. These data demonstrate an important role of mediators from activated myeloid leukocytes in the osteogenic differentiation of bone marrow-derived mesenchymal stromal cells.

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INNOVATIVE SURGICAL MANAGEMENT OF NEC - RESULTS OF 52 CONSECUTIVE CASES

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Objective: Necrotising enterocolitis (NEC) of the early born (< 1500 g) is a common and severe inflammation of small bowel and/or the colon. The process of inflammation affects the mucosal and submucosal structures and proceeds to perforation in areas with total wall necrosis. Most of the pathogenic factors are related to low weight, associated diseases (cardiac, malformations etc.), feeding problems, hypoxia, low perfusion, thromboembolia in septic conditions, bacteria and viruses. Grade I-III show beginning distension of the abdomen (I), distension, pneumatosis intestinii and perforation (II) and phlegmoneous abdominal wall, septic shock with multiple perforations or total necrosis (III). Surgery starts in grade II and leads to partial resections of bowel, application of stomata. Mostly second and third looks are planned. Gross resection of bowel leads to short-bowel-syndrome with failure of the liver after a short time combined with fatal outcome.

Methods: *a. experimental:* In a biosimulator model (CCP) fleece-bound sealing (TachoSil) is evaluated native and after impregnation with gentamicin 120 mg. Antimicrobial testing is administered on agar plates and measuring inhibition zone diameter (S. aureus, E.coli, P. aeruginosa). *b. clinical:* Indication for surgery was grade II. Perforations were closed by antimicrobially active fleece-bound sealing (TachoSil) in hemi-circular technique. No sutures were administered. Inflammatory anastomoses after tubular resection were sealed in presence of an indwelling catheter. No sutures were adminstered. Transportation and sufficient bowel wall were controlled during re-surgery.

Results: *a. experimental:* Fleece-bound sealing (TachoSil) impregnated with gentamicin showed significantly higher adhesive strength (64 hPa) than all other antimicrobially working preparations. Inhibition zones showed significantly higher diameters than all other fleece materials. *b. clinical*: 52 consecutive early borns (1998-2005, 840-1750 g) with NEC grade II were explored by laparotomy. 252 perforations were sealed by antimicrobial active fleece-bound sealing (TachoSil+gentamicin) in 112 operations. 133 stomata were created. Second/third look showed sufficient sealing of the defects or anastomoses. No insufficiency was present. No major bowel resection leading to short-bowel-syndrome was needed. In the course of the disease approx. 14 sealings with severe sclerosis of the bowel wall causing obstruction had to be resected (5.6%).

Conclusions: Antimicrobially active fleece-bound sealing (TachoSil+gentamicin) is a highly effective and efficient tool in saving bowel substance in NEC. Closure of perforations is administered in an easy way. No additional disturbance of perfusion of the highly inflammatory tissue is caused by this minimally invasive tissue management. Short-bowel-syndrome is not a fact in NEC after sealing defects or anastomoses. Sclerosis of tissue with obstructive effect will be an effect of the basic pathomechanism of NEC and not associated to the sealing material. Further studies are needed to show the bowel saving effect, reducing significantly the rate of short-bowel-syndrome and fatal outcomes.

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BURST PRESSURE, VASCULAR INTEGRITY AND COLLAGEN CONTENT OF SUTURED AND GLUED COLORECTAL ANASTOMOSES IN THE RAT MODEL

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Introduction and Objective: High insufficiency rates of up to 25% and a mortality rate of up to 50% in insufficiency of colorectal anastomoses are a clear illustration of the problems of surgical interventions. The management of such insufficiency and, if necessary, primary protection of the anastomosis by gluing it together with collagen fleece is a possible means of influencing the rate of insufficiency and the deleterious course. The objective of the study was to compare sutured and glued anastomoses in the rat model. The target parameters were the anastomotic burst pressure, the collagen content and vascular integrity over time.
Material and Method: 70 male Wistar rats were randomised into 2 groups. Group I: circular sutured anastomosis at the rectosigmoid junction. Group II: semicircular sutured anastomosis and gluing of the anterior wall with fibrin-coated collagen fleece (TachosilR). Five animals were measured at each examination point. The burst pressure was measured in situ after 6, 12, 24, 48, 72 hours, and after 5 and 10 days. After euthanasia each animal was tested for the collagen content of the anastomosis by Sirius red staining and densitometry and the vascular integrity was measured by CD-31 immunohistology and the number of vessels per visual field.
Results: 1: Measurement of the burst pressures of glued and sutured anastomoses over time
Sutured anastomses showed a significant advantage in the first 24 h. After 10 days the burst pressure was significantly better for glued anastomoses.

2: The comparison of the groups showed no significant difference as regards the collagen content or the vascular integrity.
Conclusion: The comparison between glued and sutured colorectal anastomoses showed a significant advantage for the sutured anastomoses in the first 24 hours. Thereafter, the burst pressure was significantly better for the glued anastomoses after 10 days. The burst pressure, vascular integrity and collagen content indicate comparable stability of the anastomoses in both groups. In case of insufficiency of an anastomosis, gluing together [with collagen fleece] could provide the matrix for scar formation and thus promote healing since sutures alone cannot stabilise the surgical wound.

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CLINICAL CURE RATES IN CEFTOBIPROLE- AND VANCOMYCIN-TREATED PATIENTS WITH WOUND INFECTIONS

Richard Strauss, Gary Noel

Objective: Ceftobiprole is a novel antibiotic that has activity against methicillin-resistant S. aureus (MRSA) as well as a broad-spectrum of activity against Gram-positive and Gram-negative bacteria. A large (n=784), double-blind, comparative trial to assess the safety and efficacy of ceftobiprole versus vancomycin as treatment for complicated skin and skin structure infections (cSSSI) was conducted and included patients with surgical, traumatic, and burn wound infections.

Materials and Methods: Subjects were randomized to 500mg ceftobiprole (BPR) q12h or 1000mg vancomycin (VAN) q12h. Enrollment was stratified by cSSSI type (wound, cellulitis, abscess) and included only subjects with suspected or documented Gram-positive bacterial infections. Clinical cure rates (assessed at the test of cure [TOC] visit, 7 to 14 days following end of therapy) were analyzed in both the clinically evaluable (CE) and the intent-to-treat (ITT) study populations. Microbiologic outcome was assessed at the TOC visit; pathogens were considered eradicated if no growth of a potential pathogen was observed from any culture taken at the original site of infection

Data: 259 (33%) of the 784 subjects enrolled in the trial had wound infections. Clinical cure rates in subjects in the CE and ITT populations were numerically higher in ceftobiprole than in vancomycin-treated subjects overall, and for all wound types.

Staphylococcus aureus was identified as the primary pathogen in approximately 75% of the microbiologically evaluable study subjects and MRSA accounted for approximately one-third of all S. aureus isolates. Staphylococcus aureus was identified as the primary pathogen in 157 of the 259 (60.6%) subjects with wound infections, of which 66 subjects (42%) had MRSA infections. Overall microbiological eradication rates for clinically evaluable subjects with Gram-positive pathogens isolated from the wounds at baseline were 92.3% for BPR-treated subjects, and 86.1% for VAN-treated subjects. Overall, among all patients receiving study drug, discontinuation of therapy due to treatment related adverse events (AEs) occurred in 13 (3%) BPR-treated and 17 (4%) VAN-treated subjects. Treatment related serious AEs occurred in 4 (1%) BPR-treated and 11 (3%) VAN-treated subjects.

Conclusion: Cure rates among patients with wound infections support that ceftobiprole is effective in treating patients with surgical wounds, traumatic wounds, and burns. Overall cure rate in subjects with wound infections treated with ceftobiprole was 96.3% in the CE population.

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A 305

DIAGNOSTIC PANELS IN INFLAMMATORY DISEASE AND SEPSIS

Peter Fraunberger, Autar Walli

Severe inflammation and sepsis remain a serious clinical challenge. Despite modern supportive medicine and an improved understanding of the underlying pathophysiology, mortality rates still remain high. The elevated levels of circulating pro- and anti-inflammatory mediators are a common feature in septic patients. Thus extensive search for reliable inflammatory mediators that can be used for the early diagnosis and prediction of clinical outcome is desirable. Plasma concentrations of various cytokines correlate with severity and mortality in septic patients. In addition to increased cytokine levels metabolic markers such as low cholesterol or hyperglycaemia are also associated with low outcome in septic patients. Normalisation of circulating levels of these metabolic markers indicates a better prognosis in these patients. More recently combinations of biomarkers have been suggested to improve diagnostic value of laboratory markers and possible clinical outcome. Thus an early but unspecific increase of IL-6 necessitate that PCT should also be measured as a marker of infectious disease. In addition, ratios of pro- and anti-inflammatory cytokines together with metabolic markers should prove better indicators of the inflammatory state. Therefore panels of various markers are currently under investigation. Further studies will show whether the use of biomarkers will enable us to reduce morbidity and mortality and also reduce costs in the management of septic patients.

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Table

Clinical cure rates Ceftobiprole	CE population Vancomycin	Ceftobiprole	ITT population Vancomycin	Ceftobiprole
All wounds	96.3% (77/80)	89.7% (78/87)	74.8% (95/127)	72.0% (95/132)
Surgical wounds	100% (27/27)	91.4% (32/35)	76.7% (33/43)	68.6% (35/51)
Traumatic wounds	93.0% (40/43)	86.4% (38/44)	69.4% (50/72)	69.1% (49/68)
Burns	100% (10/10)	100% (8/8)	100% (12/12)	84.6% (11/13)

A 306

BACTERIAL TOXINS AND CERAMIDE STRUCTURES AS ACTIVATORS OF DIFFERENTIAL RECEPTOR CLUSTERING IN CIRCULATING MONOCYTES

Gerd Schmitz

Bacterial glycolipid structures provoke strong proinflammatory responses that can cause fatal sepsis syndrome in humans. The lipopolysaccharide (LPS)-receptor CD14 in conjunction with toll-like receptors (TLRs) plays a major role in the initiation of primary host defense response through recognition of pathogen associated molecular patterns (PAMPs). Binding of LPS to CD14 induces a specific co-assembly of pattern recognition receptors within lipid raft microdomains, leading to a ligand specific cellular response. We have shown that the bioactive lipid ceramide (Cer), which shows structural similarity with LPS, also binds to CD14 to induce ligand dependent clustering of the CD14 receptor complex. Cer induced receptor co-assembly is overlapping in part with the LPS stimulated cluster, indicating that Cer may modulate immune cell function via CD14-CD11b/CD18 which partly differs from LPS. Our recent findings presented increased Cer and decreased lysophosphatidylcholine (LPC) levels in septic patients which showed strong correlation with outcome prediction and mortality. Plasma high-density lipoproteins (HDLs) possess anti-inflammatory properties mediated by specific lipid transfer proteins such as LPS binding protein (LBP), bactericidal permeability-increasing protein (BPI), phospholipid transfer protein (PLTP) and cholesteryl ester transfer protein (CETP) which all belong to the same gene family. Protective effects of HDL include binding and neutralization of LPS and endogenous lysophospholipids and modulation of raft microdomain dynamics in inflammatory effector cells. We previously demonstrated a profound loss of circulating mature α-HDL and increased phospholipid-rich preb-HDL precursors in sepsis accompanied by major changes in HDL apolipoprotein (apo) composition such as loss of apoAI and apoCI and increase in apoE containing HDL particles. The phospholipase A_2 inhibitor apoCI was also shown to possess a highly conserved LPS-binding motif and to improve the presentation of LPS to macrophages in early stages of sepsis. Our data support the hypothesis that Apo CI might be a physiological protector against infection by enhancing the early inflammatory response to LPS, whereas the increase in Apo E containing HDL might target LPS from macrophages to hepatocytes, finally reducing cytokine production and inflammatory responses in later stages of septic disease. Moreover, increased PLTP activity and PLTP protein were found during inflammation associated with apoE containing HDL particles, suggesting a synergistic deleterious role of LPS and PLTP in endotoxemia. Cer, LPC and other lipid mediators may play an immunregulatory role in the pathophysiology of the sepsis syndrome and predict sepsis-related mortality. The loss of protective effects of HDL, HDL-associated apoCI and LBP, and the increased PLTP activity are a poor prognostic factors and correlate directly with the severity of the illness.

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A 307

POLYMORPHISMS IN CANDIDATE GENES: A PRACTICAL (AND FEASIBLE) APPROACH TO GENETIC ASSOCIATION STUDIES USING HAPMAP DATA AND WEB-BASED TOOLS

Nicolas von Ahsen

Research in sepsis and organ failure has generated significant interest into the genetic predisposition underlying interindividual differences in disease susceptibility, severity and outcome. Single nucleotide polymorphisms (SNPs) are established genetic association markers that explain some of this variability. More recently the study of haplotypes (combination of SNPs) has gained significant interest as it overcomes the need for a priori known disease-associated SNPs and allows the researcher to study candidate genes in genetic association studies "from the scratch". In the past many genetic associations might have been missed simply because the wrong SNP was chosen for study. The use of haplotypes allows for a broader coverage of the underlying genetic profile and its association with clinical course or outcome.

A typical workflow will be illustrated in the presentation. The gene-of-interest (i.e. any human gene possibly involve in the pathogenesis, IL10 is taken as example) will be searched in the data compiled by the HapMap project (www.hapmap.org). The HapMap CEU population (Utah residents with ancestry from northern and western Europe) is usually a reliable source when a western European population is studied. SNP genotype data for the gene-of-interest are dumped from HapMap and imported in a software that extracts the tagSNPs from of the genotyping data. TagSNPs minimise the number of SNPs that must be genotyped without significantly reducing the overall information content by quality-filtering of the input SNPs and exclusion of SNPs carrying redundant information. Tagger (web-based) or Haploview (open source software) are used for tagSNP selection. The choice of a genotyping technique depends on local availability, cost, needed throughput, experience etc. Commonly used are RFLP-PCR, genotyping with hybridisation probes and MALDI genotyping by primer extension. Web-based and desktop tools are presented that help to design functional genotyping assays for these techniques. Finally, the significance of associations is tested in either 2x2 tables or by haplotype regression analysis, depending on the nature of the underlying variable (categorial vs. continuos).

In summary, the HapMap project has generated data that are extremely useful for the design of candidate gene association studies. Free available tools allow to extract tagSNPs as important predictors of common genetic variation. The study of haplotypes compared to unselected SNPs improves genetic association studies.

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A 308

IMMUNOMONITORING OF THE CRITICALLY ILL: CANCER

Dietmar Fuchs, Katharina Schroecksnadel, Gerald Brandacher, Guenter Weiss

Insufficient immunosurveillance represents an important aspect in early tumorigenesis and in the pathogenesis of malignant disease. In the later course of cancer, the development of immunodeficiency is considered to be the major reason for disease progression and death. Within the anti-tumoral host defense reaction, Th1-type cytokine interferon-γ (IFN-γ) is of particular relevance. IFN-γ stimulates several anti-proliferative and thus tumoricidal biochemical pathways in macrophages and other cells and also in tumor cell lines. They include inducible nitric oxide synthase, indoleamine (2,3)-dioxygenase, an enzyme degrading the essential amino acid tryptophan, and the production of reactive oxygen species and neopterin in human macrophages and dendritic cells. Although the anti-proliferative strategy of the immune system is directed to inhibit the growth of malignant cells, it can also affect T-cell response and thus contribute to the development of immunodeficiency.

Accelerated degradation of tryptophan and increased production of neopterin parallel the course of various malignant diseases. Moreover, a higher degree of these metabolic changes predicts poor prognosis and is associated with the development of anemia, weight loss and depressive mood in patients. As mentioned above, the monitoring of neopterin production and tryptophan degradation provides sensitive information on the immune activation status and the endogenous formation of IFN-γ in cancer patients. Data support the concept that adverse effects in patients may relate to the anti-proliferative action of IFN-γ, and results suggest that immunodeficiency in cancer patients develops as a long-term side-effect of the antiproliferative and pro-apoptotic mechanisms which are elicited within Th1-type immune response. Thereby, the enhanced production of pro-inflammatory cytokine IFN-γ seems to be critical.

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A 309

APPLYING HIGH THROUGHPUT TO CLINICAL PRACTICE - PLASTICITY OF SYSTEMIC INFLAMMATION

J Perren Cobb

Diagnosis of acute infection in the critically ill remains difficult. We hypothesized that blood leukocyte RNA profiles could be used to diagnose infection and monitor response to therapy in critical illness and injury. We demonstrate that disease trajectories derived from microarray expression profiles (what we have termed riboleukograms) can be used to diagnose pneumonia in patients and track the clinical course of acute disease. Leukocyte RNA-based diagnosis of acute infection is a new, powerful tool in the armamentarium of the intensivist faced with diverse and clinically complex patients.

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A 310

KNOWLEDGE-BASE ANALYSES OF GENOME-WIDE STUDIES

Wenzhong Xiao

High throughput, genome-wide studies of patients in clinical research pose a number of new challenges. Unlike experiments of model systems, human research is observational with many confounding variables and the manifestation and progression of disease is influenced by multiple genetic, physiological, and environmental factors. Extracting new biological insight on human diseases from genomic studies is a challenge, limited by difficulties in recognizing and evaluating relevant biological processes from huge quantities of experimental data. Here we present a structured network knowledge-base approach to analyse genome-wide experimental data in the context of the comprehensive knowledge of mammalian biology, including known functional interrelationships among proteins, small molecules and phenotypes. This method can greatly reduce the hypothesis space, enabling identification of new functional modules perturbed in the disease process. We applied this knowledge-base analysis to the studies of inflammation and the host response to injury.

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A 311

PROLONGED CATABOLIC, INFLAMMATORY, AND GENOMIC CHANGES CHARACTERIZE THE HUMAN RESPONSE TO BURN INJURY

David N. Herndon, Celeste Finnerty, Marc Jeschke, Wenzhong Xiao, Lyle Moldawer, Ronald Tompkins

The response to a burn injury is characterized by inflammation, hypermetabolism, and immune suppression involving a reprioritization of energy metabolism and protein synthesis. We hypothesized that temporal changes in the leukocyte transcriptome would provide unique insights into the initiation and resolution of severe burn injury. Genome-wide expression was analyzed from total blood leukocytes obtained from fifteen pediatric burn patients over the first year post-burn, and were compared to samples from seventeen healthy pediatric patients. Alterations in blood leukocyte mRNA abundance persisted for up to ~262 days post-burn, and only returned

to control levels by one year post-burn. Changes in leukocyte gene expression correlated temporally with proteomic and physiologic changes; expression of genes involved in increased inflammation and reduced antigen presentation and lymphocyte function have been shown for the first time to persist for a year in response to major burn.

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A 312

IMPROVING AND REGULATING LENTIVIRAL RNAI EXPRESSION IN VIVO

Patrick Stern

Our focus is improving methods for lentiviral transgenics and regulated RNAi. We have engineered a lentiviral vector with two insulator elements to prevent silencing of the integrated construct in transgenic animals. With our improved lentiviral vector, we have generated lentiviral transgenic mice expressing RNAi to silence a candidate diabetes gene in the NOD background and demonstrated protection from diabetes progression. We have further improved this vector system by incorporating miR30-based hairpins that allow Cre-recombinase regulated expression of the RNAi silencing construct. Paired with tetraploid blastocyst complementation, this vector design allows the relatively rapid production of animals that possess tissue-specific (Cre-mediated) loss of gene function. In addition to regulated RNAi, these vectors can also be used to confer Cre-regulated expression of transgenes in combination with RNAi. This vector system may be used to study tissue-specific and combinatorial expression of transgenes and/or RNAi in transgenic animals and bone marrow reconstitution experiments.

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A 313

MULTI-DISCIPLINE ANALYSIS OF GENE SILENCING: COMPLIMENTARY USE OF RT-QPCR, 2-D ELECTROPHORESIS AND WESTERN BLOTTING FOR RNAI BASED PATHWAY ANALYSIS

Francisco Bizouarn, Academia Katrina, Rubio Teresa, Ning Liu, Hamby Keith, Paulus Aran

RNA interference (RNAi) is a powerful tool used to modulate gene expression and to determine gene function. The activation of an RNAi pathway via delivery of small interfering RNAs (siRNA) into cells can result in the sequence-specific degradation of a messenger RNA (mRNA) and reduction of its corresponding protein product. The design of effective siRNA sequences in conjunction with efficient cellular delivery makes this a preferred tool for studying silencing and its effects. Analysis of gene specific silencing and subsequent changes in the expression of related genes and proteins is performed by assessing the levels of corresponding mRNAs or protein products.

In this study, we demonstrate the effective downregulation of the cytoskeleton protein, β-actin, using specific 27-mer siRNAs and siLentFect Lipid Reagent in Hela cells. Subsequently, making use of 2-dimensional gel electrophoresis (2-DGE), Capillary LC-Nanospray and MS-MS, Western blotting and RT-qPCR we examine changes of expression profiles in response to β-actin gene silencing. We will present results of our analysis, showing changes in the expression level of several proteins, either directly or indirectly associated with actin filament function. Specifically, cofilin, an actin filament-disassembling factor, is found to be highly phosphorilated after β-actin down-regulation. The complimentary use of these techniques facilitates rapid validation of 27-mer siRNA delivery and efficacy, screening for global changes in protein expression and multiplex validation of targets using qPCR techniques.

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A 314

EVIDENCE-BASED MODELING - HOW TO MANAGE

Edmund Neugebauer, Yoram Vodovotz, John Bartels, C. Anthony Hunt, Ruediger Seydel, Gary An

Introduction: Given the complexity of biological systems, understanding their dynamic behaviors, such as the Acute Inflammatory Response (AIR), requires a formal synthetic process. Dynamic Mathematical Modeling (DMM) represents a suite of methods intended for inclusion within the required synthetic framework. DMM, however, is a relatively novel approach in the practice of biomedical research. The Society for Complexity in Acute Illness (SCAI) was formed in 2004 from the leading research groups utilizing DMM in the study of acute inflammation. This Society believes that it is important to offer guidelines for the design, development and utilization of DMM in the setting of AIR research to avoid the "garbage-in garbage-out" problem. Accordingly, SCAI identified a need for and carried out a critical appraisal of DMM as currently used in the setting of acute illness.
Methods: The SCAI annual meeting in 2005, the 4th International Conference on
Complexity in Acute Illness (ICCAI; Cologne, Germany), was structured with the intent of developing a consensus statement on the methods and execution of DMM in AIR research. The conference was organized to include a series of interactive breakout sessions that included thought leaders from both the DMM and acute illness fields, the results of which were then presented in summary form to the entire group for discussion and consensus. The information in this manuscript represents the concatenation of those presentations.
Results: The output from the 4th ICCAI involved

consensus statements for the following topics: 1) the need for DMM, 2) a suggested approach for the process of establishing a modeling project, 3) the type of "wet" lab experiments and data needed to establish a modeling project, 4) general quality measures for data to be input to a modeling project, and 5) a descriptive list of several types of DMM to provide guidance in selection of a method for a project.
Conclusion: We believe that the complexity of biological systems requires that DMM
needs to be among the methods used to improve understanding and make progress with attempts to characterize and manipulate the AIR. We believe that this consensus statement will help guide the integration, rational implementation, and standardization of DMM into general biomedical research.

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A 315

SYSTEMS BIOLOGY OF INFLAMMATION

Michael Yaffe, Kevin A. Janes, H. Christian Reinhardt, John G. Albeck, Peter K. Sorger, Douglas A. Lauffenburger

Many protein kinases and their substrates function together within complex signaling networks to control inflammation. How signals emerging from these pathways are integrated and processed, as a network is unclear. To address this, we have been developing systems models of signaling where kinase activities, protein phosphorylation, binding of substrates to modular protein domains, and cellular responses such as apoptosis are quantitatively measured at densely sampled points in time, then related mathematically through partial least squares regression and principal components analysis. Using cytokine-induced apoptosis in HT-29 colonic epithelial cells, we used this approach to construct a systems-biology model of 7980 intracellular signaling events that are directly linked to 1440 response outputs associated with apoptosis. The model accurately predicted multiple time-dependent apoptotic responses induced by a combination of the death-inducing cytokine TNF with the pro-survival factors EGF and insulin, and also revealed new molecular mechanisms connecting signaling to apoptosis including the role of unsuspected autocrine circuits activated by TGF-a and IL-1a. All of the molecular signals could be divided along two primary signaling axes that constitute fundamental dimensions (molecular basis axes) within the apoptotic signaling network. Projections of different stimuli along these axes capture the entire observed apoptotic response, suggesting that cell survival is determined by signaling through this canonical stress/death-versus-survival basis set. Examination of the connection between kinase/phospho-substrate signal strength and linearity with predictions of apoptosis revealed that cells may process certain signals in a finely graded 'analog' fashion, while other signals can be processed in a coarse discretized 'digital' manner. On the basis of these findings, one particular inflammatory signaling cascade mediated by p38 MAPK and MAPKAP Kinase-2 was examined in more detail. Surprisingly, both computational modeling and in vivo experiments using cells overexpressing either constitutively active or kinase-dead MAPKAP Kinase-2 revealed that endogenous signaling through this pathway is optimally tuned to maximize the dynamic range of apoptotic signaling in response to multiple varied inputs.

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A 316

CROSSING LEVELS OF BIOLOGICAL ORGANIZATION: AGENT BASED TRANSLATIONAL MODELING OF GENOMIC/PROTENOMIC DATA

Gary An

Objectives: Biological systems exhibit structural and functional levels of organization (gene => protein=>' cell=>tissue=>organ=>organism). Research groups are oriented towards working within these levels of organization, but there are challenges in applying discovered information across their boundaries. This difficulty is related to nonlinearities between the generative mechanisms at one level and the observed phenomenon at the next. This challenge is pervasive in biomedical research, but very pronounced in the areas of genomics and protenomics. Methodological issues related to the volume of data, data noise, heterogeniety of the sample sources and the static nature of measurements further complicate the effective mechanistic interpretation of this data. Agent based translational modeling attempts to transcend these boundaries using a combination of statistical, network and dynamic discrete-event modeling based on mechanistic rules and matched to the data structure format of gene/protein chips. Herein is presented the conceptual framework of this methodology.
Materials: In-silco modeling platform Netlogo, published genomic/protenomic data, published biophysical/biochemical mechanisms of gene activation/regulation.
Methods: Theoretical model using expanded cellular automata (CA), dynamic network analysis, multi-dimensional evolving data structures and discrete-event mechanism-based constraining rules.
Data: Simulated -omic chips are demonstrated to evolve dynamically based on mutable CA neighborhoods and mechanistic rules that can be mapped to wet lab-generated information.
Conclusion: I have developed a prototype agent based translational modeling framework that can potentially simulate the dynamic evolution space of gene chip data by providing a mechanistic link between these static measurements from one time point to another. This method may not be able to identify a specific trajectory between real-world measurement intervals but can restrict the possibility space of behavior and guide further wet lab research. This method is also potentially expandable to link genenomic, protenomic and metabolomic data in a dynamic, mechanistic way.

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A 317

PREDICTION MODELING IN NEUROTRAUMA

Brahm Goldstein

Objective: Computer models of intracranial pressure (ICP) have been developed over the past 30 years that incorporate sophisticated logic (i.e. differential equations [DEs]), yet there has been an unrealized potential for application in the clinical setting. This may be because complex models are difficult to understand and use and study data often lack the clinical annotations needed to facilitate modeling (e.g. the excat timing of medication administration, ventilatory changes, cerebral spinal fluid [CSF] drainage, and environmental stimuli). We have developed a dynamic ICP model based upon a digital library of physiologic signals and clinically annotated events from patients with traumatic brain injury (TBI) and elevated ICP that may lead to subject-specific computer models of ICP dynamics and be used at the bedside to predict and optimize therapy. The objective of this presentation is to briefly review the pathophysiology of TBI and elevated ICP; summarize published models; present a patient-specific model derived from an experimental physiologic challenge protocol; present data from simple, linear prediction models and from a more sophisticated dynamic fluid compartment model; and, to identify challenges for future research.

Materials and Methods: Patient-specific physiologic challenge protocol and Physiologic Data Acquisition System (described in presentation).

Data: The dynamic fluid compartment model of ICP uses DEs to simulate ICP dynamics and curve-fitting algorithms to minimize error predicted ICP. Initial results show significant correlation of predicted ICP (+/- 2 mmHg for up to 70 minutes) with actual ICP changes in response to changes in minute ventilation, head of bed elevation, and CSF drainage. Model limitations are identified and discussed.

Conclusion: Prelimary results of a dynamic ICP prediction model are encouraging. Future challenges include: how to deal with intra- and inter-patient variability; disease nonstationarity and compexity; accurate prediction with multiple and concomitant treatments; and, determination of realistic performance expectations (e.g. shorter prediction time, predicting a patient's disease state compliance versus therapeutic response, and determination of whether a patient is 'predictable' or not).

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A 318

INSIGHTS FROM A MATHEMATICAL MODEL OF ENDOTOXIN TOLERANCE – HOW RELEVANT ARE "IN VIVO" AND "IN VITRO" STUDIES FOR CLINICAL SEPSIS?

Catalin Vasilescu, Mircea Olteanu, Paul Flondor

Objective: Endotoxin tolerance was initially described as an in vivo phenomenon whereby a sublethal injection of endotoxin (LPS) abrogated the response to a subsequent endotoxin exposure. These findings were similar to those noted after in vitro experiments with monocytes/macrophages. The immune depression expressed by the reduced cytokine releasing capacity of the whole blood in sepsis can be explained by the installation of a phenomenon similar to endotoxin tolerance.The mathematical model can provide the framework for achieving a predictive understanding of the cytokines releasing after different dosages and kinetics of LPS. This perspective on modeling of LPS signaling could show the different behaviors of the cell in "in vitro", "in vivo" (experimental animals and human volunteers) and "ex vivo" (whole blood stimulation in septic patients) experiments. We focused on the relevance of the "in vitro" and "in vivo" experiments for the immune status in sepsis.

Material and Methods: A mathematical model of ordinary differential equations of LPS signaling based on endotoxin kinetics and endotoxin tolerance was constructed. The philosophy of the model is based on the Michaelis-Menten equations for enzymatic reactions. The mathematical model was used to reproduce the TNFα production in sepsis. The mathematical model consists of a non-autonomous, non-linear, first order differential system describing the dynamics of the concentrations of TNFα and of the inhibitor, LPS being considered as an input (in various experimental and clinical conditions). A main feature (hypothesis) of this model is the independence of the inhibitor with respect of TNFα (while other factors were neglected). The main aim of the model was to analyze the relationship between LPS and TNFα using as few information as possible. It remains to be established in future work the contribution of some other actors playing a role in the development of endotoxin tolerance: IL-10, Tiff, etc. Some parameters of the system measure the various interactions between the concentration of LPS and TNF and LPS and inhibitory system production. The identification of these parameters is a difficult task and for the moment we used for that purpose some original results of one of the authors as well as published data. The clearance parameters are in general time variable and were estimated as mentioned above. The computer simulations fit well to reported behavior of TNFα. The system is flexible enough to cover most of the experimental and clinical situations and to be treated in various mathematical ways, for example, as a control system, stochastic system, etc..

Data: We tested the assumption that "in vitro" studies and animals experiments ("in vivo") to evaluate the LPS-induced response of monocytes would accurately predict the mechanisms of immunoparalysis from septic patients. Surprisingly, some results have demonstrated a lack of

correlation between results obtained in vitro experiments and those obtained from in vivo experiments. These differences can be explained, at least in part, by the kinetics and by the level (dose, concentration) of the LPS stimulation.

Conclusion: In order to gain insights on the LPS signaling machinery we simulate these experimental conditions in silico. Our hypothesis is that in LPS signaling the kinetics of LPS as well as the existence of an endotoxin-like phenomenon are crucial features. This may explain the contradictory data obtained in in vitro, in vivo and ex vivo stimulation of whole blood from septic patients. There are some particularities of the in vivo and in vitro models that fail to reproduce the conditions of clinical sepsis as seen in human disease.

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A 319

CHANGES IN BLOOD PRESSURE VOLATILITY IN A MURINE MODEL OF SEPSIS

Sergio Zanotti, Jad Skaf, Massimiliano Guglielmi, Nishant Goel, Joseph Parrillo, Steven Hollenberg

Introduction: Nonlinear analysis of hemodynamic parameters may provide insights not available from standard linear measures. However, artifact and the complexity of power spectral analysis required to evaluate nonlinear parameters poses challenges for routine clinical application. Volatility, a measure of the standard deviation variability, has been proposed as an alternative nonlinear parameter.

Hypothesis: Decreased blood pressure variability will occur in a clinically relevant murine model of sepsis and will be detectable by calculating blood pressure volatility (BPV).

Methods: In C57Bl/6 mice (8-12 weeks, 20g, n=24) radiotelemeters for noninvasive measurement of BP were implanted into the ascending aorta. After 5-7 days of recovery, baseline data were collected for 24 hours. The mice were then made septic by cecal ligation and puncture (CLP) and resuscitated with fluids and antibiotics every 6 hours; controls underwent sham ligation. Continuous blood pressure waveforms at 1000Hz, were obtained and SD were calculated on each 5 minute interval. For each animal, the SD cutoff that represented the lowest 5% was calculated, and the percentage of low SD's (representing low volatility) was defined by this cutoff. In addition, the percentage of low volatility periods was calculated for the entire experiment over 4 hour intervals to generate a time course.

Results: In control animals, low BPV was detected in 13.2% of the 5-minute periods post sham-operation. In septic animals, 31.5% of the post-CLP intervals showed low BPV ($p<0.01$ versus baseline and versus control animals). Mortality in the septic group was 60%. Survivors and non-survivors had a similar decrease in BPV early, with eventual normalization in survivors and further perturbation in non-survivors.

Conclusions: Analysis of volatility is less demanding and less susceptible to artifact as a means of expressing blood pressure variability. We have shown dramatic differences in blood pressure variability between septic and control animals in our murine model using this method. This methodology may be able to be extrapolated to critically ill patients, and has the potential to provide a novel marker of hemodynamic decompensation.

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A 320

VENOUS THROMBOEMBOLISM AND HIV INFECTION: A REVIEW

Martin Veller, Martin Veller, Jay Pillai, Dirk le Roux

Since the advent of the HIV/AIDS pandemic in Southern Africa many have reported a substantial rise in the number of patients presenting with venous thromboembolism (VTE). To date no adequate studies have been performed to address this perception yet many mechanisms by which this infection may cause a pro-coagulant state have been described (including antiphospholipid antibodies and lupus anticoagulant, deficiencies of naturally occurring anticoagulants as well as raised levels of clotting factors). These abnormalities have also been correlated with the severity of the disease as well as with the presence of associated conditions.

The resource constrained nature of medical practice in South Africa has resulted in many of such patients presenting with HIV infection and an episode of VTE receiving suboptimal therapy. In order to help address this issue it would be helpful to understand if such a VTE is as a result of the HIV infection primarily or if it was as a result of the end stage manifestations of AIDS. To elucidate this dilemma we embarked on a review of the available literature.

Conclusions:

1. The association between VTE and HIV infection per se appears to exist but has not been conclusively proven.

2. The association between VTE and the secondary manifestations of HIV/AIDS appears to be much stronger.

3. Substantial additional information is required.

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A 321

AWARENESS OF THE SURVIVING SEPSIS CAMPAIGN IN SURGICAL AND EMERGENCY MEDICINE TRAINEES

Luke Evans, Jane Evans, Mark Blunt

Introduction: Data presented at the 2006 Barcelona conference of the European Society of Intensive Care Medicine showed that where implemented, the Surviving Sepsis Campaign Guidelines have improved mortality from sepsis. However, because of overall poor adherence to the guidelines the stated aim of the campaign to reduce mortality from severe sepsis by 25% is unlikely to be met. In the UK patients with sepsis of surgical origin will typically be seen by Emergency Medicine (EM) before being admitted to a surgical ward and are unlikely to be initially managed by ICU. Both the EM and Surgical juniors should therefore be aware of the guidelines. The aim of this study was to determine the level of awareness of the SSC guidelines in Surgical and EM trainees.

Methods: A questionnaire based survey was undertaken of all EM and Surgical Trainees in the Eastern Region of the UK. Participants were recruited by post, telephone, email and in person. The questionnaire assessed whether participants had experience in critical care, were aware of the campaign or its guidelines and assessed level of familiarity of key concepts of the resuscitation bundle of the guidelines. In addition participants were encouraged to comment on any aspect of sepsis management.

Results: Summarised in table 1.There are 29 EM and 52 surgical trainees in the Eastern region, responses were obtained from 22 and 34 respectively. The responses to the key concepts of the resuscitation bundle varied greatly, even between different participants from the same speciality in the same institution, suggesting a lack of clear direction. Free text responses included "the only people that know about guidelines for sepsis are the ICU physicians" and the only time I have heard of Early Goal Directed Therapy was on ER"

Conclusion: Awareness is reasonable amongst EM trainees but poor amongst surgeons. If the aims of the SSC are to be met consideration must be given to differences in healthcare systems in different countries. In the UK educational activities should be directed towards EM and surgical trainees as well as those working in intensive care.

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HEART RATE TO PULSE PRESSURE RATIO AS AN INDEX OF CARDIOVASCULAR SUFFICIENCY IN A PORCINE MODEL OF SEVERE HEMORRHAGIC SHOCK

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Introduction: Hemorrhage induces both hypotension and sympathetically-mediated tachycardia while decreasing arterial pulse pressure (PP) through preload reduction despite increased contractility and vasomotor tone. Thus the ratio HR/PP called "S" should represent a non-linear relation determined by both volume status and physiologic/sympathetic reserve. The objective of this study was to assess the ability of S to identify decompensated shock in our established porcine model of severe hemorrhagic shock.

Methods: Six isoflurane-anesthetized female pigs were rapidly bled to a mean arterial pressure (MAP) of 30 to 40 mmHg and kept there by repeated bleedings as necessary. Volume re-expansion (Hextend) was initiated if MAP was <30 mm Hg. EKG, HR, and arterial pressure were continuously collected. The relation of HR, PP, and S at baseline, over repeated bleedings and at the decompensation point (MAP <30 mm Hg without spontaneous recovery) were analyzed. S values are in min^{-1} mm Hg^{-1}.

Results: 5 pigs survived after a blood loss of 55.6 ±3.6 % of total blood volume. One pig died despite resuscitation. Baseline HR was 88.7 ± 9.4 min^{-1} and showed a minimal increase after first bleeding (99.8 ± 12.3 / min) to a MAP of 30 mm Hg. However, HR progressively increased thereafter reaching 232.5 ± 29.4 min^{-1} at the decompensation point. Unlike HR, PP immediately decreased during the first bleed (43.6 ± 5.2 to 13.8 ± 3.1 mm Hg) and tracked the dynamic changes in repeated bleedings while the overall trend of PP remained relatively constant including at the decompensation point (13.4 ±1.9 mm Hg). S prior to bleeding was very stable and similar across pigs (2.08 ± 0.39). However S progressively increased with initial bleeding (7.9 ± 2.3) and tended to recover with cessation of bleeding. Repeated bleeding episodes were tracked by S changes, and S reached it zenith with decompensation (17.6 + 3.2, x ±SD) being always >13 at that point.

Conclusions: HR changes appear to be insensitive to initial profound hypotension at the early stages of bleeding in this model but to be specific for decompensation. PP is highly sensitive to initial massive

Table 1.

Trainees	Worked in ICU in last 2 years	Claimed to be aware of campaign	Able to name SSC	Any training on sepsis in last 2 years	Claim to be aware of research	Able to name piece of relevant research
Emergency Medicine	8 (36%)	15 (68%)	10 (45%)	13 (59%)	13 (59%)	10 (45%)
Surgery	2 (6%)	6 (18%)	3 (8%)	9 (26%)	13 (38%)	8 (24%)

Table

Heart Rate	Pig 1	Pig 2	Pig 3	Pig 4	Pig 5	Pig 6	Average	St Dev
Baseline	103	83	76	95	89	86	88.67	9.44
After 1st Bleeding	119	86	108	94	102	90	99.83	12.34
Decomp (MAP<30)	218	195	272	210	253	247	232.50	29.37
Pulse Pressure								
Baseline	43.75	48.91	49.64	40.03	35.84	43.23	43.57	5.25
After 1st Bleeding	10.00	11.94	18.50	13.03	10.93	14.69	13.18	3.08
Decomp (MAP<30)	15.65	10.89	13.30	14.58	11.50	14.49	13.40	1.87
S (HR/PP)								
Baseline	2.35	1.70	1.53	2.37	2.48	1.99	2.07	0.39
After 1st Bleeding	11.90	7.20	5.84	7.21	9.33	6.13	7.94	2.30
Decomp (MAP<30)	13.93	17.91	20.45	14.40	22.00	17.05	17.62	3.21

bleeding-induced hypotension but not specific for decompensation. The HR/PP ratio S is stable at baseline across animals and progressively increases over the entire hemorrhagic shock phase making it a more generalizable parameter of cardiovascular sufficiency than either HR or PP. Potentially this ratio could be a useful parameter to quantify cardiovascular sufficiency in humans during severe hemorrhagic shock.

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LUNG NITROXIDATIVE STRESS AS A PROGNOSTIC FACTOR IN MECHANICALLY-VENTILATED SEPTIC PA-TIENTS

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Introduction: During sepsis and mechanical ventilation Nitric Oxide (NO) is produced by endothelial and inflammatory lung cells. We study if pulmonary NO production is a prognostic factor in mechanically-ventilated septic patients.

Materials and Methods: We studied 50 patients with sepsis within the first 48 hours of sepsis. Operating room patients served as control a group (ORCG). Nitrite and nitrate (NO_x^-) and 3-Nitrotyrosine (3NT) in plasma and bronchoalveolar lavage fluid (BALF) were analyzed by the Griess/Vanadium chloride method and ELISA, respectively. Results were expressed as median and interquartile range. Receiver operator curves were constructed to compare the predictive value of NO_x^- values in BALF at admission with othter variables. Kaplan Meier analysis was used to compare survival between high and low BALF NO_x^- levels at admission. A p value less than 0.05 was considered significant.

Results: At study admission in the sepsis group nonsurvivors had higher levels of BALF NO_x^- than survivors 20(17-33) μM,27 versus 72(46-91) μM, 23, $p = 0.0001$. At day 7 BALF 3NT was higher in nonsurvivor septic patients than in survivors 1666(30-3173) pmol/mg protein versus 291(13-1908) pmol/mg protein. BALF NO_x^- had the highest area under the receiver operator curve for mortality (0.812, $p = 0.001$) in relation with other variables. Septic patients with BALF NO_x^- above 36 μM had a relative risk for mortality of 4.23 and an OR of 15.84. The difference between low bronchoalveolar NO (BALF $[NO_x^-] < 36$ μM at admission) group versus high bronchoalveolar NO group (BALF $[NO_x^-] \geq 36$ μM at admission) in ICU mortality was significant , 19% versus 78%.(Log Rank 18.19, $p = 0.00001$).

Conclusion. We conclude that during sepsis there is enhanced lung NO production that is associated with ICU mortality.

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EFFICACY OF MULTI-THERAPY IN SEPSIS CAUSED BY INFECTED PANCREAS NECROSIS

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Objective: Infected pancreatic necrosis (IPN) is the most severe form of acute pancreatitis. Association with septic conditions is the leading cause of mortality. In this study, we present a retrospective analysis of 20 years of experience involving 224 patients who underwent surgery for IPN.

Material and Methods: Since 1986, 224 patients with IPN have been treated. The mean APACHE II score was 15.5 (range 11-32). In all cases, IPN was combined with retroperitoneal abscesses. The surgical treatment was performed on average 18.5 days (range 8-25 days) after the onset of acute pancreatitis. The operative management consisted of wide-ranging necrosectomy in the total affected area, combined with widespread drainage and continuous lavage. In 109 of the 224 cases (49%), some other surgical intervention (distal pancreatic resection, splenectomy, total pancreatectomy, cholecystectomy, sphincteroplasty, gastric suture, or colon resection) was also performed. Following surgery supportive therapy was applied in all of the patients, which was the conventional

basic therapy in 97 patients (Group 1) in the period 1986-1994. In 127 patients (Group 2) in the period 1995-2006, however, this therapy was supplemented with immunonutrition (glutamine and arginine supplementation), suppression of cytokine production (TNF) by pentoxifyllin and dexamethasone, and the prophylaxis of fungal infection (fluconasole). TNF and IL-6 serum levels were measured by ELISA, and the in vitro stimulation of leukocytes was induced by E. coli LPS.

Data: Following surgery, continuous widespread lavage was applied for an average of 44.5 days (range 21-95 days), with an average of 9.5 (range 5-20) litres of saline per day. The bacteriological findings revealed mainly enteral bacteria, but Candida infection was also detected frequently. The incidence of fungal infection was 21%. Forty-eight patients (21.4%) required reoperation. The cytokine production capacity (TNF and IL-6) was shown to correlate with the prognosis. As a consequence of the pentoxifyllin and dexamethasone therapy, the TNF production generally dropped to the normal level. The overall hospital mortality was differed significantly between Groups 1 and 2: 10.3% (10 patients) and 6.4% (8 patients), respectively, died ($p<0.05$).

Conclusion: Our results clearly demonstrate that the sepsis caused by IPN responds well to adequate surgical treatment and continuous, long-standing widespread drainage and lavage, together with multi-therapy comprising immunonutrition and modification of the cytokine production, combined with adequate antibiotic and antifungal medication. Attention to all of these features can lead to a significant reduction of the mortality of IPN.

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BLOOD TRANSFUSION EXCEEDING 175 UNITS IN 24 HOURS IN A PATIENT WITH POST PARTUM HAEMORRHAGE-'WORLD'S FIRST'. ICU PRINCE OF WALES HOSPITALS, SYDNEY, AUSTRALIA

R.K. HariHaran, Thomas R. Solano

Purpose of the Study: Management of postpartum haemorrhage requiring massive transfusion, novel haemostatic agents, surgery and radiological intervention in a young patient.

Methods: Review of literature of postpartum haemorrhage and case report.

Exact Data:

Summary: Arrest of bleeding and resuscitation are cornerstones of management of haemorrhage and is also critical to the patient survival. Inspite of surgery, radiological intervention, haemostatic agents like recombinant coagulation factor V11a and aprotinin, we transfused more than 175 units of blood product in 24 hours in this patient. With massive resuscitation and coagulopathy she developed critical intraabdominal hypertension. Bedside decompression of the abdomen with 3 intercostal chest drains relieved the intra abdominal hypertension leading to immediate improvement in gas exchange and haemodynamics. Following a prolonged ICU stay with complications of bleeding, sepsis and renal failure she was eventually discharged home and remains well post discharge.

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THE GOLD STANDARD TECHNIQUE FOR INTRA-ABDOMINAL PRESSURE MONITORING IN SEPTIC PATIENTS: CONTINUOUS INTRA-ABDOMINAL PRESSURE MONITORING (CIAPM)

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Background and Goal of Study: Abdominal Compartment Syndrome (ACS) can develop within the first 12 hours of intensive care unit (ICU) admission in high-risk (shock, trauma, burn, pancreatitis, peritonitis sepsis) patients. The gold standard of intra-abdominal pressure (IAP) measurement via the urinary catheter is time consuming and its intermittent nature could prevent timely recognition of significant changes in IAP. We propose that continuous IAP (CIAP) can be accurately measured via the three-way catheter.

Materials and Methods: CIAP was measured via the irrigation port of the three-way catheter transduced to the

Table 1- Arterial Blood Gas Results

Date	Time (Hours)	pH	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	Bicarbonate (mmol/l)	Lactate (mmol/l)	Base Deficit	FiO_2
8/3/06	0139	6.39	30.4	42.1	8.1	16	23.5	1.0
8/3/06	0218	6.71	57.6	50.2	6.1	17	25.9	1.0
8/3/06	0252	6.80	518	40	6	15	25	1.0
8/3/06	1114	7.13	228	44.1	14.2	10.8	13.2	0.75
8/3/06	2100	7.03	34.5	86.5	21	4.2	7.4	1.0
8/3/06	2203	7.22	62.5	47.7	19	3.5	7.4	1.0
9/3/06	0157	7.31	74.9	36	17.7	7.3	3	0.8

Table 2 - Coagulation Profile

Date	Time Hours	Haemoglobin (g/L)	Platelets (x 10^9 / L)	Prothrombin Time (Seconds)	Activated Partial Thromboplastin Time(Seconds)	Fibrinogen (g/L)	D-Dimer µgm/ml
8/3/06	0130	82	260				
8/3/06	0215	54	133	21.8	>170	0.6	>20
8/3/06	0252	35	24	44	>170	0.6	>20
8/3/06	0815	57	46	32.1	>170	1.0	9.94
8/3/06	1330	69	51	34.9	142.2	1.2	4.78
8/3/06	1700	65	30	15.2	48.5	1.2	4.7
8/3/06	2100	92	42	17.0	65.4	1.6	5.19
9/3/06	0002	94	30	14	48	2.1	5.4
9/3/06	0157	61	22	24.9	99.2	0.9	3.96
9/3/06	0932	60	34	33.5	>170	1.1	6.74
9/3/06	1200	91	35	20.6	66.9	2.1	5.40

Table 3 - Blood Product details and rFV11a used within 24 Hours

Date	Time (Hours)	rFV11a Each Dose 9.6mg (90mµgm/kg)	Pooled Platelets	Fresh Frozen Plasma	Cryoprecipitate	Packed Red Blood Cell Volume	Total Blood- Product Between Each Dose of rFV11a
8/3/06	0245	First	1	8	20	22	51
8/3/06	0815	Second	3	8	10	26	47
8/3/06	1330	Third	0	8	10	8	26
8/3/06	1700	Fourth	3	10	10	15	38
9/3/06	1200	Fifth	1	4	10	8	23
Total	24 Hours	5 Doses	8	38	60	79	185

bedside monitor as a continuous trace without intermittent clamping of the catheter. The measurements were performed at the Department of General Surgery and ICU (ending in October 2006). Patient's demographics, severity of the injury, type of surgery, body mass index (BMI) were recorded.

Results: Two hundred patients were involved in the study. Their mean age was 65 years and BMI was 27.4 kg/m2. With our novel approach we could detect twenty patients with Abdominal Compartment Syndrome. We will show our new monitoring protocol for intra-abdominal hypertension (IAH) and ACS.

Conclusion: According to our results, CIAP measurement with a three-way urinary catheter seems to be a simple and accurate method ("gold standard") for monitoring IAP in septic patients.

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ACUTE LOWER EXTREMITY COMPARTMENT SYNDROME (ALECS) SCREENING PROTOCOL IN CRITICALLY ILL TRAUMA PATIENTS

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Objective: ALECS is a devastating complication that often presents silently in critically ill trauma patients because the physical exam (PE) is unreliable. Therefore, we developed a protocol to screen high-risk patients.

Material and Methods: This prospective observational study included all shock trauma intensive care unit (STICU) patients who met specific high-risk criteria including: pulmonary artery catheter (PAC)-directed shock resuscitation, open or closed tibial shaft fracture, major vascular injury below the aortic bifurcation, abdominal compartment syndrome, or pelvic/lower extremity crush injury. Patients were screened upon admission and every 4 hours thereafter for the first 48 hours of admission. Screening included PE and anterior/deep posterior calf compartment pressure measurements when PE was concerning or not reliable. A positive screening, defined as a delta P < 30 mmHg (where delta P is the difference between the diastolic blood pressure and the compartment pressure), mandated a four-compartment fasciotomy. Data are presented as mean ± standard deviation.

Data: Over 6 months ending July 2005, there were 2,582 admissions to our Level I Trauma Center. Four hundred and twenty-eight of these were admitted to the STICU, of which 45 (11%) met one or more of our inclusion criteria. Patient age was 38.0 ± 16.6 years, 76 % were male and the injury severity score was 29.0 ± 12.0. Eighty-seven percent of those that met inclusion criteria had a blunt mechanism of injury, with the most common cause being motor vehicle collision (51%). Twenty-six (58%) patients had one inclusion criterion, 11 (24%) two, 6 (13%) three, and 2 (4%) four. ALECS occurred in 9 patients (20% of screened patients and 2% of STICU admits). The diagnostic delta P was 20.6 ± 3.7 mmHg. The time from STICU admission to the development of ALECS was 8.9 ± 5.0 hours (range 3-18 hours). The findings at fasciotomy were all consistent with compartment syndrome (muscle bulging without necrosis).The most frequent inclusion criterion was PAC-directed shock resuscitation in 27 (60%) patients. Six (22%) of these patients developed ALECS. This subset of patients was in severe shock with a base deficit of 14.7 ± 5.1 and a lactate of 14.7 ± 4.3. The total fluid requirements in these patients for the first 24 hours of admission were 42.2 ± 21.8 L (crystalloids: 23.0 ± 12.9 L and blood products: 18.8 ± 13.4 L). A total of 11 (24%) screened patients died, including 6 (67%) who developed ALECS. In PAC-directed shock resuscitation patients, the mortality rate was 83%. None of the non-screened patients developed ALECS and 6% died.

Conclusion: We developed a sensitive screening protocol for the early detection of ALECS. Based on these results we have shortened the screening period to 24 hours. Future plans include re-evaluating the modified protocol and identifying better predictors for the development of ALECS.

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SLEEP IN THE INTENSIVE CARE UNIT: HOW WELL DO OUR PATIENTS SLEEP?

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Objective: Deep sleep (Stages 3 and 4) is a particularly important part of a normal, restorative sleep cycle. Sleep during intensive care unit (ICU) stay is frequently disrupted, and patients commonly describe symptoms of sleep deprivation. Mechanical ventilation may allow higher levels of pain control and sedation, thus facilitating sleep. The purpose of this study was to describe the quantity and quality of sleep in ICU patients with and without mechanical ventilation. We hypothesized that sleep architecture is abnormal in both groups, but that intubated patients experience less sleep deprivation.

Methods: A prospective observational cohort study was performed at our urban level one trauma center. A convenience sample of surgical/trauma patients cared for in the ICU underwent 24 hour polysomnography (PSG) in order to evaluate sleep patterns. All PSG recordings were performed and monitored by a certified sleep technician. All PSG recordings were scored and read by a single neurologist trained in PSG interpretation. Patients were grouped according to need for mechanical ventilation. Kruskal Wallis ANOVA was used to explore for differences between groups. All PSG recordings were performed greater than 24 hours after admission to the ICU or the administration of a general anesthetic. Patients with traumatic brain injury were excluded.

Results: Thirteen patients were selected to undergo PSG. Four patients were mechanically ventilated and 9 patients were breathing spontaneously. Mean age was 42.5 years (range; 20-83), 84.6% were male, and 69.2% were injured. Total PSG recording time was 268 hours (mean 20.6 hours/patient), total sleep time captured by PSG was 124 hours (mean 9.50 hours/patient). As a group the mechanically ventilated (MV) patients received more analgesia and sedation then the spontaneously breathing (SB) patients. Patterns of sleep were present in both groups; however, neither group achieved normal sleep architecture. Mechanically ventilated/sedated patients spent a longer amount of time in stage 3 non-REM sleep with a trend toward spending more time in stage 2 non-REM sleep. (TABLE) There were no other differences noted between groups.

Conclusions: Patients do achieve measurable sleep while cared for in the surgical ICU. However, sleep is fragmented with markedly abnormal sleep architecture. Although the mechanically ventilated group spent more time in stage 3 non-REM sleep, both groups had markedly depressed levels of the deeper restorative

Table

	Mechanically Ventilated (n=4)	Spontaneously Breathing (n=9)	p-Value
Age (years)	46.76 + 27.4	40.6 + 17.6	0.817
Total PSG Recording Time (minutes)	1333 + 180	1193 + 191	0.123
Total Sleep Time (hours)	13.9 + 5.40	7.55 + 6.40	0.123
Awakenings/hour of sleep	4.10 + 1.62	7.84 + 6.29	0.121
Stage 1 Time (minutes)	146 + 80.9	171 + 144	0.877
Stage 2 Time (minutes)	670 + 379	248 + 276	0.064
Stage 3 Time (minutes)	10.4 + 7.25	0	0.001
Stage 4 Time (minutes)	0	0	1.00
REM Time (minutes)	7.25 + 13.8	33.9 + 74.5	0.867

stages of sleep as well as depressed levels of REM sleep. Further studies on the effects of a strategy to promote sleep during care in the surgical ICU are warranted.

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EFFECT OF LOW ENERGY ERCHONIA LASER ON TISSUE INJURY IN ISCHEMIC ZONE OF BURN WOUND

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Introduction: Apoptotic cells are present in the ischemic zone of burn wound that extends immediately beyond the margins of visible burn injury. Reperfusion injury is thought to be contributing to apoptosis here. Mitochondrial pathway of apoptosis is activated by reactive oxygen species, severe depletion of ATP, Ca^{+2} accumulation within mitochondria and has shown to be involved in myocardial injury following ischemia reperfusion. Low Level Laser (630-640 nm wavelength) enhances ATP production in the mitochondria, which provides more energy substrate for cellular respiration and metabolism. We hypothesized that Low Level LASER (ERCHONIA) may reduce apoptotic cell death, in the zone of stasis, induced by mitochondrial mechanisms.

Methods: 18 mice (C57BL/6) were received a 30 % total body surface area dorsal scald burn. Control group (n=9) received resuscitation with 2.5 cc normal saline and analgesics. Study group (n=9) received treatment with Erchonia low-level laser (635 nm) in addition to resuscitation and analgesia. The irradiation was given for 180 sec immediately after the burn and every 12 hours until the endpoint of the study at 48 h. The LASER irradiation was given over the wound and an adjoining area of 1 inch width. Visible burn wound and adjacent tissue were biopsied for histological review. TUNEL assays and measurement of apoptotic index were carried out on adjoining area of burn wound representing the zone of stasis.

Results: Light microscopy examination of histological specimen, stained with H&E, demonstrated changes consistent with zone of stasis. Mean apoptotic index (AI) in the zone of stasis for control group and ERCHONIA groups respectively were 0.181 (± 0.02 SE) and 0.108 (± 0.06 SE) at 48 hours. The statistical analysis was done using ANOVA test. The difference between the groups is statistically significant ($p< 0.01$).

Conclusion: Our results suggest a protective role of Low Level LASER energy- Erchonia Laser- in apoptotic injury in the zone of stasis in partial thickness burn wounds. This protective effect may be due to enhanced mitochondria energy status. We plan to study exact mechanisms involved, e.g.- markers of mitochondrial pathway of apoptosis. This non-invasive technology may have clinical application for tissue preservation in the thermally injured wound.

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CORRELATION OF S100B WITH CT FINDINGS AND INTRACRANIAL PRESSURE IN PATIENTS WITH ISOLATED SEVERE TRAUMATIC BRAIN INJURY-A PROSPECTIVE STUDY

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Objective: The value of serum protein S100B in patients with traumatic brain injury (TBI) has been discussed in numerous recent studies. Previous studies have investigated its relevance in mild and severe TBI. Although it has been reported that S100B is not a reliable marker of brain damage in every setting (i.e., acute hemorrhagic shock and multiple trauma), an association with outcome in patients with TBI has been described. The relationship of serum S100B to radiological findings according to the Marshall score has been published. To date, however, neither a correlation between serum S 100B and radiological findings using volumetry instruments to quantify intracranial hematomas or contusion areas, nor a correlation between serum S 100B and intracranial pressure (ICP) in TBI have been investigated.

Material and Methods: We prospectively investigated 118 patients after severe isolated TBI. Continuous ICP monitoring was performed using a standardized intraparenchymal device. All patients had computerized tomography (CT) scans of the brain routinely on arrival and during their stay at the intensive care unit (ICU). Volume (ml) of intracranial hematomas and contusions were measured using a digital volumetry program. Midline shift and ventricular compression were quantified. Levels of S100B were obtained on admission and daily during ICU stay. When CT scans were performed, S100B was measured immediately before each CT. Samples were centrifuged and stored at -20°C. S100B was measured immunoluminometrically with Elecsys® S100-immunoassay. Levels of S100B were then correlated with ICP values and CT scans. Spearman correlation coefficient was calculated. Values of $p<0.05$ were considered significant.

Data: We found a significant correlation between S100B and dimensions of intracranial lesions ($r=0.634$; $p=0.004$). Furthermore, we found a significant correlation between S 100B and ICP ($r=0.754$; $p<0.001$), midline shift ($r=0.846$; $p<0.001$) and ventricular compression ($r=0.712$; $p<0.001$). We did not find a correlation between S100B and morphology ($p=0.142$) or localization ($p=0.094$) of intracranial lesions.

Discussion: Our data show a significant correlation between S100B and CT findings on the one hand and with ICP on the other. As a consequence, measuring S100B in patients with isolated severe TBI could be used additionally to monitor these patients. Furthermore, frequency of routine CT scans might be reduced in some patients when S100B levels are measured routinely and remain low.

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IMPACT OF CARDIOPULMONARY BYPASS ON MICROCIRCULATORY PERFUSION: A PILOT STUDY

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Background: Severe systemic inflammation, such as that seen with sepsis, is known to alter microcirculatory perfusion. Exposure to extracorporeal circulation also induces a profound systemic inflammatory response.

Hypothesis: We hypothesize that exposure to cardiopulmonary bypass (CPB) induces demonstrable changes in microcirculatory blood flow velocity.

Methods: This was a prospective observational study of cardiac surgery patients. We used Orthogonal Polarization Spectral (OPS) videomicroscopy to obtain sublingual microcirculatory perfusion images at three time points: (1) pre-operative, (2) immediately post-operative upon arrival in the ICU (post-CPB), and (3) post-recovery prior to hospital discharge. Off-line quantitative analysis of arteriolar velocity was performed in which arterioles were identified by diverging flow at branch points and velocity measured with a space-time diagram using image analysis software (MicroVision Medical, Amsterdam). The software operates on a segmentation algorithm that allows recognition of vessels and graphically represents the velocity of the red blood cells over time. We recorded comprehensive hemodynamic parameters (including vasopressor utilization) at the time of each image acquisition. We used parallel univariate plots to describe the full range of experience of microcirculatory perfusion indices in these subjects.

Results: We enrolled 6 subjects. Mean age was 62 ± 12 years. All patients were exposed to extracorporeal circulation with cardiopulmonary bypass (CPB). Mean "on-pump" CPB time was 117 ± 29 minutes. All 6 patients had vasoactive drug infusions at the time of imaging [drug(n)]: nitroglycerin (6), dopamine (1), phenylephrine (1), epinephrine (1), and nitroprusside (1). Image analysis of arteriolar blood flow velocities (microns/sec) shows a divergence in flow velocities in the post-CPB images from pre-operative and recovery images. The direction of change during the post-CPB period was not homogeneous as some patients showed an increased arteriolar velocity (n=4) and others decreased arteriolar velocity (n=2).

Conclusion: Compared to pre-operative and post-recovery measurements, the post-CPB state is associated with alterations of microcirculatory blood flow. The direction of change in arteriolar velocity was not uniform among study subjects. This heterogeneity of effect may be due to non-uniformity of global hemodynamics, vasoactive medications, or the degree of systemic inflammation. The findings of this pilot study are hypothesis generating for future studies aimed at defining the critical determinants of microcirculatory flow alterations post-CPB.

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TISSUE OXYGEN SATURATION PREDICTS THE DEVELOPMENT OF ORGAN FAILURE DURING TRAUMATIC SHOCK RESUSCITATION

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Objective: Near-infrared spectroscopy (NIRS) permits continuous, noninvasive measurement of muscle tissue oxygen saturation (StO_2) which is a potential indicator of shock severity. Our purpose was to evaluate how well StO_2, measured early after hospital arrival, compared to serum lactate in predicting the negative outcomes of Multiple Organ Dysfunction Syndrome (MODS) and mortality in severely injured trauma patients who presented in shock and required blood transfusion.

Material and Methods: This prospective, observational study enrolled 383 adults with high risk torso trauma at 7

Table: Outcome Related to Minimum StO_2 and Maximum Lactate Collected Within First Hour of Emergency Department Arrival

	MODS	Mortality	Outcome (Negative/Positive)			
Parameter	Max Lactate	Min StO_2	Max Lactate	Min StO_2	Max Lactate	Min StO_2
# patients with/without outcome	27/107	50/292	27/127	55/325	48/106	96/284
Cutoff	4 mmol/L	75 %	4	75	4	75
AUC (p-value)	.65 (p=0.0089)	.66 (p=0.0001)	.71 (p=0.0002)	.72 (p<0.0001)	.67 (p=0.0002)	.70 (p<0.0001)
Sensitivity	63%	78%	74%	91%	67%	84%
Specificity	47%	39%	46%	37%	47%	39%
Positive Predictive Value (PPV)	23%	18%	23%	20%	36%	32%
Negative Predictive Value (NPV)	83%	91%	89%	96%	76%	88%
Mean ± SD in patients with outcome	6 ± 4	56 ± 25	8 ± 6	50 ± 24	7 ± 6	54 ± 24
Mean ± SD in patients without outcome	5 ± 3	68 ± 17	5 ± 3	67 ± 18	5 ± 3	68 ± 17

US Level I trauma centers between October 2004 and February 2006. Measurement of StO_2 (InSpectra™ system, Hutchinson Technology Inc.) on the thenar eminence and collection of standard clinical parameters began within 30 minutes of hospital arrival and continued for 24 hours. Clinicians were blinded to StO_2. Serum lactate was obtained at intervals determined by clinicians. MODS was defined as a multiorgan dysfunction score ≥6 after 3 days (modified Marshall scale). Mortality was assessed between study enrollment and the subsequent 28 days.

Data: In this severely injured cohort (mean ISS =28 ± 15, 67% with blunt injury), among those eligible for analysis, 50 patients developed MODS and 55 died. StO_2 below 75% was a significant early predictor of MODS and mortality (p=<0.05 to test AUC > 0.50) and performed comparably to maximum lactate in discriminating negative outcomes.

Conclusion: StO_2 within the first hour of hospital arrival functions as well as lactate in indicating hypoperfusion in severely injured patients and discriminating those who will develop MODS and mortality, yet has advantages of being continuous and noninvasive.

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PROTOCOL-DRIVEN DAILY SPONTANEOUS BREATHING TRIALS ARE FEASIBLE AND SAFE IN INTUBATED TRAUMA PATIENTS

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Objective: Critically ill trauma patients often require mechanical ventilation in the intensive care unit. Despite their reported utility and safety, daily spontaneous breathing trials are rarely conducted or reported on trauma patients.

Material and Methods: Every mechanically ventilated trauma patient in a 24 bed academic surgical/burn/trauma intensive care unit received a daily spontaneous breathing trial (SBT) over one year. Registered nurses and respiratory therapists initiated the SBT under a standing order. The three step protocol included a safety screen, a two-minute evaluation, and a thirty minute trial. The safety screen ensured unstable patients would not undergo undue or unsafe stress. During the two minute evaluation, both a registered nurse and respiratory therapist remained at the bedside to directly observe patient response. A patient passed the spontaneous breathing trial with a rapid shallow breathing index of <100, normal oxygen saturations, and stable vital signs. Patient data was compiled prospectively.

Data: Of 748 patients admitted to the surgical intensive care unit, 189 patients were mechanically ventilated trauma patients. These 189 patients had 210 mechanical ventilation episodes. The mean patient age was 39 (range 16-86). 138 (73%) patients suffered blunt trauma, 44 (23%) penetrating trauma, and 7 (4%) burn injury. 95% of patients survived to hospital discharge (180/189patients). 1262 spontaneous breathing trials were performed, 895 on patients ventilated via endotracheal tube (ETT). There were no adverse events associated with the breathing trials. Of the 271 SBTs that were completed and passed in ETT patients, 115 (42%) were extubated. The average three month extubation rate increased from 32% in the first three months to 49% in the last three months. The most common reasons cited for not extubating a passing patient were mental status (23%), sedation (7%), and a pending procedure (41%). A reason was not mentioned 22% of the time. There were 11 uncomplicated reintubations among 153 total extubations (7%). 38 extubations occurred without passing a complete SBT. The tracheostomy rate was 27% (49 tracheostomies/182 intubated patients). 7 patients (4%) began mechanical ventilation in the ICU with a tracheostomy.

Conclusion: Routine use of protocol-driven daily spontaneous breathing trials is effective and safe in the trauma population. Trauma patients who are critically ill and survive to the intensive care unit intubated have a very low mortality in this setting. 58% of SBTs passed did not result in prompt orders to extubate, suggesting that physicians are not confident that passing a trial predicts a safe extubation.

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SCREENING OF LIVER FUNCTIONS WITH A CELL BASED BIOSENSOR IN PATIENTS WITH SEPTIC SHOCK

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Objective: The development of liver failure and the late diagnosis of this disease is a major problem in critically ill patients. Early diagnosis of liver failure can enable early onset of therapy and lead to an improvement of prognosis of these patients. Liver failure is not only a problem of primary liver diseases: nearly 19% of patients with septic shock and multi organ failure have a liver failure in the course of disease (1).

Material and Methods: We have developed a new test device for early diagnosis of liver failure (patent pending). The basic test compound are human liver cells (HepG2/C3a). In a standardised mikrotiterplate assay the toxicity of patient plasma is tested. After a incubation time of 72 hours viability of cells (XTT test), the cytochrome 1A2 activity (metabolism of etoxyresorufin) and synthesis of proteins (albumin) are measured. In a clinical study in 28 patients 3 test groups were investigated: ICU-patients with septic shock (n=10, ICU-S), ICU-patients without sepsis (n=5, ICU) and healty volunteers (n=13, HV).

Data: The 28-day mortality was 30% in the ICU-S group. All patients of ICU group survived. Mean Apache II scores were 31,3 in the ICU-S group and 11,8 in the ICU group. All patients had a plasma total billirubin below 70 µmol/liter, but all septic patients had an INR value above 1,5. Only the plasma of patients with septic shock showed a markedly decreased (and statistically significant, U-test) albumin-synthesis (ICU-S: 43.6-SD:39.6, ICU: 87.3-SD:21.3, HV: 89.8-SD:34.5; mg/l) and cytochrome 1A2 activity (ICU-S: 6.0-SD:5.9, ICU: 14.6-SD:3.5, HV: 15.7-SD:2.6; pmol/well resorufin) in the test device compared with both other test-groups. The XTT test showes a impaired viability only in the ICU-S group (ICU-S: 1.6-SD:0.5, ICU: 1.8-SD:0.3, HV: 1.8-SD:0,4; extinction/well).

Conclusion: The presented liver cell based biosensor may contribute to an early diagnosis of liver failure in septic patients. Ongoing clinical studies with septic patients and liver transplanted patients must verify the clinical relevance of the new test.

Literature: (1) Bakker J et al, Crit Care Med 2004, 32 (1): 1-12

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NEUTROPHIL-DERIVED CELL-FREE DNA (CF-DNA), A NOVEL POTENTIAL IMMEDIATE EARLY MARKER IN DAMAGE CONTROL, CORRELATES WITH SEVERITY OF INJURY AND MAY PREDICT SIRS

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Objective: In patients with multiple trauma, hyperactivated neutrophils are closely related to pathophysiologic sequelae such as capillary leakage syndrome, haemodynamic disorders, SIRS, and sepsis. The prediction of these neutrophil-mediated sequelae currently is not possible. Recently, it has been shown that activated neutrophils may form "neutrophil extracellular traps" (NETS) consisting of neutrophil DNA, histones, and proteases (Science, 303:1532-5). Since NETS may severely impair microvascular circulation and thus may lead to posttraumatic organ dysfunction we evaluated a novel assay that allows the rapid quantification of cell-free DNA (cf-DNA) in the patient's blood.

Material and Methods: The quantification of cf-DNA was performed according to a novel assay (Leukocare AG, Munich) based on a fluorescent dye that intercalates into DNA. Blood samples (serum/plasma) can be analyzed within minutes with a fluorescence plate reader. In vitro tests with isolated neutrophils from healthy donors and animal experiments were carried out to define neutrophil cf-DNA characteristics and kinetics. Moreover, blood samples from patients with multiple trauma were used to study time kinetics and correlations with patient's outcome.

Data: Ex vivo, neutrophils but not other blood cells were shown to form NETS upon stimulation with PMA or following mechanical stress. cf-DNA values increased up to 10-fold in stimulated versus non-stimulated blood or isolated neutrophils. In porcine ischemia/reperfusion experiments cf-DNA values increased up to 15-fold immediately after reperfusion. In a clinical study with 15 severely injured patients (ISS >16) cf-DNA kinetics correlated with the severity of trauma and outcome. Compared with cf-DNA from healthy donors the cf-DNA values were significantly increased in patient's blood samples obtained in the shock room. A rapid decrease of cf-DNA values until day 2 after trauma with subsequent low values was associated with a good prognosis whereas slow decrease and secondary increase within a weak was associated with the development of SIRS, sepsis, and death.

Conclusion: cf-DNA seems to be an immediate early marker for the severity of the injury and in addition may predict second hits such as ischemia/reperfusion-related inflammation, development of posttraumatic SIRS and sepsis. However, a large clinical trial with patients suffering from multiple trauma ought to be conducted in order to confirm the value of cf-DNA in damage control.

Table 1 - Patient demograhics by treatment and timing of exchange procedure (*=p<0.05)

Group	N	Age	Sex Male	Mean NISS	Open #	Gd 3 Open	Delay 1° proc.	Duration 1° proc.	ICU stay	Died	Non-Union
ETC	81	33.3	60	25	23	1	4.4h	175m	9.9d	1	6 (8%)
DCO	111	32.3	73	36*	33	12*	3.6h	94m*	23d*	8	2 (2%)
<7days	31	31.2	21	31.2	9	1	3.7h	103m	10.6d	0	-
7-14 days	28	31.5	19	33.2	10	4	3.8h	104m	22.1d	0	-
>14 days	53	32.6	33	40.3*	13	6	3.6h	94m	29.1d	8*	-

Table 2 - Infective complications by treatment group and timing of exchange procedure (*=p<0.05)

Group	N	Timing IMN (d)	Contamination		Superficial		Deep		ROMW		Any infection	
ETC	81	<1	3	3.7%	5	6.1%	3	3.7%	2	2.5%	9	11.1%
DCO	111	14.1	14*	12.6%	4	3.6%	6	5.4%	2	1.8%	12	10.8%
<7d	31	4.3	1	3.2%	1	3.2%	3	9.6%	0	0%	4	12.9%
7-14 d	28	11.1	1	3.6%	0	0%	2	7.1%	2	7.1%	4	14.2%
>14 d	53	23.8	12*	22.6%	3	5.6%	1	1.9%	0	0%	4	7.6%

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DAMAGE CONTROL IN FEMORAL SHAFT FRACTURES A RISK OF LOCAL INFECTION?

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Hypothesis: In polytrauma patients with femoral shaft fractures, damage control orthopaedics (DCO) entails primary external fixation and subsequent conversion to an intramedullary device (IMN). Sub-clinical contamination of external fixator pin sites is relatively common and it is argued that a DCO approach may risk subsequent local infective complications. We aimed to determine the rate of wound infection following DCO procedures and primary IMN for femoral fracture stabilisation in severely injured patients.

Materials and Methods: An evaluation of a prospective patient database of patients treated in our institution between 1996 and 2002 was performed. Inclusion criteria were femoral shaft fracture, New Injury Severity Score (NISS) 20 points or more, age more than 16 and survival more than 2 weeks. Two groups, damage control (DCO) and early total care (ETC) (1° Nail), were formed. Contamination was defined as positive culture from the wound or fixator pin-sites without clinical signs of infection. Superficial infection was a combination of positive bacterial swabs and local or systemic signs of infection. Deep infection defined as was any case requiring surgical intervention with a sub-group requiring removal of femoral metal work (ROMW) also defined.

Results: 173 patients met the criteria for inclusion, with 192 fractures (19 bilateral). The mean follow up was 19 months. Table 1 illustrates patient demographics.

Patients in the damage control group were more severely injured than those undergoing primary intramedullary nailing. There were also more severe (Grade 3 A,B or C) local soft tissue injuries in this group. 98 of the 111 DCO patients underwent subsequent IMN. The others either died after the initial 2 week period without conversion being appropriate, or it was elected to complete treatment with external fixation due to local or systemic complications. The mean time of exchange an ex/fix to a nail was 14.1 days. Table 2 shows rates of infective complications.

Though contamination rates were higher in the DCO group, there was no excess of infective complications. Contamination increased significantly in patients who underwent conversion to IMN after 14 days. Grade 3 open injury was significantly associated with infection irrespective of treament.

Conclusion / Significance: This study demonstrates that infection rates following DCO for femoral fractures are not significantly different to those observed following primary intramedullary nailing. The overall risk of deep infection in the DCO group did not show any correlation with the timing of converting the external fixator to a nail. The risk of contamination was higher in patients where the exchange nailing was performed after a period of 2 weeks.

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PERIOPERATIVE EVALUATION OF CARDIAC FUNCTION IN BURN-INJURED CHILDREN

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Introduction: Early cardiac dysfunction following burn injury is a well-described consequence of inflammation in experimental models (1), yet clinical evidence following

burn injury is less characterized and somewhat controversial. Perioperative burn patients have large fluid shifts, high levels of catecholamines and other mediators and general anesthesia, which can alter preload, afterload and contractility, respectively. The present study addressed the extent of systolic and diastolic dysfunction in perioperative burn-injured children. An understanding of the impact of burn injury on cardiac function may better direct perioperative burn management to reduce complications following major burn injury and surgery.

Methods: Intra-operative transesophageal echocardiography (TEE) (Vivid 7, GE Medical Systems, Milwaukee, WI) was performed during the first 60 days following burn injury (perioperative ICU period) in 28 children, ages 2-18 yr, with burns greater than 40% TBSA. Systolic function was determined by volumetric measurements of the left ventricle in the transgastric long axis view at the end of diastole (EDV) and systole (ESV). Ejection fraction (EF%) was calculated by (EDV – ESV)/(EDV). Diastolic data was obtained from pulsed wave Doppler measurements across the mitral valve and tissue Doppler measurements of the mitral annular velocity during diastole (e'). A ratio of mitral inflow velocity (E–wave) to tissue Doppler (e') was used as an index of diastolic function. Based on historical data, an ejection fraction (EF%) > 60% was considered normal (2) and an E/e' ratio < 8 = normal, 8-15 = impaired function and > 15 = severe diastolic dysfunction (3).

Results: Systolic function: EF% was 48 ± 2 (Mean ±SEM). Severe systolic dysfunction (EF %< 40%) was observed in 6/28 burn children. Diastolic data were obtained in 22/28 burn children: E/e' was 13 ± 2 (Mean ±SEM). Evidence of severe diastolic dysfunction (E/e' >15) was observed in 4/22 patients. A weak positive correlation between systolic and diastolic dysfunction was also observed (r2 = 0.18)

Conclusion: Children with severe burn injury have reduced systolic and diastolic function during their perioperative ICU course. A subset of burn-injured children appear to have severe cardiac dysfunction. Specific perioperative clinical implications of cardiac dysfunction in perioperative burn patients include high sensitivity to preload, afterload and contractile depressive agents. Knowledge of cardiac function can be used to better optimize cardiac performance in perioperative burn patients in order to reduce the risks of hypoperfusion and pulmonary and peripheral edema.

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OPERATIVE MORTALITY AND MORBIDITY RATES AMONG SURGEONS: COMPARISON OF POSSUM SCORING SYSTEM AND COSTS RELATED IN ABDOMINAL SURGERY

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Purpose: The original Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity predictor equation for mortality scoring systems was developed to provide risk-adjusted mortality rates in general surgery. The aim of this study was to compare crude and risk-adjusted operative mortality rates among six surgeons using the above scoring systems and assess their applicability for patients scored retrospectively.

Methods: A total of 725 consecutive patients undergoing major gastrointestinal surgery were analyzed; 60 percent underwent colorectal, 22.5 percent underwent upper gastrointestinal, 7.5 percent underwent small-bowel surgery, 6 percent hepatobiliopancreatic and 4 percent other (peritonectomy, nephrectomy...). The observed:-predicted mortality and morbidity ratios using the POSSUM predictor equation and analytical costs for each patient were calculated for each surgeonand grouped by Diagnosis Related Groups.

Results: The actual overall operative mortality rate was 11.1 percent (elective was 3.5 percent, and emergency was 12.1 percent). The POSSUM score was found to over-predict death by a factor of 1.2: 14.5 percent ($P < 0.01$). Mortality rates among the four surgeons varied from 7.6 to 14.7 percent but depended on the proportion of elective vs. emergency surgery. The observed:predicted morbidity ratio for POSSUM y was close to unity (0.912-1.037), and morbidity rates (23 percent) were accurately predicted by the Possum score. When mortality and morbidity scores were correlated to cost-induced by each surgeon those with more complex cases showed lower mortality, morbidity and induced costs when perioperative scorings adjusted each ratio.

Conclusion: The POSSUM predictor equation for mortality equation seems not to be an accurate predictor of mortality in gastrointestinal surgery in well-trained surgeons. But it would seem to provide a good choice for analyzing operative mortality and morbidity rates and induced costs for individual surgeons, taking into account variation in case mix. This has important implications for the future assessment of surgeons' clinical standards, assessment of quality of surgical care and economic implications for the induced costs of each treatment.

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IMMUNE MONITORING OF THE CRITICALLY ILL - MAJOR TRAUMA

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In the multiply traumatised patient, the relative contribution to the systemic inflammatory response by the first hit and by the subsequent surgery (second hit) continues to be debated. Although primary operative stabilisation of femoral fractures reduce the overall frequency rate of posttraumatic complications, adverse changes in inflammatory mediators have been described with respect to primary intramedullary stabilisation of fractures. It is recognised that IL-6 plays a pivotal role in determining the insult induced both by surgery and trauma. Moreover, surgery following trauma increases the immune response. This may trigger the development of SIRS, which puts the patient at risk to develop MODS. Therefore, the impact of different surgical procedures was investigated alone and in the context of multiple trauma together with the time point of secondary surgery. Patients with multiple injuries were prospectively studied. Inclusion criteria were ISS>18 without severe traumatic brain injury. Multiply injured patients were followed up for 14 days. Samples were immediately drawn and in the intensive care unit every day. IL-6 and LBP were measured. The Denver-score was used to define the incidence of MODS. In addition, these multiply traumatised patients were split into an early secondary surgery group (ESS, surgery at days 2-4) and a late secondary surgery group (LSS, surgery at days 5-8). Perioperatively, IL-6 serum levels were evaluated. Interleukin-6 values on admission were highest in group +MODS. Multiply traumatised patients with highest levels after surgery stayed longer on the ICU, had longer ventilation times and showed a higher incidence of SIRS, sepsis and MODS. Sepsis could be differentiated using LBP. Patients developing sepsis expressed significantly increased LBP levels up to 400 ug/ml. Patients undergoing ESS developed more often MODS than those with LSS (46.5% vs. 15.7%). IL-6 secretion during surgery is more pronounced in those multiply traumatised patients undergoing ESS. During the posttraumatic course, both groups show a comparable early secretion of IL-6 with similar declines over the first two posttraumatic days. Thereafter, patients with ESS demonstrate a further increase in the concentrations of IL-6, which is not observed in those with LSS. A combination of initial IL-6 values >500 pg/ml and surgery on day 2-4 positively correlated with the development of MODS ($r=0.96$, $p<0.001$), whereas surgery on days 6-8 did not ($r=0.57$, $p<0.07$). This study showed that surgical intervention for femoral fractures induces an adequate, but small, response as seen by transiently increased IL-6 serum levels. The increase was not as prominent as observed after surgical intervention in the multiply traumatised patient. Thus, surgery adds to the proinflammatory cytokine release induced by the initial injury. These increases are associated with the development of complications (SIRS and MODS). Especially during days 2-4, patients are very vulnerable. On the one hand, this may be associated with the proinflammation, on the other cellular dysergia may play an important role. Cellular dysergia gives microorganisms the opportunity to survive. Those worsen outcome and increase the already existent risk for developing MODS. On a later time point, the body has a recovered immune system (days 5-8) and secondary surgery can be performed with less risk. Although the clinical condition may appear stable, it is necessary to evaluate and respect the inflammatory burden of the patient. The time and type of primary and secondary surgery has to be evaluated carefully in this light.

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IMMUNE MONITORING OF THE CRITICALLY ILL - TRANSPLANTATION

Gerald Brandacher

Since immune responses directed against transplant allografts are the main drivers of rejection, the ability to accurately quantitate antidonor immunity is an important goal in clinical transplantation. However, accurate diagnosis of acute rejection still relies on the invasive procedure of graft needle biopsy, which can entail various complications and sampling errors. Due to the fact that during acute rejection lymphocytes and macrophages are rapidly activated and release large amounts of cytokines and other inflammatory mediators upon activation an alternative approach to graft biopsy could be to measure such molecules or their soluble receptors in biological fluids. However, despite a multitude of studies on virtually all cytokines, there are still almost no convincing data so far on which of the many potential factors to focus on. Because no tests are available to accurately and consistently predict the risk for allograft rejection, the development of less invasive diagnostic methods that additionally provide insights into the pathophysiology of rejection would be of considerable value. As a consequence, immunosuppressive therapy could be individualized and the adverse effects of inadequate or over-immunosuppression minimized.

The immunomodulatory enzyme indoleamine 2,3-dioxygenase (IDO) is activated by interferon-γ (IFN-γ) and via tryptophan depletion and the production of proapoptotic metabolites suppresses adaptive T cell-mediated immunity in inflammation, host immune defence and maternal tolerance. The rate of tryptophan degradation expressed by the ratio of product (kynurenine, kyn) and substrate (tryptophan, trp) kyn/trp was seen to be a good estimate of biological IDO-enzyme activity. Close correlations exist between markers of immune activation like neopterin and kyn/trp. Neopterin, a pteridine derivative secreted by monocyte-derived macrophages upon stimulation with IFN-g, is a sensitive marker of cellular Th1-type immune responses. In previous studies neopterin measurement in serum and urine has been shown to be of clinical value in predicting immunological complications such as acute rejection in patients following kidney transplantation. The current study shows that the reliability and the advantages of such a non-invasive

approach could be further increased by simultaneously measuring neopterin and kyn/trp. These initial observations allow us to hypothesize that changes in tryptophan metabolism hold the potential for developing a novel prognostic test for acute rejection of renal allografts. Analyzing IDO activity immediately after transplantation could help define the subgroup of patients more likely to experience acute rejection with additional implications for immediate implementation of graft-saving therapy.

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PHARMACOGENETICS OF ANESTHESIA

Kirk Hogan

The prevalence and severity of many perioperative complications are strongly associated with genetic predispositions that are often unknown in advance of surgery, including drug toxicity and inefficacy, aberrant thrombosis, prolonged paralysis, bronchospasm, and sepsis. Although many genetic variations conferring heightened risk fail to manifest phenotypes in the absence of drug exposure or surgical stress, they are readily detectable by DNA-based methods and their consequences may be avoided by selection of alternative interventions. We've found that substantial genetic heterogeneity is present in most patients before surgery, and it cannot be accounted for using contemporary tools for detection, e.g., a family medical history check-box. Because the surgical environment is heavily monitored and controlled, profiling in the perioperative interval provides an ideal opportunity to introduce genomics to clinical practice. There are few scientific barriers, cost barriers are falling, legal precedent favors taking safety steps when available, regulatory agencies are adjusting to multiplexed applications, and payers will realize savings with reduced mortality and morbidity. In addition to conventional measures (e.g., environmental engineering and enforcing human performance standards), non-invasive genetic screening prior to anesthesia and surgery holds significant promise for the prevention of perioperative complications and the promotion of patient safety.

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PHARMACOGENETICS OF NOCICEPTION

William R. Lariviere

Discovery of the genes underlying distinct types of pain is critically important for the understanding not only of the pain, but also of the responsiveness to analgesic treatments. To quantitatively determine the genetic relatedness of common mouse models of nociception and hypersensitivity, we have performed a genetic correlation analysis of standard inbred mouse strain surveys. We have identified 5 clusters of positively correlated assays indicating at least five genetically fundamental and distinct types of nociception and hypersensitivity: 1) baseline thermal nociception; 2) spontaneous responses to noxious chemical/inflammatory stimuli; 3) thermal hypersensitivity; 4) mechanical hypersensitivity; 5) afferent input-dependent hypersensitivity (Lariviere et al. 2002). Paradoxically, pharmacogenetic mechanisms of analgesic sensitivity are not strictly dependent on the class of the analgesic drug, but are specific to the type of pain being inhibited. We and others have shown that the analgesic effectiveness of drugs of distinct drug classes is moderately to highly correlated in inbred strains of mice assessed with the same pain assay, but is not correlated across types of pain assay even for a single drug (Chesler et al. 2003; Wilson et al. 2003a). Furthermore, analgesic effectiveness can be negatively correlated with baseline sensitivity in the assay used to assess the analgesia such that strains of mice that are most sensitive to the assay are generally least sensitive to its analgesic modulation (Wilson et al. 2003b). The possible clinical significance - that patients may be relative non-responders across classes of drugs against their particular clinical painù- makes it imperative to have a thorough understanding of the underlying genetic mechanisms of each fundamental type of pain. Sex-specific genetic mechanisms mediating analgesic effectiveness have been detected in mouse models and have led to human translation of the findings to implicate genetic mediation of female-specific kappa-opioid mediated analgesia by the melanocortin-1 receptor gene (Mogil et al. 1997). Chesler EJ, et al. Pain 2003a; 106: 325-335. Lariviere WR, et al. Pain 2002; 97: 75-86. Mogil JS, et al. Journal of Neuroscience 1997; 17: 7995-8002. Mogil JS, et al. Proc Natl Acad Sci USA 2003; 100: 4867-4872. Wilson SG, et al. Journal of Pharmacology & Experimental Therapeutics 2003a; 304: 547-559. Wilson SG, et al. Journal of Pharmacology & Experimental Therapeutics 2003b; 305: 755-764.

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WARFARIN SENSITIVITY AND CYP2C9 AND VKORC1 GENES

Bonny Lewis Bukaveckas

One example that is thought to be by many "low hanging fruit" in the field of pharmacogenetics is anticoagulation with warfarin (Coumadin). Over 400,000 individuals in the U.S. per year treated with warfarin show either hypersensitivity or resistance to this medication with potentially life threatening complications. There is up to a 60-fold variation in the therapeutic dose of warfarin between individuals, from 0.5 - 60 mg/day with an average dose of ~4-6 mg/day. Even with high intensity monitoring of patient response to warfarin, typically measured as prothrombin time and expressed as an international normalized ratio (INR), determining the correct dosage

is complex and extremely difficult to predict. This is due to a wide variation between patients in their sensitivity to warfarin, and this is in large part determined by differences in rate of drug metabolism and variability of vitamin K-dependent clotting factors. Common variations of the gene for the warfarin-metabolizing enzyme cytochrome P450 2C9 (CYP2C9) may cause warfarin hypersensitivity, making a 5mg dose of warfarin too much for these people. Warfarin exerts its anticoagulant effect by inhibition of the vitamin K epoxide reductase protein complex (VKORC1). Rare variations of VKORC1 may result in warfarin resistance, making 5mg of warfarin too little to achieve the desired therapeutic effect. However common genetic polymorphisms in both CYP2C9 and VKORC1 genes have been found to decrease warfarin dose requirements. Despite long familiarity and ubiquitous clinical use, initiation of warfarin therapy in many centers is still in large part empiric, with errors being potentially life threatening bleeding. In November 2005, The Clinical Pharmacology Subcommittee of the US FDA Advisory Committee for Pharmaceutical Sciences strongly advocated, in an 8 to 2 vote, for the inclusion of genotype information in the prescribing of warfarin stating, "Sufficient mechanistic and clinical evidence exists to use lower doses of warfarin for patients with genetic variations in CYP2C9 that lead to reduced activity and genotyping patients in the induction phase of warfarin therapy would reduce adverse events and improve achievement of stable INR in patients with genetic variations in CYP2C9" and "Sufficient mechanistic and clinical evidence exists to use lower doses of warfarin for patients with genetic variations in VKORC1 that lead to reduced VKORC1 activity and genotyping patients in the induction phase of warfarin therapy would reduce adverse events and improve achievement of stable INR in patients with genetic variations in VKORC1." Clinical assays are available in these genes and the US FDA is considering a change in the warfarin prescribing information to include genotyping. There are currently prospective studies underway by our group as well as others to incorporate this genetic information into a new warfarin dosing paradigms, with the goal of improved clinical care and decreased resource utilization being the goal.

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OUTPATIENTS WITH END STAGE RENAL DISEASE ON HEMODIALYSIS HAVE ENDOTOXIN LEVELS COMPARABLE TO CRITICALLY ILL PATIENTS

Daniel Levine, Roxana Bologa, Thomas Parker, Debra Foster, David Klein

Patients with end-stage renal disease (ESRD) on hemodialysis (HD) have a high mortality rate, a chronic inflammatory state and elevated levels of serum inflammatory markers. We used the endotoxin activity assay (EAA) to investigate the possible role of endotoxemia as a trigger of the inflammatory response in a cohort of ESRD patients on HD. We enrolled 40 ambulatory patients on chronic HD with no recent hospitalizations or acute medical problems. Blood was drawn immediately pre-HD for assay of endotoxin activity (EAA, Spectral Diagnostics), inflammatory markers (IL-6, Il-1, TNF-? and endotoxin binding proteins (LBP, BPI, sCD14, total and endotoxin core-reactive IgG and IgM (EndoCAb, Hbt, The Netherlands)). The majority of patients (32/40; 80%) had high EAA levels, similar to values that predict poor outcome and sepsis on admission to the ICU (EAA > 0.6 units). However, proinflammatory cytokines were only moderately elevated and well below the septic range: median and [range]: IL1? 0.5 [0.2-0.9], IL6 5.4 [2.9-10.3], TNF-? 17 [13-23]. EAA was negatively related to serum BPI ($R=-0.436$, $p < 0.005$) and cholesterol ($R=-0.324$, $p > 0.05$). LBP, sCD14 and EndoCAb IgG and IgM antibodies were high, but not linearly related to EAA. High EAA and high EndoCAb IgG and IgM without severe cytokinemia may reflect adaptation to repeated exposure to endotoxemia. Potential long term consequences from this exposure remain to be determined. Further, a better understanding of the origin of the endotoxemia in patients with ESRD may assist in the management of these patients through the course of a critical illness.

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CURRENT CONCEPTS OF THE ROLE OF ENDOTOXIN IN SEPSIS AND THE SIGNIFICANCE OF ENDOTOXIN REMOVAL THERAPY

Steven Opal

Bacterial lipopolysaccharide comprises up to 75% of the outer leaflet of gram-negative bacteria and is essential for the viability of virtually all clinically relevant, gram-negative bacilli. This phylogenetically ancient, microbial structure is the most important pattern recognition molecule inducing profound changes in host transcriptional programs. Up to 12% of the entire human transcriptome is up-regulated or down-regulated within 24 hours of exposure to a single dose of LPS. An elaborate series of signaling pathways and regulatory networks attest to the critical significance of LPS recognition for survival against early microbial invasion. Essential structure function relationships of LPS signaling pathways both at the level of cell surface receptors and intracellular signal transduction networks are now increasingly well understood. The integration, regulation, dynamics and interplay between LPS signaling pathways and other microbial mediators remain a major challenge for systems biology. While it has been repeatedly demonstrated that very high levels of endotoxin is injurious to patients with severe sepsis, it is less clear that removal of endotoxin signaling after sepsis has already started will be beneficial. Considerable animal data and suggestive clinical findings in human sepsis indicate that removal of endotoxin may be therapeutically relevant even after the onset of severe

sepsis. A new generation of anti-LPS molecules is now available for clinical experimentation. The outcome of these current clinical trials will determine the clinical relevance of high LPS levels in the course of human severe sepsis/septic shock.

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UTILITY OF THE ENDOTOXIN ACTIVITY ASSAY (EAA) TO ASSESS ENDOTOXEMIA IN BURN PATIENTS

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Purpose: The American Burn Association estimates that more than 1 million burn injuries and approximately 4500 fire and burn related deaths occur each year. The acute response to burn injury and resuscitation have been hypothesized as secondary to circulating endotoxin. Endotoxemia can result in a systemic inflammatory response syndrome (SIRS), and subsequent Multiple Organ Dysfunction (MOD) in other injury models including trauma and sepsis. The exact mechanism by which burn injury leads to the development of systemic inflammation and MOD has not been fully elucidated. However, burn injury has been shown to induce leukocyte activation, expression of adhesion molecules and the release of inflammatory mediators. Which of these factors has the predominant effect upon burn pathophysiology is unclear, although there is evidence that endotoxin translocation may play an early role in driving this response. To further assess this hypothesis, we sought to measure circulating endotoxin levels among a sample of burn patients using the Endotoxin Activity Assay (EAA; Spectral Diagnostics; Toronto, CA).

Methods: We enrolled 12 patients admitted to the Parkland Hospital Burn Unit with burns over 15 to 40% total body surface area (TBSA) under a protocol approved by the University of Texas Southwestern Medical Center and Parkland Memorial Hospital Institutional Review Boards. Clinical data were collected prospectively into a burn registry concomitant with patient enrollment. In addition, blood was drawn and EAA was conducted at baseline ($\leq$24 hours of injury), and again on post-burn days 1, 2, 3 and 7.

Results: Endotoxemia was common in burn patients with 15-40% TBSA burns. On average and in particular cases, EAA was relatively low upon admission, rose rapidly to a maximum on post-injury day 2-3 and resolved by day 7. A characteristic pattern of endotoxemia over time in this patient population was noted. EAA scores did not appear to be directly correlated with the degree of burn injury, as indicated by percent of total body area burned in this patient sample. In ex-vivo studies of whole blood inoculated with varying concentrations of BPI, the EAA was sensitive to titration of LPS out of solution by increasing amounts of BPI.

Conclusions: Endotoxemia is common in patients with 15-40% TBSA burns. Levels rose in general from baseline perhaps reflecting translocation of endotoxin during burn resuscitation and the inflammatory response to burn. The lack of association between burn size and EAA may have been precipitated by a number of factors, including an individualized host response, degree of resuscitation and other patient comorbidities. Further studies are warranted to assess whether the pattern of EAA rise in burn patients may provide clinically useful prognostic information and may guide resuscitation. The ability of the EAA to monitor progress in a trial of bactericidal permeability increasing protein (BPI) may have important implications for the guidance of future specific antiendotoxin treatment of burn patients.

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LIPOTEICHOIC ACID - THE GRAM-POSITIVE ENDOTOXIN?

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Our understanding of sepsis and host response to bacterial infection has been shaped by about 50.000 scientific articles on Gram-negative endotoxin. While these lipopolysaccharides are available in high quality for 50 years, the nature of the Gram-positive counterpart became clearer only over the last few years. Lipoteichoic acids (LTA) have been under discussion to play such a role for several decades. The chemically more labile structure required a novel purification scheme and appropriate experimental handling. Highy-purified preparations (>99.9%) have beeen carried out from about 20 species and structures were elucidated. The activity of these molecules has been confirmed by chemical synthesis and a minimal active structure defined. Monocyte/macrophage activation proceeds via CD14, TLR-2 and CD36. LTA induce a variety of biological activities overlapping with but distinct from LPS. For example, they are capable to induce via L-ficolin the lectin pathway of the complement system and induce a more anti-inflammatory cytokine pattern as well as a very strong chemokine release. LTA induces liver injury in galactosamine sensitised mice and (cross-)tolerance to various immune stimuli. When presented on a solid phase, LTA potency was amplified 1000x. Thus, the first chemically defined immune stimulus abundant in most Gram-positive bacteria and sufficiently potent to account for the endotoxic properties of Gram-positive bacteria has been identified.

The limulus assay for LPS has allowed to detect LPS in various settings. The detection of LTA as a pyrogenic threat in parenterals and air or on medical devices is increasingly recognised. The development, international validation and current implementation in pharmacopoeia of a novel pyrogen test based on the human fever reaction enabled the development of new safety measures. Similar

to LPS, it is tempting to determine levels also in biological fluids for diagnostic purposes. ELISA-based approaches to measure LTA have been used for liquor. For detection in blood, novel approaches based on binding to human serum albumin coated beads, subsequent washing and pyrogen testing are currently furthered. In conclusion, the perspective to detect Gram-positive pyrogens and possible expansion to diagnostics broadens our LPS-centric approach.

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THE ROLE OF COAGULATION ACTIVATION IN SEPSIS-ASSOCIATED TISSUE HYPOPERFUSION

Marcel Levi

Sepsis is frequently associated with multiple organ dysfunction, associated with significant morbidity and mortality. Besides other mechanisms, thrombotic obstruction of the microvasculature may contribute to the pathogenesis of organ failure in sepsis. This widespread dysfunction of the microcirculation is a consequence of systemic intravascular activation of coagulation and endothelial cell perturbation.

Knowledge on important pathogenetic mechanisms that may lead to sepsis-associated coagulopathy and microvascular dysfunction has resulted in novel preventive and therapeutic approaches to patients with sepsis. The trigger for the activation of the coagulation system is mediated by several pro-inflammatory cytokines, expressed and released by mononuclear cells and endothelial cells. Thrombin generation proceeds via the (extrinsic) tissue factor/factor VIIa route and simultaneously occurring depression of inhibitory mechanisms, such as antithrombin III and the protein C and S system. Also, impaired fibrin degradation, due to high circulating levels of PAI-1, contributes to enhanced intravascular fibrin deposition.

In addition, rather than being a unidirectional relationship, the interaction between inflammation and coagulation involves significant cross-talk in which again the endothelium appears to play a pivotal role. Activated coagulation proteases, such as the tissue factor-factor VIIa complex, factor Xa and thrombin can bind to protease-activated receptors on various cells and the ensuing intracellular signaling leads to increased production of pro-inflammatory cytokines and chemokines. Activated protein C can bind to the endothelial protein C receptor, thereby affecting NFkB nuclear translocation and subsequently influencing inflammatory gene expression and inhibition of tissue factor expression on mononuclear cells. This intricate relationship between inflammation and coagulation on the endothelial cell surface may have major consequences for the pathogenesis of microvascular failure in patients with sepsis

Based on the knowledge of the pathogenesis of microvascular failure and coagulation activation in sepsis, strategies aimed at the inhibition of coagulation activation were developed and have been found favorable in experimental and clinical studies. In particular, restoring the function of the protein C system by administration of activated protein C, was shown to be of benefit in patients with sepsis and organ failure. Other strategies comprise inhibition of tissue factor-mediated activation of coagulation or restoration of other physiological anticoagulant pathways, for example by means of the administration of antithrombin concentrate or recombinant TFPI.

Detailed knowledge on the pathogenesis of microvascular dysfunction in sepsis, resulting in tailored treatment strategies to restore the defect, appears to result in a better outcome of patients with sepsis.

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SHOULD WE TARGET THE MICROCIRCULATION IN SHOCK RESUSCITATION?

Jean-Louis Vincent

Tissue hypoxia is believed to play a key role in the development of multiple organ failure associated with shock. The microcirculation delivers oxygen to the tissues and experimental and human studies have demonstrated reduced microcirculatory flow, and increased heterogeneity of flow, in various shock states, including septic shock. In addition, the degree of microvascular disturbance and its persistence has been associated with poorer outcomes. Restoring microcirculatory flow, may, therefore, be a useful target for resuscitation. Until relatively recently, we have had to rely on surrogate markers of regional oxygenation, including blood lactate levels and mixed venous oxygen saturation (SvO_2), but techniques are now available that allow direct visualization and quantification of microcirculatory changes at the bedside. Using one such technique, orthogonal polarization spectral (OPS) imaging, various therapeutic strategies, including acetylcholine, intravenous dobutamine, nitroglycerin, and drotrecogin alfa (activated) have been shown to improve microcirculatory patterns in patients with shock. The mechanisms underlying microcirculatory dysfunction in shock states are not fully understood, but likely include a combination of reduced functional capillary density, reduced red blood cell deformability, endothelial cell dysfunction with increased permeability and apoptosis, altered vasomotor tone, increased numbers of activated neutrophils, and activation of the clotting cascade with fibrin deposition leading to the formation of microthrombi. The continued development of techniques to directly visualize and monitor changes in the microcirculation will help determine the precise role of this organ in the organ dysfunction associated with shock states. Early results suggest that the microcirculation may be a useful target to assess the adequacy of, or need for

ongoing, resuscitation during shock, but further clinical trials are needed to determine how best to quantify microcirculatory changes such that the effects of therapeutic interventions can be monitored. Whether adjusting therapies according to microcirculatory parameters influences outcomes also needs to be determined.

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DISEASE SPECIFIC IMAGING WITH WHOLE BODY MRI

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Purpose: Recently introduced dedicated whole-body MRI protocols allow for comprehensive assessment of complications from macro- and microangiopathy in long standing diabetes [1]. At 1.5 Tesla the multi-organ assessment still imposes limitations in spatial and temporal resolution compared to single-organ studies. The purpose of this study was to implement and evaluate a high-resolution protocol on a 3 T whole body scanner and evaluate the protocol in asymptomatic patients suffering from diabetes for more than ten years.

Materials and Methods: 65 asymptomatic patients with type 1- or 2-diabetes lasting for more than ten years (mean age 63 years) were examined on a 1.5 T (12 pts) or 3.0 T (33 pts.) whole body MR system (Magnetom Avanto/Magnetom Trio, Siemens Medical Solutions), both equipped with 32 receiver channels. Imaging of the brain with axial T2-w-images, T2*-w-images, diffusion weighted images and a FLAIR-sequence was performed. For the time-of-flight-MR angiogram of the cerebral arteries a spatial resolution of 0.7 x 0.5 x 0.7 mm was acquired. At 3 Tesla, cardiac function was assessed with a dual breath-hold multi-slice Cine TrueFISP technique with PAT (T-SENSE acceleration factor 4) allowing for a temporal resolution of 48ms [2]. For delayed contrast enhancement imaging, single-shot TrueFISP phase sensitive inversion recovery (PSIR) images were performed within a single breathhold 15 min after administration of contrast media [3]. 3D-Gd-MR-angiography of the carotids (resolution 1.0 x 1.0 x 1.0, iPAT factor 3), the abdominal aorta (1.4 x 1.1 x 1.2, iPAT factor 3), the thighs (1.1 x 1.1 x 1.1, iPAT factor 2), the calves and pedal arteries (1.0 x 1.0 x 1.0, iPAT factor 2) was obtained. A TR-CE-sequence of the lower calf and pedal arteries (1.4 x 1.4 x 1.5, iPAT factor 3, temporal resolution 3.7 s / frame) was performed after repositioning of the patient in order to compensate for a shortened range of table movement in the Magnetom Trio. Finally, high spatial resolution native and contrast-enhanced images of the feet were acquired. Correlation to a group of 200 healthy adults (mean age 55 years) who received a whole body MRI for cardiovascular screening was performed [4]. Image quality was assessed by two experienced radiologists.

Results and Discussion: All images were of good to excellent diagnostic quality without major artifacts, such as venous overlay, severe dielectric or frequency shift artifacts. Vascular pathologies were substantially more often found in the diabetes group than in the healthy control group. Carotid artery stenosis was observed in 28% of diabetes patients compared to 6% of healthy adults. Renal artery stenosis was seen in 10 diabetic patients compared to 0.25% in the healthy control group. Stenoses of peripheral arteries were seen in approx. 50% of patients. In the group of healthy adults, stenoses of the peripheral arteries were observed in 14%. In 10 patients lesions requiring intervention were found. In 14 diabetes patients myocardial infarctions were detected by positive late enhancement and hypo- to akinetic cardiac segments. In 200 healthy adults, only 2 myocardial infarctions (incidence of 1%) were detected. Two acute embolic cerebral infarctions were noted. Also, chronic ischemic lesions of the brain above the age range and small lacunar defects of the brain were found significantly more often in the diabetes group than in the group of healthy patients. Micro-bleedings were observed in 6 % of patients. Soft tissue edema of the foot was seen in 4 patients, osteomyelitis was detected in 0.6 % of patients, neuropathic foot diseases were found in 11% of patients.

Conclusion: Diabetes causes multiple complications such as vascular stenoses, silent myocardial infarctions, lesions of the brain and foot complications. Compared to a healthy control group, pathologies are substantially more often found in patients with longstanding diabetes. Due to the integration of several technical advances, whole body MRI with PAT, especially at 3T, is a highly promising method for comprehensive disease specific imaging since typical pathologies in diabetics can be identified with high spatial resolution and patients can potentially be treated earlier.

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IMAGING OF BRAIN RECEPTORS

Wolf-Dieter Heiss

Receptors have a prominent role in brain function, as they are the effector sites of neurotransmission at the postsynaptic membrane, have a regulatory role on presynaptic sites for transmitter reuptake and feedback, and are modulating various functions on the cell membrane. Distribution, density, and activity of receptors in the brain can be visualized by radioligands labeled for SPECT and PET, and the receptor binding can be quantified by appropriate tracer kinetic models, which can be modified and simplified for particular application. Selective radioligands are available for the various transmitter systems, by which the distribution of these receptors in the normal brain and changes in receptor binding during various physiologic activities or resulting from pathologic conditions can be visualized. The quantitative imaging for several receptors has gained clinical importance-for example, dopamine (D2)) receptors for differential diagnosis of movement disorders and for assessment of receptor occupancy by neuroleptics drugs; serotonin (5-hydroxytryptamine, 5-HT) receptors and the 5-HT transporter in affective disorders and for assessment of activity of antidepressants; nicotinic receptors and acetylcholinesterase as markers of cognitive and memory impairment; central benzodiazepine-binding sites at the gamma-aminobutyric acid A (GABAA) receptor complex as markers of neuronal integrity in neurodegenerative disorders, epilepsy, and stroke and as the site of action of benzodiazepines; peripheral benzodiazepine receptors as indicators of inflammatory changes; opioid receptors detecting increased cortical excitability in focal epilepsy but also affected in perception of and emotional response to pain; and several receptor systems affected in drug abuse and craving. Further studies of the various transmitter/receptor systems and their balance and infraction will improve our understanding of complex brain functions and will provide more insight into the pathophysiology of neurologic and psychiatric disease interaction.

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CINE-MRI FOR DETECTION OF INTRAABDOMINAL ADHESIONS: CORRELATION WITH INTRAOPERATIVE FINDINGS IN 89 CONSECUTIVE CASES

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Purpose: MRI imaging was correlated with the intra operative findings. To evaluate the accuracy of cine-MRI

Material and methods: Patients with adhesion related complaints after previous abdominal surgery underwent anamnesis and clinical examination. A cine-MRI in transverse and sagittal orientation was used for a dynamic examination of induced visceral slide. An abdominal map consisting of nine segments was created to document the location and extention of adhesion. Cine-MRI and intraoperative findings were correlated.

Results: Eighty-nine (89) surgeries (59 laparotomies and 30 laparoscopies) were performed. The use of cine-MRI-scan in the detection of adhesions has an overall accuracy of 90%, sensitivity of 93% und positive predictive of 96%. The stronger the adhesions, the more accurate the scan findings. Of 44 patients with second degree MRI scan findings, 50% had second degree intraoperative findings. Of 35 patients with third and fourth degree of adhesions on MRI scans, 74% had exactly the same intraabdominal findings on surgery. MRI scan revealed the following locations of adhesions: small intestines 75%, large intes-

tines 35%, abdominal cavity 42% and reproductive organs 32%. Intraoperatively adhesions were found in the following locations: small intestines 70%, large intestines 40%, abdominal cavity 42 % and reproductive organs 28%.

Conclusion: Cine-MRI provides relevant preoperative informations with respect to localizing the extent of intra-abdominal adhesions.

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INTRAVASCULAR OPTICAL COHERENCE TOMOGRAPHY: CHARACTERIZATION OF ATHEROSCLEROTIC PLAQUES

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Objective: Intravascular optical coherence tomography (OCT) is a new and promising imaging modality providing micro-structural information on atherosclerotic plaques. Based on the principles of interferometry, OCT uses the back-reflection of low coherent infrared light to create cross-sectional images with a spatial resolution of 10 to 20 μm. This is at least an order of magnitude higher than the resolution of intravascular ultrasound (IVUS), the current standard of reference. In our studies, we compared OCT with IVUS and histopathology in terms of its ability to differentiate atherosclerotic plaques and to quantify vascular dimensions.

Material and Methods: All studies were performed using the first commercially available intravascular OCT system (Lightlab Imaging, Westford MA, USA). More than 200 atherosclerotic arterial segments were obtained from coronary and below-the-knee-arterial specimens. OCT imaging criteria for different plaque types (fibrous, lipid-rich, calcified) were established and compared to histopathology. A comparison of OCT with IVUS addressed the parameters luminal area (LA), vascular wall area (VA) and plaque area (PA).

Data: Sensitivity and specificity for OCT criteria were 86% and 86% for fibrous plaques, 78% and 93% for lipid-rich plaques, and 84% to 95% for calcified plaques (overall agreement, 84%) Interobserver and intraobserver reliabilities of OCT assessment were high (κ values of 0.84 and 0.87, respectively). Analyzing VA, LA, and PA, a high and significant correlation between vessel parameters derived by IVUS and those derived by OCT (r=0.80-0.95) were shown. OCT overestimated VA by only 1% and underestimated LA by only 2% compared to IVUS. Plaque area was underestimated by 4%.

Conclusion: The ability of OCT to accurately characterize atherosclerotic plaques may hold promise for a better understanding of the progression or regression of atherosclerosis. Additional information on plaque composition and monitoring of reaction to different therapeutic approaches may lead to more sophisticated and individually tailored treatment strategies for vascular disease in the future.

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FUNCTIONAL AND MORPHOLOGICAL EVALUATION OF THE STRESSED MYOCARDIUM

Konstantin Nikolaou, Bernd Wintersperger, Armin Huber, Christoph Becker, Stefan Schoenberg, Maximilian Reiser

In recent years, technical advances and improvements in cardiac computed tomography (CT) and cardiac magnetic resonance imaging (MRI) brought along an increasing interest in the potential clinical role of these modalities in the non-invasive work-up of patients with various cardiac diseases. In different cardiac pathologies, CT and MRI each have specific advantages and strengths. Cardiac dysfunctions are major complications in patients with advanced viral or bacterial infection, severe trauma and burns accompanied with multiple organ failure - collectively known as systemic inflammatory response syndrome (SIRS). The striking association between inflammation and cardiac dysfunction not only prognoses likelihood of survival in patients with SIRS but also prompts the necessity of understanding the pathophysiology of cardiac dysfunction in these patients, so that effective therapeutic regimen may be identified. The fairly uniform response of the myocardium indicating cardiac dysfunction is surprisingly constant. Systolic performance, as measured by stroke volume or cardiac output and pressure work as estimated by ventricular pressure, are impaired when myocardial contraction is compromised. At times, diastolic function, assessed by ventricular relaxation and filling, is impaired. In addition to the dysfunction that occurs, there is a longer term response of the myocardium to sepsis, and this response is similar to that which is elicited in the heart by multiple brief ischemia/reperfusion episodes. Comparing cardiac CT and MRI to assess these pathologic cardiac changes, MRI seems to have the greater potential. While MDCT is strong in assessing morphological structures such as the coronary arteries and the shape and size of the myocardium, MRI offers a more functional assessment of the heart and is superior to CT due to its inherent superior soft tissue contrast. An essential feature in the assessment of the stressed myocardium includes the measurement of global and regional myocardial contractile function. Myocardium may become dysfunctional as a result of either acute reversible ischemic insults (myocardial stunning) or chronic gradual decrease in blood supply (hibernating myocardium). Functional ("Cine-") MR has been shown to provide superior interstudy reproducibility in the assessment of clinically relevant changes in left ventricular dimensions and function as compared to echocardiography. For perfusion imaging of the myocardium, standard MR contrast-agents such as gadolinium chelates (eg, Gd-DPTA) can be useful as contrast and

perfusion agents at the same time. Non-ischemic myocardium exhibits gradual signal enhancement and washout with the passage of the T1-enhancing contrast agent. Administration of adenosine accentuates the baseline perfusion defect in ischemic myocardium and helps differentiate ischemic from non-ischemic myocardium. Analogous to the detection of contractile reserve with low-dose dobutamine administration, vasodilator-induced perfusion defects detect myocardial perfusion reserve. A valuable feature of cardiac MRI may be its ability to differentiate between different myocardial layers because of its high spatial resolution. This could allow differentiation between sub-endocardial and transmural perfusion defects. Sub-endocardial ischemia is believed to be the first indication of compromise of myocardial blood flow. Finally, imaging of cardiac viability, i.e., detection of myocardial infarctions, can easily be performed by MRI, showing the location and extent of persisting myocardial ischemic injuries with great accuracy. In conclusion, MRI seems more suitable for myocardial imaging in patients with sepsis or shock, as compared to CT. An assessment of myocardial function, perfusion and viability can be performed in a single examination.

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ALARM ALGORITHMS: RECOGNIZING TRUE FAILURE

Michael Imhoff, Ursula Gather, Roland Fried

Introduction: The disease state of the critically ill is described by complex data from multiple sources. For the clinician the challenge is to distinguish clinically relevant changes from noise and artifacts. The overreaching goal must be the early detection of true failure with high sensitivity and specificity. In physiological monitoring today most alarm systems are based on simple thresholds only. This leads to an unacceptably high rate of false positives alarms, while the actual rate of false negatives remains unknown. In this contribution we will give an overview and present some examples of advanced alarm algorithms.

Requirements and approaches: Alarm algorithms have to be robust against artifacts and missing values. Real-time application requires efficient and fast algorithms with adequate scalability of memory and computational demands. Online use demands instantaneous updates of alarm calculations with every new incoming value. Alarm algorithms have to provide predictable behavior and meet methodological rigor. Many different alarm algorithms have been published and several have been investigated in simulation and clinical studies. Univariate algorithms, i.e., algorithms that handle one variable at a time, include median filters, Kalman filters, different robust filters, autoregressive models, phase space models, dynamic linear models, and trend detection methods. For the handling of multiple variables multivariate algorithms, e.g., graphical models, dynamic factor models, multivariate regression, have been employed. Also approaches from artificial intelligence, for instance, knowledge-based systems, neural networks, fuzzy logic, Bayesian networks, or decision trees, were investigated in the context of alarm detection. While some of these approaches showed promising results, none has gained universal acceptance or even commercial implementation.

Examples from vital signs monitoring: Robust regression techniques represent one approach to univariate signal extraction. Different regression methods yielded satisfactory results for extracting the underlying signal from noisy simulated data and from real clinical monitoring time series. Repeated median regression seems to be the best choice for intensive care monitoring because of the quality of signal extraction and the favorable computational demands. Different multivariate algorithms were applied to complex monitoring time series. Graphical models were successfully used to identify "lead" variables that were representative of groups of variables. Dynamic factor models were applied to identify latent variables as representations of the underlying data generating processes.

Conclusions: Recognizing true failure in the critically ill requires fast and reliable interpretation of complex data with high sensitivity and specificity. Currently commercially available methods are often inadequate for this task. Statistical research has made new univariate alarm algorithms available that can provide better and more robust alarm detection. Also, new multivariate alarm algorithms are being developed and show first promising results. Further research is still needed. In summary, new advanced alarm algorithms will help to detect true failure in the critically ill.

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MECHANISMS OF PHYSIOLOGICAL VARIABILITY

Sven Zenker

The measurement of physiological variability in health and disease has received growing attention over the last years. A particularly well studied example is heart rate variability, which has been shown to have diagnostic and prognostic utility in a number of experimental and clinical settings ranging from primary cardiac ailments to various pathophysiological states relevant to critical illness. While these phenomenological observations of variability phenomena, as well as their alterations in disease, are quantifiable and relevant, an understanding of the mechanisms underlying physiological variability is crucial for guiding interpretation of the observations. Using variability in the cardiovascular system as an example, this talk will illustrate generic mechanisms underlying many variability phenomena in biological systems through the use of mathematical models.

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COMMUNICATIONS IN PHYSIOLOGY: SHORT-TERM AND LONG-TERM TIME SCALES

Dirk Hoyer, Phyllis K. Stein, Hendrik Schmidt

Objective: The cardiovascular controller incorporates mechanisms at different time scales, such as the heart period (around 0.5-1 s), vagal (around 1-2 s), sympathetic (around 10 s), renin-angiotensin-aldosterone system (scale of minutes), renal volume pressure regulation (scale of hours), and circadian rhythm (which is present in all autonomic and humoral activities). Autonomic Information Flow (AIF) measures, treated as functions of the time horizon of the prediction of heart rate patterns (time scale of communication), can improve the assessment of particular aspects of complex cardiovascular mechanisms which themselves operate over different scales. We have confirmed in clinical studies that AIF of different time scales, from seconds to minutes, identifies pathophysiologically disturbed autonomic function in several patient groups. Furthermore, outcome in these patients was related to particular AIF measures. We investigated the hypothesis that complex pathological dysfunction and outcome can be assessed by disease-specific AIF time horizons.

Material and Methods: Holter recordings were assessed in 50 healthy controls, patients with multiple organ dysfunction syndrome (MODS) (26 survivors, 10 non-survivors), after abdominal aorta surgery (AAS, 32 length of stay in hospital (LOS) > 7 days, 62 LOS >= 7 days), and with heart failure (14 low risk - without history of aborted cardiac arrest (CA), 13 high risk - with history of CA). In all groups, 24 h Holter recordings, as well as 6 h data sets selected during day (in between 6 a.m. and 3 p.m.) and night (in between 10 p.m. and 6 a.m.) were analyzed using AIF methodology which was based on the HRV Task Force guidelines, Furthermore, the circadian rhythms of the AIF measures were investigated. The area under curve (AUC) of receiver operating characteristic with 95% confidence interval as measure of predictive accuracy was calculated.

Results: We found time horizon specific risk markers, namely decreased HF related AIF in MODS, increased VLF related AIF after AAS, and decreased AIF over one heart beat period in the heart failure patients were associated with higher risk. The circadian patterns were abnormal in all patient groups.

Discussion: As for the MODS prognosis, an intact vagus may be a major precondition for the body's rapid anti-inflammatory action in sepsis and multiple organ dysfunction syndrome. Thus, a loss of vagal information flow may be pathophysiologically and prognostically relevant. As for the LOS prognosis after AAS the long term AIF had the highest discriminatory value. Here, mechanisms of all shorter time scales are reflected in the resulting long term predictability. Complex communication is better maintained in the lower risk group. In the heart failure patients the reduced heart beat interval related AIF was the only prognostically significant index, which is in line with the cardiac origin of the sudden cardiac death. This result might reflect cardiac destabilisation because of disturbed (less divergent) attractor-like behavior.

Conclusion: We conclude that different time scales (horizons) of AIF and their circadian variation represent specific physiological and pathophysiological aspects of altered autonomic communication. In addition to potential predictive implications, this knowledge might be useful for the development of comprehensive therapeutic strategies.

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INFERRING COUPLING BETWEEN PHYSIOLOGICAL SYSTEMS FROM DATA

Michael Rosenblum

We discuss the usage of the coupled oscillators approach in analysis of bivariate physiological data, taking the cardio-respiratory interaction and analysis of brain activity as examples. Analysis of bivariate or, generally, multivariate measurements is a standard problem of physiological data analysis. We discuss several data analysis tools, based on the assumption that the multivariate data originate from two coupled self-sustained oscillators. These tools are designed to provide the solutions for the following tasks: (i) to detect and quantify an interaction between the systems, (ii) to reveal the direction of coupling, and (iii) to estimate delay(s) in coupling. These problem can be solved, provided the following assumptions are fulfilled: (i) we deal with several active oscillatory systems, each capable of producing its own rhythm; these rhythms are influenced by rhythms of other oscillators due to weak coupling between the sources, (ii) we know how to ascribe the signals we measure to systems we want to analyze, and (iii) the signals are appropriate for phase estimation.

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THE BRAIN, THE HEART AND THE VASCULATURE: COMPLEX BUT UNCOMPLICATED INTERACTIONS

Aneta Stefanovska

Contemporary measurement techniques enable noninvasive monitoring of cardiovascular functions, both from the central and peripheral points of view. Modern analytical

techniques based on nonlinear and stochastic dynamics show that cardiovascular dynamics is characterised by several distinct frequency components in the interval from 0.005 to 2 Hz. Moreover, it was indicated that these are present at each site of the system. The corresponding oscillatory processes are mutually dependent via couplings that lead to amplitude/frequency fluctuations of the characteristic peaks. The origin of the low frequency oscillations (<0.01 Hz) and their potential connection to endothelial function and the immune system will be discussed.

Neuronal oscillations span an even wider frequency interval, covering frequencies from 0.025 Hz to 600 Hz. New studies have been initiated and interactions between the cardiovascular oscillations and some of the brain waves have been demonstrated for the first time. The results indicate the existence of a functional linkage between certain oscillations of the two systems, possibly mediated by glial cells. The work will be reviewed and causal relations will be discussed with a special emphasis on quantification of the depth of anaesthesia.

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CEREBROVASCULAR MODELING IN THE ICU

Mauro Ursino, Massimo Giannessi, W. Bosseau Murray

Objective: The mathematical relationships between cerebral perfusion pressure (CPP), intracranial pressure (ICP) and cerebral blood flow (CBF) as well as the autoregulatory control of these quantities by the normal brain, have been well described. However, the individual relationships are not available in single models usable by researchers, students and clinicians. Furthermore, in clinical practice, only cerebral perfusion pressure and intra-cranial pressure can be routinely monitored, while cerebral blood flow (the most important parameter) can only be indirectly "deduced." It is therefore difficult to teach trainees to think in terms of cerebral perfusion pressure when it is not actually measured. Developing an intuitive understanding and "feel" for the relationships of the measured and non-measured parameters is also difficult, as the relationships are not linear, do not necessarily change in the same directions, and might not be auto-regulated normally in cases of brain trauma. We developed a comprehensive cerebrovascular and intracranial pressure model to calculate and display these complex relationships.

Material and Methods: Published mathematical formulae (from animal studies, and human studies where available) were programmed into MatLab. The main variables that could influence brain perfusion are blood pressure, carbon dioxide concentration, and ICP. We added parameters such as intracranial hemorrhage (variable in volume and duration), head up position, decreased autoregulation, etc. that could influence the relationships.

Data: The model was stable for extremes of parameters. It performed clinically realistically given inputs of published traumatized patients, as well as cases known to clinicians. Results indicate that the model would be useful to study clinical questions such as the optimal head-up position (in terms of CBF) in a given patient, the effects of intracranial bleeding on cerebral hemodynamic, the optimal carbon dioxide concentration to reach the best compromise between ICP and perfusion, the results of decompressive craniectomy in head injury patients. For instance, we found that in certain cases of persistent hypotension and raised intra-cranial pressure (and low cerebral blood flow), it might be useful to maintain a normal carbon dioxide concentration (or even slightly elevated), in contrast to the present practice of maintaining all head injured patients in a state of slight hypocapnia

Conclusion: The model is suitable for developing a curriculum for trainees to understand the complex relationships between intracranial pressure and perfusion. Clinicians and researches can use the model to study controversies in patients with intracranial hypertension and to develop optimization strategies for complex head injured patients.

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USE OF A TRAUMA REGISTRY TO MONITOR LENGTH OF STAY AND DISCHARGE DISPOSITION BY PAYOR SOURCE AT A US LEVEL I TRAUMA CENTER

John Kirby, William Carroll, Sean Glasgow, Julie Nash, Timothy Buchman, Douglas Schuerer

Objectives: We wished to assess the associations between payor status, severity of injury, length of stay, and discharge disposition in patients brought to an urban, level 1 academic trauma center in the US.

Material and Methods: A retrospective review of all trauma registry data for patients admitted longer than 48 hours over a 24 month period. For each of the 5 payor categories, the patients' length of stay, ISS, and destination at discharge were tabulated.

Data: We identified 1931 patients with LOS greater than 48 hours. Despite similar ISS's, Medicaid patients stayed longer than all other groups except Workman's Comp (WC). (Table 1) Medicare patients were less likely to go home or to jail, and more likely to go to inpatient rehab than all other groups. SP were less likely to go to inpatient rehabilitation or home with nursing than those with CI. (Table 2)

Conclusions: Medicaid patients stay longer than other insurance types. SP patients are less likely to get home nursing or go to inpatient rehabilitation. Older Medicare patients often cannot go home after trauma. These

Table 1: Payor vs. LOS and ISS (*p<.001 for all compared to Medicaid length of stay)

Payor	Number of pts.	Mean LOS(Range)	Median ISS (Range)
Self - Pay (SP)	559	9.2 (3-87) *	10 (1-75)
Commercial Insurance (CI)	638	9.0 (3-128) *	10 (1-75)
Medicare	503	8.8 (3-71) *	10 (1-75)
Medicaid	133	10.5 (3-125)	10 (1-75)
Workman's Comp (WC)	98	8.2 (3-34)	10 (1-66)

Table 2: Disposition based on insurance (* p<0.001 for Medicare compared to all other groups. #p<0.01 CI vs. SP, p<0.05 CI vs. Medicare. † p<0.025 for SP vs. CI)

Disposition	Self- Pay	Com. Ins.	Medicare	Medicaid	WC
Home or jail	375 (67%)	325 (51%)	111 (22%) *	72 (54%)	63 (64%)
Inpatient Rehab	126 (23%)†	199 (32%)	313 (62%) *	42 (32%)	19 (20%)
Home w nurse care	34 (6%)	70 (11%) #	35 (7%)	14 (11%)	11 (11%)
Home w rehab	6 (1%)	12 (2%)	6 (1%)	0 (0%)	1 (1%)
Acute Care Hosp	3 (1%)	9 (1%)	6 (1%)	0 (0%)	2 (2%)
Other	15 (3%)	23 (4%)	32 (6%)	5 (4%)	2 (2%)

inequities may worsen as the US population ages and Medicaid programs are cut. Earlier planning for a patient's disposition given his payor source may help equalize length of stays.

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OUTCOME AFTER POLYTRAUMA: THE EFFECTS OF SOCIAL AND DEMOGRAPHIC FACTORS AT THE TEN YEAR FOLLOW-UP IN 637 PATIENTS

Christian Probst, Martin Panzica, Boris Zelle, Nicola Sittaro, Christian Krettek, Hans-Christoph Pape

Background: Over the last decades, improved emergency care has decreased the mortality of multiply injured patients. Therefore, the long-term outcome in terms of morbidity and rehabilitation has gained importance in the evaluation of these patients. In addition to requiring extensive orthopedic rehabilitation, we hypothesized that multiple trauma also exerts numerous adverse effects on the patients' economic and social status. Furthermore, we hypothesized that social and economic determinants might influence the overall outcome from multiple trauma.

Methods: We included all surviving polytrauma patients (aged 3 to 60 years) of our level one trauma center, injured between 1973 and 1990. Patients were assessed by a questionnaire and a clinical exam. Main outcome measures were the Hannover Score for Polytrauma Outcome (HASPOC) and the Short-Form 12 (SF-12). We additionally evaluated several social and economic parameters.

Results: 637 Patients were re-assessed at 17 ± 5 years after polytrauma. The average HASPOC was 64.2 ± 44.6, the average SF-12 physical 44.1 ± 10.6. Considerable change for the worse was found for income, overall financial situation, employment, profession and retirement as well as for interpersonal relationships and recreational activities. Among the determinants for an unfavorable outcome were old age, high injury severity, female gender, blue collar working class, work related injury and a close relationship to a spouse. 76.4% of patients achieved a satisfactory subjective outcome. 26.9% of patients retired due to physical disability from injuries to the lower extremities (34.4%) and severe head injuries (39.3%). 64.2% of the patients required rehabilitation for more than two years after the injury.

Conclusions: A high percentage of patients remain in the rehab process for more than two years. Besides injury related limitations for a successful rehabilitation, female gender and blue collar working class are the most significant social determinants of worse outcome. Superior outcomes can be achieved in young patients. The rate of patients with a favorable outcome is higher than suggested by previous reports.

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UPDATE ON BLUNT TRAUMA DOCUMENTATION

Christian Probst, Thomas Paffrath, Christian Krettek, Hans-Joerg Oestern, Hans-Christoph Pape

Introduction: The role of trauma documentation systems for trauma research has continuously increased since the

first trauma registries were developed in the late eighties. Data acquisition and processing improved highly, partly because modern computer and network technologies offer new approaches. International comparison is important for the learning process and the investigation of differences in the mechanisms of injury, rescue systems and treatment protocols. We demonstrate key points of the learning curve thus supporting a further spreading of trauma registries.

Methods: Seven exemplary trauma registries from the U.S., Canada, Victoria (AUS), the U.K., France, Germany and the new EuroTARN registry were analysed according to their development until the current status. Special investigations were conducted for data acquisition, inclusion criteria and the volume and characteristics of patient data.

Results: We found a clear over-all beneficial influence of the documentation systems on the respective trauma system. Data acquisition displayed a wide range of difference from paper forms being entered into a centralized database by hand to direct entry of the data into the database by a local user via an internet platform. Some systems copy computerized patient data from local hospital systems. Two registries are available in two languages. One has the option to add further languages as demanded. Datasets are comparable in terms of general data and a compulsory trauma diagnosis. Still, the details of the documented period of care and the inclusion criteria differ considerably.

Discussion: We describe the important role of several trauma registries within a trauma care system. Although the success is hard to measure, related publications, continuous growth, the official use for quality control and the demand to participate by other countries stress their wide spread acceptance (secondary internationalization). These advantages make trauma registries a valuable tool in many countries.

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POSSUM SEQUENTIAL PHYSIOLOGY SCORING FACILITATES OBJECTIVE ASSESSMENT OF COST-EFFECTIVENESS IN PATIENTS WITH AN INTRA-ABDOMINAL EMERGENCY

Carlos Emparan, Pablo Soriano, Roger Cabezali, Gerardo Palacios

Background: Patients who present with an intra-abdominal emergency often require extreme measures in surgical care. The assessment of response to surgery and physiologic recovery leads a critical role in medical and economical efforts performed in these patients. This study examined the role of sequential physiology scores in assessing the response to intensive medical efforts and their cost-effectiveness objectively.

Methods: Sequential physiology scores were recorded in 213 patients with abdominal pathology that subsequently required urgent or emergency surgery and required admission to the Surgical Intensive Care Unit. The physiology component of the Physiological and Operative Severity Score for enumeration of Mortality and morbidity (POSSUM), Acute Physiology and Chronic Health Evaluation (APACHE) II and III, and Simplified Acute Physiology Score (SAPS) II were determined at presentation, immediately before surgery, and daily after surgery as long as the patient was discharged from the Hospital. Analytical costs of each patient were estimated daily and correlated with each one of the ICU scores. For statistical analysis a Markov-Montecarlo chain with Bayesian simulation of each scoring system was used.

Results: There were 190 survivors; 23 patients died. All scoring systems showed a positive correlation between score and economical investment in the patient. The POSSUM, and APACHE II and III physiology scores differentiated more effectively between survivors and patients who died than SAPS II, and precisely showed when cost-effectiveness was adequate for each patient (correlation between scoring and effectiveness with a $p< 0,01$). Above every scoring system, physiologic Possum showed a better correlation between evolution, intensiveness of care and economic effectiveness of the treatment.

Conclusion: POSSUM sequential physiology scores may facilitate the assessment of patients' response to intensive care efforts and economic effectiveness of therapies. Patients who fail to respond to improve to physiologic score after surgery can be identified as patients with negative clinical and economic outcomes.

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BIOLOGICAL INTERACTION BETWEEN HEAD INJURY, ABDOMINAL INJURY AND THORACIC INJURY INCREASES MORTALITY

Rolf Gedeborg, Rolf Gedeborg, Karl Michaelsson

Objective: To estimate the effect of biological interaction between (a) head injury and abdominal injury and (b) head injury and thoracic injury on 90-day mortality. While synergy between different injuries in patients with multiple injuries appears likely in a clinical perspective, most mortality prediction models for injury do not account for possible biological interactions between different injuries.

Material and Methods: Incident injury admissions to hospital during 1998-2003 were identified from the Swedish National Hospital Discharge Register (SHDR) and prehospital injury deaths were identified from the Swedish Cause of Death Register (SCDR). Injuries were classified according to the Centers for Disease Control Injury Matrix based on ICD-10 diagnoses. Injury matrix categories were used to define head injury, abdominal

injury and thoracic injury but admissions with a diagnosis of concussion (S060) were excluded from the head injury category. Categorical variables indicating the presence of isolated head injury, isolated abdominal/thoracic injury and combination of head injury and abdominal/thoracic injury were entered into a logistic regression model together with age and sex. Ninety-day mortality was used as outcome measure. The relative excess risk due to interaction (RERI) [with 95% confidence interval] was calculated.

Data: There were 571 353 injuries identified during six years: 38 645 with head injury, 7 863 with abdominal injury and 33 558 with thoracic injury. 595 patients had combined head and abdominal injury and 2 826 patients had combined head and thoracic injury. For the combination of head injury and abdominal injury, the RERI was 5.40 [3.65 to 7.15], and 61% of the mortality risk was attributed to synergy between injuries. For the combination of head injury and thoracic injury, the RERI was 1.60 [1.16 to 2.04], and 35% of the mortality risk was attributed to synergy between injuries.

Conclusion: For the combination of head injury with abdominal or thoracic injuries, a substantial proportion of the mortality risk appears to be due to synergy between injuries. This may partly be explained by differences in the causing mechanism and energy content of trauma. Biological interaction should be considered in mortality prediction models for injury.

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ADDING DIAGNOSTIC INFORMATION FROM AUTOPSY TO HOSPITAL DISCHARGE DIAGNOSES REDUCES INJURY SURVIVAL RISK RATIOS

Rolf Gedeborg, Rolf Gedeborg, Ingemar Thiblin, Karl Michaelsson

Objective: Hospital discharge diagnoses and survival risk ratios for different diagnoses are important and reliable tools for injury epidemiology. Previous studies indicate that findings at autopsy can influence the classification of injuries, compared to classification based on hospital discharge diagnoses alone. We measured the impact of these two diagnostic approaches on survival risk ratios by use of the Swedish central nationwide registers.

Material and Methods: Incident injury admissions during 1998-2004 were identified from the Swedish National Hospital Discharge Register (SHDR). Injuries were classified according to the Centers for Disease Control (CDC) Injury Matrix for ICD-10 based on ICD-10 diagnoses in SHDR. For comparison, injuries were also additionally classified using ICD-10 diagnoses from autopsy in the Swedish Cause of Death Register (SCDR). Absolute differences in hospital survival risk ratio derived from these two different ways of classification were calculated for each diagnosis category in the injury matrix.

Data: Among 639 603 incident injury admissions there were 6 569 hospital deaths (1.0 %), and 1 679 of these had cause of death diagnoses based on autopsy. Compared to survival risk ratios calculated exclusively from SHDR, adding autopsy diagnoses from SCDR resulted in 12/152 of the CDC diagnosis categories having a survival risk ratio reduced by more than 5 %. Of these twelve categories, seven were specified categories while the rest were non-informative, unspecified categories. Notable categories where autopsy diagnoses lowered survival risk ratios were (a) internal organ injury in abdomen, lower back and pelvis (from 0.90 to 0.77), (b) fracture in lower back and pelvis (from 0.97 to 0.88), (c) multiple injuries in thorax (from 0.80 to 0.55) and (d) crushing in thorax (from 0.97 to 0.91).

Conclusion: Adding information from autopsy data may alter survival risk ratios for some diagnosis categories compared to information based on hospital discharge diagnoses alone. This is mainly due to undiagnosed injuries in (a) abdomen, lower back and pelvis and (b) thorax. This should be considered when using hospital discharge data in injury epidemiology. However, survival risk ratios for most diagnosis categories are unaffected by autopsy diagnoses.

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COMPARISON OF LONGITUDINAL LEUKOCYTE GENE EXPRESSION AFTER BURN INJURY OR TRAUMA/HEMORRHAGE IN ANIMAL MODELS

John A. Mannick, Henry Baker, Bernard Brownstein, Irshad Chaudry, J. Perren Cobb, James A. Lederer

The goal of the present study was to compare the dynamics of leukocyte gene expression in circulating white blood cells (WBC) and splenocytes at multiple time points in animal models of burn injury or trauma/hemorrhage. A further goal was to use contemporary pathway analysis to identify functional modules influenced by genes whose expression was altered by either or both forms of injury

Male C57BL/6 mice in groups of 6 were subjected to 25% scald burn injury or trauma/hemorrhage or sham procedures under general anesthesia. Groups of injured or sham mice were re-anesthetized and exsanguinated by cardiac puncture at 2 hours, 1 day, 3 days or 7 days after injury. After centrifugation of blood samples, the platelet-rich plasma was discarded and red blood cells were lysed. RNA was extracted from the remaining WBC population. Spleens were removed from the animals at the time of sacrifice and splenic leukocytes, prepared in similar fashion,

were subjected to RNA extraction. Single-stranded antisense cDNA was prepared from each RNA sample and hydridized to Affymetrix MOE430e microarrays.

Microarray data were normalized using dChip software. Probe sets whose signal intensities showed significant ($p<0.001$) variation between injured and sham groups were identified by f-test using the class comparison tools and time series algorithms in BRB array tools. Burn and trauma/hemorrhage mice were compared with one another at the 4 time points to determine genes differentially expressed in common in WBC and splenocyte populations. Finally, burn and T/H animals were compared with their respective shams at all time points with regard to genes up or down regulated in common in WBC and splenocytes. Ingenuity pathway analysis software was used to explore gene-gene interactions and functional modules of interest from the experimental data sets.

RNA samples from 4 individual mice of the 6 injured or sham animals sacrificed at each time point were selected for hybridization. Therefore, a total of 128 microarrays were available for analysis. Time sequential analysis of 4,627 probe sets differentially expressed in injured vs. sham animals at the $p<0.001$ level showed large numbers of probe sets were differentially expressed in injured vs. sham animals in both blood and spleen at all time points (118 to more than 4,000). While a modest number of genes were up and downregulated in common in both WBC and splenocytes between the two models of injury at the 4 time points studied, the vast majority of differentially expressed genes appeared to be specific for the type of injury. In blood samples on day 1 the greatest number of genes were differentially expressed in common between the two injury models (130 genes).

In the spleen, the time of greatest commonality was seen at day 7 when 434 genes were differentially expressed in common, 428 of which were upregulated. The functional pathways most significantly associated with the commonly expressed genes on day 1 were cellular development, cell death and cell-to-cell signaling. The pathways most significantly associated with commonly expressed genes in the spleen at day 7 were immune response, anti-apoptosis, cell cycle control, chromosome segregation, DNA replication, chromosome condensation and pyrimidine metabolism. These commonly upregulated genes in the spleens of both models appeared to be directing a "recovery" program for immune reactivity, cell division and resistance to apoptosis.

While some genes were up and down regulated in common between WBC and splenocytes in both models throughout the time course of the study the majority of genes differentially expressed in the two compartments were not expressed in common, thus emphasizing the compartmentalization of the response to injury. Future studies to determine the biologic significance of altered gene expression in several of the molecular pathways identified in the present study would seem highly appropriate if clinical applications of these findings are to be sought.

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COMPARATIVE GENOMIC ANALYSIS IN HUMAN AND MURINE BURNS

Henry Baker, David Herndon, John Mannick, James Lederer, J. Perren Cobb, Ronald Tompkins

As part of the large-scale collaborative research project known as Inflammation and the Host Response to Injury, we sought to validate, in a murine burn-injury model, the human genomic response to massive burn injury that we observed overtime in a pediatric burn population. The genomic response to burn injury was assayed in peripheral leucocytes in humans and mice using Affymetrix U133 plus 2 and U430 plus 2 GeneChips, respectively. For each species burn-responsive genes were identified at a significance level of $p < 0.001$ using time-series analysis. In the pediatric dataset, 3025 probe sets representing 2397 genes were identified. These genes assorted into four clusters that could be used to define three distinct post-burn genomic response patterns among subjects. Genes in subcluster a increased in expression and remained elevated for 68 days before returning to control levels. Genes in subclusters B and C increased in expression and remained elevated for up to 262 days; whereas genes in subcluster D decreased in expression levels and remained depressed for up to 68 days. In the murine system 1925 probe sets were identified representing 1523 burn-responsive genes. The significant genes identified in each species were used to identify burn-responsive pathways and networks. The relevant pathways and networks were largely overlapping between the species. Furthermore, human orthologs of murine burn-responsive genes revealed the same three post-burn genomic response patters that were observed with burn-responsive genes identified in humans. Likewise, the murine orthologs of human burn-responsive genes yield similar temporal patterns of genomic expression as did burn responsive genes identified in mice. These results demonstrate that the genomic response to burn injury is largely conserved between mice and humans.

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COMMONALITY OF THE GENOMIC RESPONSE TO INJURY, ENDOTOXIN AND BURNS

Irshad Chaudry, Bernard Brownstein, James Lederer, Henry Baker, Daniel Remick, John Mannick

The National Institute of General Medical Sciences (NIGMS) provided us the opportunity to examine whether there was commonality or differences in the gene expression profiles of leukocytes from blood (WBC) and spleen harvested after injury. This was a collaborative program and the studies were performed at three different sites, i.e., University of Alabama at Birmingham, Birmingham, AL; University of Michigan, An Arbor. MI; and Brigham and Women's Hospital,

Boston, MA. Mice underwent trauma-hemorrhage (T-H), burn injury or endotoxin (LPS)-infusion. Appropriate sham controls were included with each procedure. Two hrs following injury or endotoxin infusion, mice were sacrificed, blood and spleen were harvested. Spleens were processed to prepare single cell suspension and leukocyte populations from blood and spleen were recovered after RBC lysis. RNA was extracted from the isolated leukocyte population; complementary RNA was synthesized from each leukocyte RNA sample and hybridized to microarrays. Our results suggest that a large number of genes were differentially expressed at the 2-hr time point in injured or LPS-infused vs. sham animals. We also found that 13 of the differentially expressed genes in blood, and 46 in the spleen were upregulated or down regulated in common among all three animal models and thus may represent a common, early transcriptional response to systemic inflammation from different injuries. An analysis of the data further revealed that the majority of these genes could be assigned to pathways participating in immune response and cell death. Furthermore, the up- or downregulation of a cohort of 23 of these genes was validated by RT-PCR. In summary, the microarray analysis showed that there was a significant alteration in leukocyte gene expression profile in the models of injury and inflammation. While some of these genes were commonly expressed among the three models of injury and endotoxin infusion, the majority of the differentially expressed genes appear to be uniquely associated with the type of injury and/or the inflammatory stimulus. We hope that analysis of the gene expression in individual leukocyte subsets will provide important novel information concerning the effects of injury and inflammation on immune cell types known to mediate both the host defense and inflammation (supported by USPHS NIGMS Grant 1U54-GM-62119-03). The additional investigators in the Large-Scale Collaborative Research Program "Inflammation and Host Response to Injury" also participated in this study.

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CELL SPECIFIC GENOMIC RESPONSES TO THERMAL INJURY

James Lederer, Henry Baker, Buddy Brownstein, Perren Cobb, Irshad Chaudry, John Mannick

Sequential leukocyte mRNA expression after thermal injury, as revealed by microarray analysis supported by the large collaborative project "Inflammation and the Host Response to Injury - Glue Grant" has demonstrated increased or decreased expression of a substantial number of genes, some of which were not previously known to be associated with the injury response. The next series of studies will identify changes in mRNA expression in highly purified blood and spleen leukocyte populations from burn-injured or trauma/hemorrhage (T/H) mice. Blood neutrophils, T-cells, and monocytes or splenic macrophages and T-cells were purified from sham and burn or T/H mice at days 1 and 7 after injury by a magnetic nanoparticle procedure. RNA was isolated and labeled for hybridization to Affymetrix mouse U430 genechips. Thus far, we have completed the analysis of blood T cell RNA prepared from sham and burn mice. We discovered that 83 probe sets were differentially expressed by sham versus burn T cells at day 1 after injury, $p<0.001$. Of the 83 genes, 28 were upregulated and 55 were downregulated. Among the genes whose expression was downregulated in blood T cells were c-Jun, important in T cell activation, the complement regulatory elements, Daf 1 and Daf 2, the T cell activation antigen, CD69, and Stat 1, important in IFNγ signaling. Upregulated genes included the T cell costimulatory receptor ICOS, associated with anti-inflammatory Th2-type cytokine production, CD47, the receptor for anti-inflammatory signaling by thrombospondin 1, and SOCS3 important in modulating IL-6 signaling. Therefore, the findings from this study suggest that circulating T cells display a counter-inflammatory type gene expression phenotype by day 1 after injury. We anticipate that the overall results of this large-scale genomic-based study will provide new insights into how specific cell types respond to injury.

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VARIABILITY AND UNIFORMITY-CHALLENGES OF COMPLEXITY AND MODEL ORGANISMS

J Perren Cobb

In preclinical models of human disease, we have studied the use of circulating blood transcriptomics and proteomics to characterize the host response to abdominal and pulmonary sepsis. Our data confirm that high-throughput, multiplexed technology can diagnose bacterial sepsis apart from other sources of systemic inflammation (endotoxin) with high accuracy in de-identified, genetically identical animal cohorts. Moreover, these gene and protein expression profiles can differentiate between the host responses to different types of bacteria. Prospective clinical trials are indicated to determine the value of this new approach and to optimize gene selection methods.

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NATURALLY BOUND AND LINKED GROWTH FACTORS IN FIBRIN MATRIX TO REDUCE SKIN FLAP NECROSIS

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Introduction: Several pathologies are accompanied by impaired vascular supply thus leading to necrosis and

tissue loss. The concept of therapeutic angiogenesis deals primarily with the induction of vessel growth by exogenous application of various substances including growth factors. We investigated the potential of local VEGF and PDGF.AB liberated from a fibrin biomatrix to reduce flap necrosis. As fibrin(ogen) has a specific binding site for $VEGF_{165}$, it was simply added to fibrin (naturally bound). In contrast, fibrin with covalently linked PDGF.AB (via transglutaminase; Kuros, Baxter AG, Vienna) was used for controlled local factor release.

Material and Methods: Two different rodent flap models were used to study the influence of FS alone, fibrin-$rhVEGF_{165}$ (200ng) and fibrin-covalently linked with TG-PDGF.AB (10, 100, 1000ng) on flap necrosis compared with control (sutures). Planimetric analysis (flap necrosis, shrinkage) and histology/immunohistochemistry were parameters of effectiveness.

Results: In both models the vehicle group with FS alone showed better results than the control group. Fibrin-VEGF resulted in a significant reduction of dorsal random flap necrosis, and a significant increase in vessel density as shown by immunohistochemical analysis. Following fibrin – TG-PDGF.AB there was also a reduction in the amount of tissue necrosis in the epigastric flap model. Lower concentrations (10ng and 100ng) showed superior results.

Summary: Although fibrin sealant is effective in sealing flaps, wound healing may be enhanced through the addition of growth factors such as VEGF or TG-PDGF.AB.

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HEMOPERFUSION WITH TORAYMYXIN« IN PATIENTS WITH SEVERE SEPSIS DUE TO INTESTINAL PERFORATION

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Introduction: Toraymyxin® was developed to diminish the presence of endotoxin in the circulatory torrent by adsorption to polystyrene fibres with immobilized polymyxin B. Efficacy for adsorption and safety of this device have been proven in experimental and clinical studies and has been approved by the Japanese health system in 1994 for use in clinical practice. Gut perforation with severe sepsis is a process with a high gram negative prevalence. Its precocious diagnosis associated with a decisive surgical intervention becomes an ideal indication for hemoperfusion with Toraymyxin® (HPT).
Objective: To evaluate safety and effect on hemodynamic function of HPT for treating severe sepsis secondary to intestinal perforation.
Design: Observational cohorts with intervention, prospective multi-centre study.
Setting: 5 critical care units in 5 university hospitals.
Period for preliminary report: Sept. 06-Dec. 06.
Inclusion criteria: Patients with severe sepsis due to intestinal perforation with a SOFA score between 5 and 16 before the intervention and severe sepsis for more than 6 hours post- decisive surgical procedure.
Treatment: two HPT of 2 hours in the first two days with a 24 h interval.
Results (expressed as mean ±SD or ±CI, alpha error = 0,05): after informed consent 13 patients with gut perforation were included, 8 primary and 5 secondary to suture dehiscence. 54% female. Mean age 71 years (SD 8). Mean APACHE II 24 (SD 6). Mean SOFA 9,5 (SD 2). Patients with positive culture: 61%, 100% gram negative and appropriate antibiotic (AB) treatment, 30% plus other germs 90% with appropriate AB treatment. Norepinephrine dose dropped from the beginning of the 1st HPT to the finish of 2nd HPT: 0,89±0,30 to 0,32±0,23 (p<,05). Three patients with low cardiac index (<2,5 L/min/m2) increased it after the first HPT day 2,1±0,32 to 3,43±0,37 (p<,05). Stroke volume variation dropped during the first HPT from 18,8±2,8% to 11,6±3,1% (p<,05). Base excess raised from -9±3 to -3±3 (p<,05) during the 1st HPT day. Platelet count dropped from 214000±75000 to 91000±21000 (p<,05) from 1st to end of 2nd treatment. The difference between levels through both HPT was not statistically significant. Neither haemorrhagic nor other complications were detected. Mortality rate was 46%. Negative cultures were greater in deaths (50 vs 28%) The only one patient with inappropriate AB treatment died.
Conclusions: The HPT is a safe treatment and results in a good hemodynamic and metabolic response in this case series preliminary report. More cases and comparison with contemporaneous series is needed for more robust information.

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CONTINUOUS HEMODIAFILTRATION WITH PMMA HEMOFILTER IN THE TREATMENT OF SEVERE SEPSIS AND SEPTIC SHOCK

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It has been widely recognized that cytokines play a pivotal role in the pathophysiology of septic shock and subsequent organ failure. We have reported that continuous hemodiafiltration (CHDF) using polymethyl methacrylate (PMMA) membrane hemofilter (PMMA-CHDF) could effectively and continuously remove various cytokines from circulating blood mainly through adsorption to the hemofilter membrane and that such cytokine removal with PMMA-CHDF was effective for the treatment of hypercytokinemia-related pathophysiology, such as ARDS and severe acute pancreatitis. In April 2000, we introduced rapid measurement system of interleukin-6 (IL-6) blood level using automated chemiluminescent enzyme immunoassay (CLEIA), which can measure IL-6 blood level within 30minutes in the clinical

laboratory. Since then, we can scientifically determine the indication and the timing of the initiation and termination of PMMA-CHDF for cytokine removal and started to apply PMMA-CHDF on severe sepsis and septic shock. In a preliminary study, we applied PMMA-CHDF on septic patients immediately after the onset of shock regardless of renal function, and evaluated changes in hemodynamic parameters, indices of tissue oxygen metabolism, IL-6 blood levels as an indicator of hypercytokinemia and cellular injury score (CIS) as a severity index of organ dysfunction. Blood pressure and urine volume significantly increased 2 hours after initiation of PMMA-CHDF and these changes were accompanied with increased systemic vascular resistance index (SVRI) and unchanged cardiac index (CI). IL-6 blood levels and blood lactate were significantly decreased after initiation of PMMA-CHDF and CIS was also significantly improved. Taking those results, we now apply PMMA-CHDF in combination with early-goal directed therapy for patients with septic shock and severe sepsis who showed sustained high IL-6 blood level (> 1000pg/ml) despite adequate supportive care. With these treatment strategies, survival of severe sepsis and septic shock has been gradually improved. These results suggest that cytokine removal with PMMA-CHDF would be effective for the treatment of severe sepsis and septic shock.

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S.A.F.E. (BT): A NOVEL SYSTEM FOR EXTRACORPOREAL REMOVAL OF BACTERIAL TOXINS (LPS AND LTA)

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Recently we investigated in a pilot study whether the use of an apheresis system based on a DEAE-cellulose cartridge is capable of reducing plasma concentration of endotoxins (lipopolysaccharides, LPS) in patients with severe sepsis (1). We enrolled 15 intensive care patients with plasma LPS concentrations higher than 0.30 EU/ml and processed about 1.7 volumes of plasma (6000 ml) in each session.

A significant reduction in plasma LPS levels from a median of 0.61 to 0.39 EU/ml could be achieved. Long term comparison of the initial and post-treatment levels after a series of five to six individual apheresis treatments also showed a statistically significant decline in IL-6, CRP, fibrinogen and an increase in cholesterol levels.

In the meantime we further improved this extracorporeal apheresis system by using the so-called S.A.F.E.^{BT} (Selective Adsorber For Elimination of Bacterial Toxins) adsorber developed by B.Braun Melsungen. This adsorber is composed of especially woven hollow-fibres which are modified with tentacle-like DEAE-ligands. Besides its improved rheology and smaller size this adsorber is unique in that it also adsorbs and eliminates toxins originating from gram-positive bacteria, i.e. lipoteichoic acids (LTA).

(1) Bengsch S, Boos K-S, Nagel D, Seidel D, Inthorn D Shock 23 (2005) 494-500

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ACUTE ISCHEMIC COLITIS

Jan Horn

Acute ischemic colitis presents with a wide range of causes often categorized as mesenteric occlusive (arterial thrombosis, arterial embolism, cholesterol-embolism, post-aortic reconstruction, post-traumatic, vasculitis, radiation enteritis, diabetic vasculopathy, or venous thrombosis from hypercoagulable status or pancreatitis) or more commonly mesenteric non-occlusive (idiopathic, shock-induced, medication-induced or accompanying colonic mechanical obstruction). These entities can produce patchy versus diffuse necrosis that can be localized or pan-colonic with varying degrees of transmural damage. Presenting findings often include visceral tenderness, diarrhea, mild hematochezia, distention and less often nausea with vomiting. Diagnosis is usually possible with computed tomography and colonoscopy. The majority of often elderly patients present with non-transmural necrosis and therefore respond to conservative treatment with hydration, correction of underlying contributing factors and careful observation. Septic deterioration (fever, leukocytosis, and hypotension) or development of peritonitis should prompt immediate exploration. Affected areas are sometimes difficult to gage because the mucosal ischemia may not be visible on serosal inspection. Resection of involved areas with adequate margins gives best results, with diversion often required for high-risk patients. Recovery from conservative treatment occurs in one-half to two-thirds of patients and is rarely complicated by strictures or recurrent ischemia. If surgery is required, mortality often approaches 50% from sepsis and complications of their co-morbid conditions.

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INFECTED PANCREATIC NECROSIS

E. Patchen Dellinger

Patients with infected necrotizing pancreatitis have a high morbidity and mortality. Regarding timing and technique for operative source control, almost no controlled trials have been conducted. Patients with greater degrees of retroperitoneal necrosis and/or a more severe physiologic disturbance (measured by APACHE II or Ranson scores or an organ failure score) have a higher incidence of infection. Operation on clinical grounds alone results in intervention in sterile necrosis as often as 60% of the time

with the risk of secondary infection following operation. The only reliable preoperative diagnostic test for infection is CT or ultrasound-guided percutaneous aspiration of necrotic retroperitoneal tissues. This technique is approximately 90% accurate and can be repeated as necessary in prolonged cases. Ninety percent of errors with this technique occur during the first week of the disease, a time when aspiration can usually be avoided. The following elements are components of successful source control for a patient with infected necrotizing pancreatitis. (1) The presence of infection of retroperitoneal tissues must be recognized. This is most commonly done with cultures and gram stains of percutaneous aspirates guided by CT scan. Whether to operate and debride patients with sterile necrosis who are not responding to nonoperative management is unresolved. It should be done rarely and late in the course of the disease. Debridement of infected necrotic peripancreatic and pancreatic tissues is more likely to be nearly complete and to avoid the need for subsequent debridements if at least 2 weeks have passed since the onset of acute pancreatitis. There may be a role for percutaneous drains as temporizing procedures in combination with antibiotics for patients who are diagnosed with infected peripancreatic tissue during the first two weeks. (2) When the debridement is performed it should be as complete as possible, accessing all affected areas and removing as much necrotic tissue as possible. Debridement has a high risk of causing bleeding or bowel injury, and these complications can be very serious and should be avoided if possible. The correct balance in aggressiveness of debridement is difficult to put into words and depends substantially on the operative experience of the surgeon. The published literature cannot be used to support one technical approach over another in regard to choice of incision, use of drains, closure of the abdomen, mandatory re-exploration, or other such options. It is likely that an increasing experience will be available in the literature using a variety of "less invasive" percutaneous and videoendoscopic approaches. (3) Whatever approach and schedule are employed, the surgeon must be prepared for additional interventions, the details of which will depend on the circumstances found in the individual patient. Most of the controversies in the management of infected pancreatic necrosis are amenable to prospective multicenter trials.

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ABDOMINAL COMPARTMENT SYNDROME COMPLICATING INFECTION

Zsolt Balogh, Zsolt Bodnar, Frederick Moore

The epidemiology, risk factors, independent predictors of the postinjury abdominal compartment syndrome (ACS) were described during the last decade. Current evidence suggests that the incidence of postinjury ACS could be decreased with timely hemorrhage control and judicious fluid resuscitation. The characterization of the etiologically diverse group of ACS patients with abdominal infection and with sepsis is much more difficult and still has to be done. To date the pancreatitis group is one of the better characterized patient populations where the presence and the grade of intra-abdominal hypertension has a proven association with the adverse outcomes. The infection related ACS can be grouped as primary ACS (intestinal perforations, pancreatitis, and generalized peritonitis) and secondary ACS (non-abdominal sepsis). In primary ACS the management should be focused on the surgical solution of the abdominal catastrophe. The secondary ACS patients uniformly receive massive fluid resuscitation and undergo whole body ischemia reperfusion injury. In these patients treating the septic source outside of the abdomen has a pivotal role together with fine-tuned fluid resuscitation. Abdominal decompression is evident in the primary ACS cases and was so far the only treatment for secondary ACS patients. There is growing evidence on non-surgical or non-operative management of secondary ACS patients where the organ dysfunctions are not immediately life threatening, but surgical decompression is still a standard therapy in patients with progressive organ failure who do not respond to conservative treatment. In the future, with awareness, monitoring of the intra-abdominal pressure and the liberal use of open abdomen, the incidence of ACS should decrease in the septic general surgical patients' population like it decreased in the trauma population. But the management of open abdomens will remain a significant challenge with high morbidity and mortality rates.

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OVERVIEW OF PROSPECTIVE RANDOMIZED TRIALS IN TRAUMA

David Hoyt

Several recent attempts to define the trauma research agenda have been very effective in identifying potential strategies for prospective clinical trials. A recent effort by NIH created consensus over five major areas that should be evaluated. These include evaluation of pre-hospital airway and ventilation strategies to avoid potential damaging affects of early airway and ventilation control; evaluation of pre-hospital fluid treatments with regard to whether fluid should be given, and what type of fluid should be given, what is opportunity for hemoglobin solutions or manipulating the inflammatory response through initial fluid treatment; evaluation of systemic and local hemostatic therapy to treat early bleeding and coagulopathy by ; evaluation of the use of antioxidant therapy or other pharmacologic or metabolic adjuvant therapy to manipulate the post shock metabolic or immune response that occurs following injury; and evaluation of body temperature modulation to exploit the potential value of hypothermia or "suspended animation". In addition studies to evaluate complex strategies to treat head injury and multiple organ failure need

further clinical trial design. Recent efforts by the NIH have created a Multi Center Resuscitation Outcomes Consortium which will explore some of these trials or potential studies. The need for additional clinical trial groups to explore multiple questions in a contracted time frame is needed.

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PREHOSPITAL TRIALS: EXCEPTION FROM INFORMED CONSENT

Ernest Moore

Clinical investigation of potentially life-threatening interventions for emergent conditions outside the hospital, by definition, cannot be achieved with traditional informed consent as originally defined by the Nuremberg Code in 1947. International consensus to codify regulations for such exceptions to informed consent are rooted in the Helsinki Declaration of 1964, which recognized that specific reasons exist that justify involving research subjects with a condition that renders them unable to give informed consent. In the United States, deliberation by a number of federal agencies providing oversight for research ultimately culminated in the 1996 Food and Drug Administration (FDA) regulations defining "Exception from Informed Consent Requirements for Emergency Research", commonly referred to as 21 CFR 50.24 (Title 21, Code of Federal Regulations, Section 50.24). Similar guidelines have been implemented in other parts of the world; this discussion will be limited to the US perspective. The fundamental criteria to qualify for 21 CFR 50.24 are: 1) a life-threatening condition, available treatment unproven or unsatisfactory, 2) informed consent not feasible because of incapacitating medical condition, proxy decision-maker contact not feasible within the therapeutic window, and identification of prospective subjects is not reasonable, 3) participation holds prospect of direct benefit to the research subject, 4) research could not be carried out without the wavier, 5) attempts to contact the research subject's legally authorized representative within the therapeutic window, 6) consent documents exist for prospective informed consent, and 7) additional protection of research subjects to include community consultation, public disclosure of the research plans, independent data monitoring committee (IDMC), and public disclosure of the study results. Protocols using an exception to informed consent must be performed under a separate investigational new investigational drug application (IND) that identifies the potential need for a waiver. Community consultation is a challenging aspect of the 21 CFR 50.24 criteria; the goals are to provide the opportunity for discussions with, and soliciting opinions from, the communities in which the study will transpire. The consultation is to be a dialogue, not simply eliciting opinions regarding the study. The process is multifaceted and involves public news media, Web sites, telephone hotlines, public meetings, community advisory boards, and meetings with community groups. The process must also entail an opt-out mechanism for potential subjects; bracelets have been employed for some prehospital trials. During the conduct of clinical trials employing the wavier, the local institutional review board (IRB) provides ongoing assessment to ensure protection of the research subjects' rights. In sum, the US FDA 21 CFR 50.24 offers an ethically sound mechanism to conduct prehospital emergency research with exception from informed consent.

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PRELIMINARY RESULTS OF FABP FOR EARLY DIAGNOSIS OF ABDOMINAL TRAUMA

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Background: Fatty acid binding proteins (FABPs) are relatively small cytoplasmic proteins that are abundantly expressed in tissues with fatty acid metabolism. The intestinal (I)-FABP exhibits a specific localization in the small bowel epithelium, whereas the liver (L)-FABP is predominantly expressed in the liver. There is evidence that FABPs are released to the circulation after organ damage, hence FABPs can provide a relatively specific clinical tissue injury marker. This study determined levels, kinetics and correlation of the induction of procalcitonin (PCT), L-FABP and I-FABP during multiple trauma.

Methods: In a prospective case study 67 adult trauma patients admitted to the intensive care unit were evaluated and divided into 4 groups regarding the Injury Severity Score (ISS) and the control group (n=10): ISS<25 without (wo) abdominal injury (AI) (n=25), ISS>25 wo AI (n=13), ISS<25 with (w) AI (n=13) and ISS>25 w AI (n=16). The plasma serum samples were directly obtained after admittance to the emergency room, and on the following two days in a daily manner. 10 healthy volunteers served as control. PCT was measured by the Kryptor-Assay and FABPs by ELISA-technique. Data are presented as mean values±standard error of the mean. Wilcoxon-test was used for statistic evaluation. A $p<0.05$ was considered significant.

Results: The plasma concentrations of L-FABP (298±67 ng/ml) and I-FABP (1395±438 pg/ml) were significantly enhanced in patients with ISS>25 w AI compared to other groups on admission (L-FABP: ISS<25 wo AI: 27±7, ISS>25 wo AI: 94±32, ISS<25 w AI: 87±43 ng/ml, respectively I-FABP: ISS<25 wo AI: 221±46, ISS>25 wo AI: 309±67, ISS<25 w AI: 531±202 pg/ml). On the following 2 days the values declined. The PCT values were significantly increased on the first (3.4±0,9 ng/ml) and the second day in the group ISS>25 w AI compared to all other groups (day 1: ISS<25 wo AI: 0.24±0.06, ISS>25 wo AI: 0.79±0.26, ISS<25 w AI: 1.44±0.85), but PCT was not increased at the admittance.

Conclusion: The plasma level of L-FABP, I-FABP and PCT were associated to trauma severity and -localization. In contrast to PCT both I-FABP and L-FABP can

distinguish between the groups already on the admission of the patients. These results suggest that both measured FABPs could be useful early markers for the detection of tissue specific injury in acute multiple trauma.

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THE LUNG AS A CENTRAL COMPARTMENT OF ACTIVE CMV INFECTION

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Objective: Human cytomegalovirus (HCMV) is well known to increase morbidity and mortality in the transplant setting. In non-immunosuppressed patients in the intensive care unit (ICU) sepsis has been identified as a risk factor for HCMV reactivation. We present quantitative data from a recent clinical study on the blood and lung as sites of compartmentalized HCMV reactivation in critically ill patients.

Material and Methods: We present data from 99 non-immunosuppressed patients with severe sepsis and documented simplified acute physiology score (SAPS) II. Seropositive sepsis patients were monitored weekly in blood by qualitative and quantitative HCMV PCR using COBAS Roche Amplicor test. Additionally, tracheal secretions were weekly screened for qualitative and quantitative HCMV DNA and qualitative HSV DNA as well as for quantitative detection of viral infectivity in a rapid microculture system.

Data: 38 patients with severe sepsis out of 99 patients in total reactivated HCMV during their clinical stay in ICU. Parameters for HCMV reactivation in blood were positive leukoDNAemia in 24/38 (63.2%) patients and positive plasmaDNAemia in 12/38 (31.6%) of all patients with HCMV reactivation. Only 7/38 patients showed viral DNA levels above 1000 copies/ml. In all seven cases plasmaDNAemia was low-level. In contrast, 27/38 (71%) patients reactivated HCMV in lung. About 50% of all reactivating patients had DNA levels above 1000 copies/ml and 7/38 (18.4%) had extremely high viral load in tracheal secretions. HSV DNA was found in 24/38 patients in tracheal samples. Both, infectious HCMV and HSVcould be isolated from lung. Surprisingly, 14/38 patients with viral reactivation did reactivate only in lung and not in blood or initially in lung.

Conclusion: Herpesviral reactivation is a frequent phenomenon in ICU patients with severe sepsis. 38% of all HCMV seropositive patients reactivated HCMV in blood or lung. We observed a striking disconcordance between blood and lung in terms of HCMV viral load. While the HCMV plasmaDNA load ranged from 1000-5000 copies/ml, the viral load in tracheal secretions was superior to 100 000 copies/ml in 18.4% of sepsis patients with maximum viral load > 1 000 000 copies/ml in a few cases. In these cases also infectious virus could be detected. We did not observe a strict correlation between the viral isolation of HCMV from lung in terms of quantitative infectivity to the DNA load. In all cases of drastic virologic lung affection the clinical definition of HCMV pneumonia was not fulfilled. However,statistical evaluation of further clinical data of ICU patients with severe sepsis is in progress. The most important finding in relation to viral pathogenicity is a strongly compartmentalized pattern of HCMV reactivation in lung without involvement of peripheral blood. This phenomenon can contribute to a better understanding of the initially often contradictory role of HCMV in criticall ill patients, since several studies monitored exclusively blood and not lung for herpesviral reactivations.

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CMV INFECTION IN SEPSIS PATIENTS- THE EXPERIENCE AT TUEBINGEN UNIVERSITY

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Introduction: CMV reactivation is well known to increase morbidity and mortality in immunosuppressed patients such as transplant recipients. In nonimmunosuppressed patients in the intensive care unit (ICU) setting sepsis has been identified as a risk factor for CMV reactivation. Here we present a double-blinded prospective study assessing the consequences of CMV reactivation in nonimmunosuppressed patients with severe sepsis.

Methods: In 3 (2 surgical, 1 medical) ICUs of a German university hospital adult patients were screened for severe sepsis. Patients with recently occurring severe sepsis (< 72 h) were enrolled, if their anti-CMV IgG titer was positive. The exclusion criterion was a manifest immunodeficiency. At enrollment the simplified acute physiology score (SAPS) II was assessed. Patients were monitored for CMV reactivation weekly until death or hospital discharge by qualitative and quantitative PCR from plasma, leukocytes and tracheal secretions (TS), and virus isolation from TS. CMV reactivation was defined as positive CMV DNA detection or virus isolation. Patients with (CMV+) and without CMV reactivation (CMV-) were compared regarding to in-hospital mortality, duration of mechanical ventilation, length of stay (LOS) in the ICU and the hospital. Data were analysed using the Wilcoxon score rank sum test and chi square test. The level of significance was set to .05.

Results: CMV reactivation was observed in 38 out of 99 patients. Both groups (CMV+ / CMV- patients) were quite similar in regard to gender and age at study enrollment. Interestingly, the median SAPS II was higher in CMV- patients (47 vs. 42; $p < 0.013$). Accordingly, a lower mortality rate was anticipated for CMV + patients compared to the CMV- group. Contrary to expectations there was no difference be-tween the both groups

regarding to in-hospital mortality (CMV +: 36.8% vs. CMV - 42.6%; p >0.67). This may point to a relatively increased mortality in CMV + patients, although CMV disease did not occur. There was a striking difference between the groups in respect to the period on ventilator: 21.5 days vs. 8.0 days (median) in CMV+ and CMV- patients, respectively ($p < 0.005$). Similarly, CMV+ patients had a longer median LOS after enrollment either in the ICU (29.5 days vs 10, $p < 0.001$) and in the hospital (49 days vs 23, $p < 0.001$). This difference was further assured when the analysis was re-stricted to survivors.

Conclusion: Our data suggest that CMV reactivation leads to increased morbidity and additional treatment expen-diture independently from CMV disease. Further analysis of the data points at a crucial role of pulmo-nary pathology due to CMV reactivation in this context.

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DEAD BACTERIA DO NOT DEVELOP ANTIBIOTIC RESISTANCE: THE CASE FOR TREATING COLONIZED PATIENTS TO REDUCE ANTIBIOTIC RESISTANCE DEVELOPMENT

Steven Opal

The progressive emergence of antimicrobial resistance among microbial pathogens threatens the very foundation of modern chemotherapy against infectious diseases. One long held strategy designed to reduce the development of antibiotic resistance is the elimination of colonizing bacteria before they have had an opportunity to cause invasive disease in the host. The idea of decolonizing patients before major surgery with selective decontamination of the digestive tract (SDD) or removing methicillin-resistant Staphylococcus aureus from skin sites before surgical placement of medical devices has been the subject of discussion and debate for decades. If this strategy is to be undertaken, the timing, duration and dosing of antibiotics is of critical importance. Pharmacokinetic/pharmacodynamic parameters need to be followed to eliminate potential bacterial pathogens and avoid the inevitable generation of resistant subpopulations. This is particularly problematic when large concentrations of microbial pathogens have accumulated. Subinhibitory dosing of antibiotics is to be discouraged owing to the risk of inducing transient hypermutation. This process promotes transmission of bacterial resistance genes and unwittingly contributes to antimicrobial resistance development. It is possible to decolonize patients to prevent spread of antibiotic resistance but this needs to be done with great care. The goal is to eradicate specific pathogens without jeopardizing subsequent therapeutic choices if actual infection develops.

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A 386

THE PREVENTION OF CATHETER RELATED BLOODSTREAM INFECTIONS USING ANTIMICROBIAL IMPREGNATED CENTRAL VENOUS CATHETERS

Tom Elliott

Central venous catheters (CVCs) continue to be associated with infections either at the site of insertion or systemic. Various strategies have been implemented to reduce the risk of these infections including use of aseptic barrier techniques and educational programmes.

Despite all of these approaches infections still arise. This is partly due to the inability to sterilise the skin both before and after cannulation and that access to the devices is required allowing entry opportunities for microorganisms. It seems therefore logical to develop catheter polymers which contain antimicrobial agents potentially offering further protection from microbial colonisation and infection. Several antimicrobial devices are now available and their role in the ICU will be presented.

To assess the various antimicrobial CVC studies we have carried out an extensive computerised Medline literature search from 1966 to 2006. The Centre for Disease Control definitions for catheter related infections were utilised. Two antimicrobial catheters have been widely investigated. These are the silver sulfadiazine chlorhexidine device (SS-CH) and a minocycline rifampicin (M-R) CVC. Chlorhexidine and silver sulfadiazine act synergistically with the chlorhexidine disrupting the cytoplasmic membrane and the silver salts preventing DNA replication. The ARROW g+ard Blue® (ARROW International Inc. USA) was initially only coated on the external surface with CH-SS. More recently a second generation device has been produced which has increased concentration of chlorhexidine on the external surface and chlorhexidine on the intraluminal surface including the hubs. (ARROW Blue +, ARROW International Inc. USA). Of thirty eligible trials associated with the first generation CH-SS CVC eight demonstrated significant reductions in the colonisation rates of CVCs compared to controls. Of the thirteen trials only two were associated with a significant reduction in catheter related bloodstream infections (CRBSI). However, overall analysis demonstrated a significant reduction in catheter related bloodstream infections. Only three trials evaluating the second generation CH-SS CVCs have been carried out. These demonstrated a significant reduction in colonisation but not in CRBSI.

The M-R CVC has also been extensively studied. In our meta analysis five eligible trials assessed colonisation rates and of these three achieved significant reductions in comparison to the controls. Similarly overall analysis demonstrated a significant reduction in CRBSI.

Antimicrobial CVCs have also been directly compared in clinical trials. The first generation CH-SS CVC has been compared to the MR CVC in two studies. The results of one investigation demonstrated a significant reduction in

both colonisation and CRBSI with the MR CVC. The other study also showed a similar trend but statistical significance was not reached. The reason for the enhanced efficacy of the MR CVC as compared to the CH-SS CVC may have been due to the first generation CH-SS being evaluated which is only coated on the external surface.

If antimicrobial CVCs potentially reduce CRBSI, then why are they not more fully used? Concern has been raised as to the possibility of emergence of resistance, however, to date this has not been detected in clinical practice. Questions have also been raised regarding potential methological flaws with some of the studies. For example in several studies the definitions of CRBSI were variable, and the molecular relatedness of organisms recovered from CVC tips and blood has only been evaluated in a limited number of studies. These issues need to be considered in determining efficacy and potential application of these catheters.

What is the value of antimicrobial CVCs in the Intensive Care Unit (ICU)? Several studies have assessed these CVCs in the ICU situation. In one study[1] 200 patients received M-R CVCs and 237 patients control catheters. The antimicrobial devices resulted in a significant decrease in colonisation with coagulase negative staphylococci but there was an increase in candida species colonisation. There was also a decrease in CRBSI although the 30 day survival rate was not increased. In a further study the M-R CVC was compared to a silver platinum carbon impregnated CVC again on an Intensive Care Unit[2]. Both catheter types had low rates of CRBSI. It was suggested that further studies requiring many hundreds of CVCs may be necessary to demonstrate significant differences in catheter related bloodstream infections. The CH-SS CVC has also been assessed in the ICU[3]. The CH-SS catheters reduced colonisation rates, however, there was no difference in CRBSI. These results were confirmed by a further study[4] on the ICU. In comparison[5] the CH-SS catheter was evaluated on a Medical-Surgical ICU and there was a significant reduction in colonisation and CRBSI with the antiseptic catheters. Similarly, Brun-Buisson[6] also described with the CH-SS catheter in the ICU, where there was a low infection rate, a significant reduction of catheter colonisation, a reduction of overall infection episodes but not bloodstream infections. This was with the second generation of CH-SS coated both on the inside and outside. Rupp et al[7] in a large multi-centre randomised trial assessed the second generation CH-SS catheter. The antiseptic catheters were found to be significantly less likely to be colonised than control catheters. There was however not a significant decrease in CRBSI probably due to the low infection rates and the relatively low number of devices studied.

Colonisation of catheters is recognised as one of the key requisites to development of bloodstream associated infections. If antimicrobial CVC's reduce colonisation then it is likely even in the ICU situation that associated sepsis may also be reduced. It is however difficult to prove as many studies have been underpowered in terms of numbers of patients required to reach significance. Similarly, it could be argued that on ICU, the aseptic care which CVC receive is of a high standard and that further reductions in sepsis rates will not be as significant as compared to other clinical areas. It has however been clearly demonstrated that antimicrobial catheters, in situations where the underlying infection rates are high, will significantly reduce both colonisation and infection. In a recent surveillance report[8] differences in CRBSI were reported according to the type of ICU with burns and trauma having the highest rate of sepsis. It would therefore seem reasonable to use antimicrobial CVCs in the ICU situation where CRBSI are high which in turn would reduce antibiotic use thereby minimising the risk of the emergence of resistance.

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CON: ANTIMICROBIAL COATED CATHETERS PREVENT INFECTIONS AND SAVE LIVES IN THE ICU

Thomas Glueck

Central venous catheters (CVC) are an indispensable tool for the treatment of critically ill patients, as they allow simultaneous administration of nutrition, antibiotics, vasopressors, fluids and other medication. Unfortunately, infectious complications of such devices are still a major

problem, especially if these are in place for periods > 10 days. The US National Nosocomial Infection Survey program (NNIS) gives CVC infection rates of approx. 5/1000 device rates. In contrast, the German KISS program, using the same criteria, found CVC infection rates of only 1.8/1000 device days. While there are abundant data on CVC infection rate, there is less information on the attributable morbidity and mortality of CVC-related bloodstream infections: uncontrolled studies reported an increase in mortality up to 35%, but studies that controlled for severity of illness found less increases in mortality.

CVC colonization and subsequent infection may develop by different routes: a) transmission of pathogens from the skin surface into deeper tissues at the time of catheter insertion, b) transfer of circulating bacteria to the external catheter surface, and c) infection of the internal catheter lumen by contaminated infusate. Each of these pathogenic concepts implicates strategies for prophylaxis, among which prevention of CVC contamination at the time of catheter insertion seems to be most effective.

The CDC/HICPAC guidelines for the prevention of CVC infections recommend for all catheter types optimal skin disinfection and maximal sterile barrier precautions during CVC insertion. Catheter insertion and care should be done by specially trained personnel.

Antimicrobial coating of CVCs as a measure to prevent infectious complications has been introduced in the late 1980s and early 1990s. Antimicrobial coated CVCs are up to 2 times more expensive than standard CVC. Chlorhexidine/sulfadiazine-impregnated CVCs retain their antimicrobial activity only for 6-10 days, minocycline/rifampin-impregnated CVCs for longer periods. Acute allergic reactions were observed with both catheter types. Induction of resistance to minocycline and rifampin has been demonstrated in in vitro models using such catheters.

Several clinical studies have shown that antimicrobial coated CVC can reduce colonization and infection rates, but the results were not consistent in all studies due to different definitions for CVC infections and different study design. Cost effectiveness analyses have found that antimicrobial coated CVC are cost effective only if the infection rate exceeds 3.3/1000 device days. There are no robust data on the effect of antimicrobial coated CVC on hard clinical endpoints such as prolongation of hospital stay or death. It has been suggested that a similar reduction as observed with antimicrobial coated CVC can be achieved by rigorous skin disinfection prior to insertion - unfortunately this has not yet been tested in an appropriately designed clinical study.

Following the CDC/HICPAC recommendations and other recent publications, antimicrobial coated CVC cannot be recommended unconditionally. They should be reserved for patients a) expected to need a CVC in place for > 5 days, and b) known to have a high risk for infection (e.g. burn patients) or belonging to a patient population with a CVC infection rate > 3.3/1000 device days despite following generally recommended infection control measures. All other patients should continue to receive standard CVC.

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WE CAN USE BIOMARKERS TO GUIDE CARE IN CAP: PRO

Jean-Louis Vincent

Community-acquire pneumonia (CAP) is a leading cause of morbidity and mortality in developed countries and may patients with CAP will have disease severe enough that admission to the intensive care unit (ICU) will be necessary. Early diagnosis and treatment are crucial, but diagnosis can be difficult as signs and symptoms are often non-specific. Once antimicrobial treatment has been initiated, how long it should be continued is a matter of debate, with no accurate means of assessing resolution of the CAP. A marker, which could be used to diagnose CAP, monitor its progress, and guide treatment would therefore be invaluable. Various biomarkers have been suggested for this purpose, including procalcitonin and pro-adrenomedullin. Although not specific for CAP, levels of these 'markers' are raised in patients with CAP and increased levels have been associated with increasing disease severity and worse outcomes. A recent randomized study used procalcitonin levels to guide the antibiotic treatment of CAP, and reported that procalcitonin guidance reduced total antibiotic exposure, antibiotic prescriptions on admission, and antibiotic treatment duration compared with patients treated according to standard current guidelines. Further multicenter clinical trials are needed to confirm these single center data, and to validate the cut-off values used. In addition, other biomarkers may prove to have greater sensitivity and specificity. Nevertheless, the ability to reduce antibiotic prescriptions, and hence reduce costs, side effects, and selective pressure for the emergence of antibiotic resistance, is clearly an exciting prospect, and strongly supports the use of biomarkers to guide care in patients with CAP.

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PATHOPHYSIOLOGY OF PERITONTIS

William Cheadle

Peritonitis refers to inflammation of the visceral and parietal peritoneum and the peritoneal cavity itself. The disease has been divided into primary, secondary, and tertiary. Primary peritonitis was seen commonly in the pre-antibiotic era and is usually caused by Staph aureus. Spontaneous bacterial peritonitis in cirrhotics and peritonitis in those with peritoneal dialyisis catheters are examples. Secondary bacterial peritonitis is a polymicrobial infection caused by both aerobic and anaerobic Gram negative enteric organisms. The most common cause is a

perforated hollow viscus, and treatment is surgical with adjuvant antibiotics. Tertiary refers to cases of secondary bacterial peritonitis that are persistent or recurrent after initial surgical treatment. Intraperitoneal bacteria are initially cleared by resident peritoneal macrophage phagocytosis and through the subdiaphragmatic lymphatics into the mediastinum and thoracic duct. Peritoneal mast cell degranulation, and coagulation and complement cascade activation occurs, with subsequent neutrophil influx into the peritoneum. The end result of this local host defense response is either resolution of the infection, abscess formation, or generalized peritonitis. The local peritoneal immune response is characterized by early activation of the innate immune system, including macrophages, neutrophils and natural killer cells, and subsequent migration into the peritoneum enhanced by chemoattractant production, such as LTB_4, IL-8, C5a and C3a. Activation of neutrophils, and their subsequent inappropriate sequestration in organs remote from the site of infection, appears to be one of the key events in the development of organ failure associated with peritonitis. We have shown that organ failure and not recurrent peritonitis was associated with mortality from intra-abdominal infection. There are low levels of circulating endotoxin, tumor necrosis factor (TNF), and interleukin (IL)-1 during peritonitis in the mouse, and mortality was similar in endotoxin sensitive and endotoxin resistant mice. Cytokine manipulation in peritonitis models has been associated with both beneficial and detrimental results, while inhibition of leukocyte apoptosis and complement C5a has improved survival in this model. An abnormal early innate immune response leading to remote organ PMN sequestration is likely responsible for organ failure during peritonitis rather than enteric bacterial endotoxin.

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MICROBIOLOGY OF BACTERIAL PERITONITIS

Carl Erik Nord

Spontaneous primary bacterial peritonitis is seen in patients who have liver disease and ascites. The pathogenesis has the following sequence: (i) translocation of microorganisms into the mesenteric lymph node, (ii) bacteremia arising from the lymphatic system, (iii) growth of bacteria in ascites secondary to bacteremia, and (iv) growth of bacteria in opsonin-deficient ascites. The same microorganisms are found in the blood and the ascites of approximately 50% of patients who have liver cirrhosis and culture-positive ascites. The incidence of bacterial peritonitis is high in patients who have cirrhosis and impaired reticuloendothelial system. Phagocyte activity and susceptibility to bacterial peritonitis is higher in patients if there is decreased opsonin activity in the ascites. Escherichia coli is most frequently isolated from the ascites, followed by Klebsiella pneumoniae, pneumococci, streptococci and enterococci. Staphylococcus aureus is not often found in primary peritonitis. Anaerobic bacteria are isolated in 5% of patients. Primary peritonitis is rarely caused by Chlamydia pneumoniae, Neisseria gonorrhoeae and Mycobacterium tuberculosis.

Secondary bacterial peritonitis is often caused by perforation of the intestine resulting in spillage of intestinal contents into the abdominal cavity. The magnitude of bacterial contamination depends upon several factors such as the site of the perforation, the cause of the perforation and the local defense system limiting the infection. The number and types of microorganisms depend upon the site of gastrointestinal perforation. When the stomach is perforated, rather few types of acid-resistant microorganisms such as Helicobacter pylori, lactobacilli, streptococci and Candida are recovered. The proximal small intestinal microflora also contains fewer microorganisms, whereas the number of microbial species is greater in the distal ileum. Perforation of the upper small intestine leads to the isolation of lactobacilli, streptococci, enterococci and clostridia. When the large intestine perforates, many different microbial species are spilled into the peritoneal cavity. In most cases when an infection develops, E. coli, enterococci, Bacteroides fragilis, peptostreptococci, and clostridia predominate. In compromised patients, Pseudomonas aeruginosa and Acinetobacter baumanni can also be isolated. With colonic perforation, E. coli is the most frequently recovered aerobic microorganism and B. fragilis is the most frequently isolated anaerobic microorganism. Severely ill hospitalized patients may have an altered lower intestinal microflora with increased numbers of P. aeruginosa, Enterobacter spp., and multidrug-resistant nosocomial pathogens such as Enterococcus faecium, E. faecalis, and Candida.

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ACTIVATED PROTEIN C: IS THERE A ROLE IN THERAPY OF INTRA-ABDOMINAL INFECTIONS?

Philip S Barie, Lynn J Hydo, Jian Shou, Soumitra R Eachempati

Background: The efficacy of therapy with drotrecogin alfa (activated) (recombinant human activated protein C [APC]) for surgical patients with severe sepsis has been questioned There is also concern that patients who have undergone surgery recently may be at increased risk of bleeding complications from therapy. Recent data and clinical trends in the management of patients with intra-abdominal infection and severe sepsis are analyzed with respect to the efficacy and safety of therapy with APC.

Methods: Review and synthesis of the pertinent English-language literature.

Results: Surgical source control is the mainstay of therapy for intra-abdominal infections, whereas antibiotics, fluid resuscitation, and support of visceral organ function are necessary adjuncts. Therapy with APC can be given to

surgical patients, albeit with some delay (most protocols specify a 12-h wait after major surgery to mitigate the perceived increased risk of bleeding). In the pivotal PROWESS clinical trial, APC therapy did not appear to be efficacious for surgical sepsis, but rigorous scrutiny of surgical indications and adequacy of source control by blinded re-appraisal of the PROWESS data suggested that APC therapy may be effective for surgical patients at high risk of death (Acute Physiology and Chronic Health Evaluation [APACHE]-II score > 24 points). Several comparable studies aggregated in the INDEPTH database show a significant reduction in mortality (OR 0.66, 95% CI 0.45-0.97) for therapy with APC of surgical patients with severe sepsis and a high risk of death, although the risk of bleeding is higher in surgical patients compared with APC-treated non-surgical patients, In contrast, surgical patients at a lower risk of death do not benefit from APC treatment, but are at risk for bleeding.

Conclusions: Surgical patients with severe sepsis and a high risk of death (APACHE II > 24 points) have significantly lower mortality if treated with APC. The increased risk of bleeding with APC therapy is acceptable given the clearly improved survival. Surgical patients with sepsis at lower a risk of death do not appear to benefit from therapy with APC, which should be withheld in most circumstances because of the risk of bleeding.

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GRAM-POSITIVE GERMS IN SURGICAL INFECTIONS: SETTING THE STAGE

Stefanos Geroulanos

Surgical infections have a major impact on the surgical patient. They may be present as clinical syndromes requiring operative intervention for cure or as complications in the postoperative period.

Many of the classical infections that plagued patients needing or undergoing surgery in the past like abscess formation, empyema, tetanus, gaseous gangrene and others are now rare, but this improvement brought about also negative effects: Antibiotic use over the past 50 years has caused a profound change in the epidemiology of surgical infections. The formerly prevalent gram-positive cocci of the 60′ ties have given way to gram-negative bacilli in the 80′ ties. However, in the 90′ ties a major come back of the Gram-positive cocci like MRSA, MRSE, VRE and others has been observed.

For example, the background course of Staph. aureus resistance is spectacular: In 1950 Staph. aureus became Penicillin resistant, in 1961 Oxacillin and in 1969 Gentamycin resistant. In 1977 Staph. aureus became Vancomycin intermediate, while in 2001 Vancomycin resistant. The prevalence of MRSA ranges now in the USA and southern Europe between 40-60% reaching in some hospitals 89%. Contrary to this, in northern European countries like Scandinavia, the prevalence was kept by strict antibiotic and infection control policies under 2% and in Central Europe under 10%.

In mid 80′ ties, Plasmidia crossed over from Staph. aureus to Staph. epidermidis making aslo this saprophytic germ a major problem in patients with implanted prosthetic devices and catheter related infections. The ability of this germ to produce slime around plastic material and be hidden in it, made this germ also untouchable for most antibiotics.

In the early 90′ ties Vancomycin resistant etnerococci (VRE) came to the forefront and today there are reports of patients carrying both MSRA and VRE. This situation made necessary the development of new antimicrobial and antifungal drugs.

In the new century Gram-negative rods -resistant to all antibiotics available- like Acinetobacter, Pseudomonas, Stenotrophomonas and Serratia are emerging.

It looks as if once a new developed drug is effective against one group of germs, another group of germs overwhelms the previous one up to the moment that the previous group of bacteria becomes resistant to the new drugs. Next to it, the uncontrolled use of antimicrobial agents alters the normal intestinal flora causing again a major swift in the resistance pattern. Last, -but not least- modern medical care has promoted violation of man's natural defenses on several fronts, like performance of major operative procedures, insertion of prosthetic devices, breaking the natural barriers by catheter insertion, immunosupression induced by age, adipositas or cachexia, concomitant diseases, medicaments, drugs and others. In addition, today it is not uncommon for a patient to be compromised by all these factors simultaneously.

Therefore, one should not wonder that despite major progress in the arsenal against infections, the postoperative infection rate does not decrease. More and more sophisticated and expensive drugs have to be developed and new combinations of drugs have to be tried in order to combat infections from resistant germs.

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MANAGEMENT OF MRSA SURGICAL INFECTIONS: RECENT LESSONS THAT HAVE BEEN LEARNED

E. Patchen Dellinger

Management of surgical infections by definition always involves an intervention that is, by convention, called "source control." For a simple abscess this is incision and drainage. For a superficial surgical site infection the incision may be as simple as removing the skin sutures and letting the wound fall open. For necrotizing soft-tissue infections source control involves a much more extensive operation with debridement, and often with

removal of substantial amounts of tissue. By convention, antibiotics are often given at the same time as the source control for surgical infections, but there are few studies to help us understand which infections actually require antibiotics and very little information regarding how much antibiotic should be given and for how long. By convention we have assumed that if antibiotics are to be given they should be active against the pathogens recovered from the infection. This is well established for pneumonia, bacteremia, and intra-abdominal infections among others but has rarely been studied for surgical infections. Over the past 30 years the incidence of MRSA among soft-tissue infections has been growing steadily and has thus called into question the common practice of administering a penicillinase-resistant beta lactam antibiotic such as one of the semisynthetic penicillins (oxacillin, etc) or first generation cephalosporins. For patients without a significant systemic response to the infection what little evidence exists suggests that incision and drainage alone without antibiotic may be sufficient. A randomized trial of antibiotic vs placebo for incisional surgical site infections by Huizinga (1986) in South Africa and a large observational study at San Francisco General Hospital of abscesses with MRSA treated with first generation cephalosporins compared to "appropriate" antibiotics by Paydar (2006) observed no benefit for the addition of antibiotic therapy when appropriate source control was provided. With the increasing incidence of community-associated MRSA throughout the world, it is clear that if antibiotics are going to be administered that serious consideration should be given to the use of vancomycin or one of the newer antibiotics effective against MRSA. However, the growing pattern of MRSA also increases the value of avoiding antibiotic therapy when that is safe for the patient. What level of MRSA in the community should trigger a change in prophylaxis patterns is also unknown. One of the few studies to compare vancomycin with a cephalosporin for prophylaxis of cardiac cases found an excess of MSSA infections in the vancomycin group with no overall benefit to vancomycin (Finklestein, 2002). Lastly, it is very important to be aware of some new patterns in MRSA surgical infections. Historically, S. aureus was considered to cause primarily abscesses and surgical site infections confined to the incision, in contrast to beta-hemolytic streptococci and mixed infections that had the potential to cause rapidly spreading necrotizing soft-tissue infections, commonly called necrotizing fasciitis. Recent reports have made clear that the new community-acquired MRSA is fully capable of causing these serious, life-threatening infections. Accordingly, the initial antibiotic management of a necrotizing soft-tissue infection should always include an agent known to be effective against MRSA until the identity and sensitivity of the pathogens are known.

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THE CLINICAL IMPLICATIONS OF SUSCEPTIBILTY TESTING OF GRAM-POSITIVE COCCI

Susan Rehm

Introduction: In this era of rising rates of resistance among gram-positive bacterial isolates, clinicians should recognize the importance of accurate and complete antimicrobial susceptibility testing.

Data: Rates of infections due to methicillin-resistant Staphylococcus aureus (MRSA) are rising in most parts of the world. Susceptibility testing plays a vital role in the detection of MRSA and particularly for community-associated MRSA (CA-MRSA). Since inducible clindamycin resistance is common among CA-MRSA isolates, routine use of the D-test is important for guidance in the choice of appropriate therapy. The poor clinical outcomes associated with infections due to glycopeptide-resistant, glycopeptide-intermediate, and heteroresistant vancomycin-intermediate Staphylococcus aureus (hVISA) are receiving increasing attention, in part because of delays in recognition of the resistance of the isolates. More than a decade ago similar problems in detection of glycopeptide resistance among enterococcal species, particularly with use of disk diffusion tests, led to changes in methodology. Unfortunately, the convenience of automated methods for determining MICs is offset by the possibility that they may not detect certain types of antimicrobial resistance. This leads to requirements for further testing that delays final results and incurs additional costs. In addition, standards defining susceptibility have come into question. The designation of MIC cut off's is particularly challenging because of the emergence of antibiotic-tolerant isolates and the recognition of the significance of low-level antibiotic resistance.

Conclusions: Acknowledgment of poor clinical outcomes associated with certain strains of gram-positive cocci has led to ongoing examination of methods for determining appropriate antibiotic therapy. In the future, molecular detection of genetic elements conferring resistance may become a useful adjunct to traditional antimicrobial susceptibility tests.

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DIAGNOSIS OF FUNGAL INFECTIONS: ROLE OF NEW NON-INVASIVE TOOLS IN THE CRITICALLY ILL PATIENT

Oscar Marchetti

Fungi, mainly Candida and Aspergillus, have emerged as important causes of infections in immunocompromised and critically ill patients. Multiple risk factors, clinical and radiological presentation are not specific for the diagnosis of invasive mycoses. Microbiological documentation is often possible only at an advanced stage (dissemination, sepsis syndrome) and late antifungal therapy results in high failure rates (severe complications, mortality). Antifungal prophylaxis and pre-emptive/empirical therapy in persistently febrile high risk patients are attractive approaches to prevent or early treat invasive mycoses, respectively. However, the lack of strict selection criteria results in the treatment of large numbers of patients, which increases toxicity, risk of emergence of resistant species and costs. These aspects highlight the importance of promptly identifying patients who need antifungal therapy. Conventional culture techniques lack sensitivity (blood) or specificity (non-blood sites). Invasive tissue sampling is often problematic and yield after prolonged therapy is low. Radiological signs appear often late in the course of infection. The EORTC-MSG diagnostic classification of invasive mycoses in immunocompromised patients based on clinical, microbiological and radiological criteria does not apply to other types of patients. Thus, diagnosis of systemic fungal infections in critically ill patients remains a major challenge. The colonization by Candida is a major risk factor which can be assessed with the colonization index. The increasing growth of Candida at multiple body sites can predict the risk of invasive candidiasis. However, a prospective screening on a broad basis is work-intensive, expensive, and difficult to perform. The need for sensitive and specific diagnostic tools has led to intensive research on detection of circulating fungal metabolites, antigens, antibodies and DNA. Laboratory tests for measurement of circulating antigens such as mannan, galactomannan and beta-glucan, major components of the fungal cell wall, and of anti-mannan antibodies have been developed. Clinical studies have reported interesting results on their diagnostic performance and have suggested that these assays may be useful for early diagnosis and assessment of response to antifungal therapy. Issues such as choice of the cut-off values, number of positive samples, need for sequential measurements, false-negative results during antifungal prophylaxis or therapy, false-positive results in patients receiving synthetic penicillins and combination of various tests have been addressed. Some of these markers are now integrated in the EORTC-MSG diagnostic criteria of invasive mycoses. Molecular diagnostic methods for detection of fungal DNA in blood or tissues have been mainly studied in onco-hematological patients and variable results have been reported. Lack of standardization, automation, and inter-laboratory reproducibility has limited their implementation in clinical practice. In summary, new rapid, sensitive and specific non-invasive laboratory tools are promising for early diagnosis of invasive mycoses and targeted antifungal treatment, which would prevent severe infectious morbidity and mortality as well as toxicity and costs related to unnecessary therapy. Prospective clinical studies are needed to evaluate the impact of these diagnostic markers on the clinical management of patients at high risk of invasive mycoses.

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THE IMPORTANCE OF HEMODYNAMIC MONITORING AS PART OF THE SURVIVING SEPSIS GUIDELINES

Phillip Dellinger

Hemodynamic monitoring plays a key role in the sepsis resuscitation bundle of the Surviving Sepsis Campaign (SSC) performance improvement program. This bundle (figure below) includes seven indicators or goals for the first 6 hours of management of the patient with severe sepsis. Initial fluid resuscitative therapy is performed empirically in the presence of hypotension (systolic blood pressure <90, mean arterial pressure <65 or decrease in systolic pressure of 40 or more). A fluid bolus of ^{3}20 ml/kg of crystalloid or colloid equivalent should be administered. The same fluid bolus is also empirically administered to patients with lactate >4 mmol/L (36 mg/dl). The second empiric component is the administration of vasopressors to maintain a mean arterial pressure (MAP) ^{3}65 mm Hg. This is typically administered following fluid resuscitation when MAP remains <65 mm Hg. However, based on appearance of patient and how low the initial mean arterial pressure is, vasopressor therapy may be initiated concomitantly with fluid therapy.

The hemodynamic monitoring portion of the resuscitation bundle begins when the patient remains hypotensive following fluid bolus (hypotension refractory to initial fluid resuscitation), or has an initial lactate >4 mmol/L. In that circumstance a central venous catheter is inserted in the neck or chest in order to monitor both central venous pressure (CVP) and central venous oxygen saturation ($S_{CV}O_2$) in the superior vena cava. Once the catheter is placed fluid resuscitation is targeted to a central venous pressure of ^{3}8 mm Hg. Resuscitation is also targeted to achieve a $S_{CV}O_2$ ^{3}70%. The latter is accomplished with a combination of fluid resuscitation, packed red blood cells (if hematocrit is <30), and dobutamine. CVP target of ^{3}8

mm Hg is for a typical patient, but clinical acumen should also be integrated into this decision process. For example, for a patient with known thickened left ventricle or who is being mechanically ventilated, a CVP target of $^{3}12$ mm Hg or even higher may be a more appropriate target. One study suggests a pulmonary artery catheter measured $S_V O_2$ of 65% would be an acceptable surrogate for a $S_{CV}O_2$ of 70%. It should be noted that the failure to obtain a lactate in patients presenting with sepsis may prevent not only the diagnosis of severe sepsis (lactate >2 mmol/L), but also lead to the failure to give a fluid bolus and insert CVP catheter if no hypotension was present (lactate >4 mmol/L.

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PRELOAD, VOLUME RESPONSIVENESS, EXTRAVASCULAR LUNG WATER - TOOLS FOR CARDIAC OUTPUT OPTIMIZATION

Azriel Perel

Although many new hemodynamic monitoring techniques have emerged in recent years, it seems that in some instances critically ill patients are still not monitored adequately. This reluctance may be due in part to the many reports on the inability of the pulmonary artery catheter (PAC) to improve patient outcome. Another factor which determines current use of hemodynamic monitoring technique is the search for hemodynamic variables that are 'simple' to understand and 'simple' to measure. It is my view that this approach may at times lead to inaccurate and even misleading hemodynamic assessment, since all hemodynamic variables have limitations that are very often underestimated or ignored.

- The history, physical signs, and routine laboratory tests have limited sensitivity and specificity in hemodynamic evaluation.

- Heart rate and arterial blood pressure are nonspecific signs.

- Estimates of intravascular volume based on any given level of filling pressure (CVP, PAOP) do not reliably predict a patient's response to fluid administration. Hence using filling pressures as target values during fluid therapy may at times lead to either incomplete resuscitation or to fluid overload and pulmonary edema.

- A low cardiac output (CO) value, by itself, will tell you that something is wrong but not what is wrong and what should be done about it (fluids? inotropes?).

- A low central venous oxygen saturation ($ScvO_2$) value will also tell you that something is wrong, but not what is wrong. In addition, when the $ScvO_2$ is normal or high one cannot assume that all is well (e.g., CO normal) since in septic patients the $ScvO_2$ may be elevated due to an abnormally low O_2 extraction.

Optimal fluid resuscitation and its end-points remain a matter of hot debate. This is due in part to the reliance on single and/or 'simple' hemodynamic parameters. A comprehensive assessment of CO, preload (volumetric measurement), fluid responsiveness (PPV or SVV in ventilated patients), $ScvO_2$, extra-vascular lung water (for the quantification of lung edema and as a precaution measure against fluid overload) and microcirculatory/cellular function indicators, is necessary for a complete hemodynamic evaluation and management. Making hemodynamic monitoring in critically ill patients any simpler may result in incomplete or excessive resuscitation.

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L-ARGININE

Martin K. Angele

The vascular endothelial cells plays a key role in the control of vascular smooth muscle tone via the release of nitric oxide (NO). Following adverse circulatory conditions, i.e. trauma and blood loss associated shock, endothelial cell dysfunction occurs leading to a decrease in the release of endothelium-derived NO which may contribute to further alterations in tissue perfusion and organ function. Early administration of L-arginine, the precursor of NO and the substrate for NO synthase (NOS) in vascular endothelial cells, however, has been found to restore the depressed organ blood flow and to reduce tissue injury following shock. In this respect, administration of L-arginine following trauma-hemorrhage during resuscitation restored the depressed organ blood flow in various organs, i.e. liver, splanchnic organs as well as the lung and improved the depressed cardiac output. The maintenance of cardiovascular responses has been found to be associated with restoration of the depressed cell-mediated immune responses and attenuation of the massive inflammatory response encountered under such conditions. Furthermore, the excessive infiltration of the liver with neutrophils following trauma-hemorrhage was decreased by L-arginine administration thereby reducing hepatic injury. In addition, L-arginine treatment decreased the inflammatory response at the site of trauma and improved wound healing process following blood loss.
Thus, administration of L-Arginine might represent a novel and useful approach for maintaining organ blood flow thereby decreasing tissue damage and reducing immunodysfunction following adverse circulatory conditions. Despite those promising results in animal models at present none of the published clinical trials has demonstrated efficacy of L-arginine at doses above standard dietary practices on the outcome in critical ill surgical patients beside the reduction of infectious complications. Some clinical trials even report worsened shock parameters and diminished organ function following L-arginine treatment. In summary, the attitude of experts in the field concerning L-arginine enriched diets seems to be fairly

variable, ranging from elixir to violent poison. The truth is probably somewhere in the middle. Prospective randomized trials are required investigating the effect of L-arginine surgical and septic patients exactly defining the time-point of L-arginine administration.

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CYSTEINE: AN ESSENTIAL AMINO ACID DURING THE SYSTEMIC INFLAMMATORY RESPONSE?

Robert Grimble

Cysteine is normally considered to be a semi-essential amino acid due to the ability for it to be synthesised from the essential amino acid methionine.

Major alterations in the intensity and characteristics of protein and amino acid metabolism occur during the systemic inflammatory response. During the response amino acids are released from peripheral tissues to support the metabolic events in visceral tissues and the immune system which are essential for survival of the patient. Paradoxically, the profile of amino acids released may not match the precise demands. Early evidence for this phenomenon came from the studies of Sir David Cuthbertson who showed a fall in the ratio of urinary S to N in a young male, after orthopedic surgery, indicating preferential retention of sulphur amino acids during the response. Further evidence in support of this phenomenon has come from animal model studies and observations in surgical patients. During the inflammatory response molecules which are rich in sulphur amino acids and those metabolically related to them (methionine, cysteine, serine and glycine) are synthesised in increased amounts e.g. acute phase proteins, glutathione and connective tissue. Cysteine availability is rate limiting for GSH synthesis. Unfortunately there is over a one hundredfold difference in the Km for cysteine utilisation by the rate limiting enzymes for GSH and protein synthesis respectively. Thus as intracellular cysteine concentration falls, GSH synthesis will decrease well before protein synthesis is affected. Decreases in tissue GSH content have been observed in a wide range of conditions in which the systemic inflammatory response is occurring (critical illness, cancer, burn injury, HIV, aging and chronic inflammatory diseases). Glutathione is not only a key component of anti-oxidant defence but essential for many processes that are modulated during the systemic inflammatory response (T cell function, apoptosis, leukotriene synthesis).

Pharmacological (N-acetyl cysteine and procysteine) and nutritional methods (whey protein products) exist for exogenous provision of additional cysteine. These have been shown to be beneficial in a number of situations in which the systemic inflammatory response is operative. Thus evidence for the essentiality of cysteine during the systemic inflammatory response comes from basic biochemistry and metabolic and intervention studies.

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HIGH-FAT NUTRITION INHIBITS INFLAMMATION AND PRESERVES GUT BARRIER FUNCTION VIA CHOLECYSTOKININ, NICOTINIC-RECEPTORS AND THE VAGUS NERVE

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Rationale: High-fat enteral nutrition was shown to reduce inflammation and preserve gut barrier function following severe blood loss. Dietary fat is a potent stimulator of the autonomic nervous system via cholecystokinin leading to several responses such as satiety. Recently was shown that mechanical stimulation of efferent vagus nerve fibres reduces endotoxin-induced inflammation via nicotinic receptors. Here, the role of CCK receptors, the vagus nerve and nicotinic receptors in the protective effect of high-fat enteral nutrition was studied.

Results: Vagotomy and administration of antagonists for CCK receptors and nicotinic receptors significantly blunted the inhibitory effect of high-fat enteral nutrition on hemorrhagic shock-induced tumor necrosis factor (TNF)-α ($p<0.05$) and interleukin (IL)-6 ($p<0.05$). Furthermore, vagotomy and inhibition of CCK and nicotinic receptors reversed the protection of high-fat enteral nutrition on intestinal integrity as measured by bacterial translocation ($p<0.05$ for each group compared to controls) and endotoxin levels ($p<0.05$ for each group compared to controls).

Methods: A non-lethal hemorrhagic shock model was used in which 30-40% of the total blood volume was withdrawn. In experimental groups rats were subjected to vagotomy or injected with CCK-A and -B receptor antagonists and a nicotinic receptor antagonist intravenously.

Conclusions: These data reveal a novel neuro-immunological pathway in which high-fat enteral nutrition directly regulates inflammatory cells via the vagus nerve. This mechanism sheds a new light on functionality of nutrition and offers new potential protective treatment modalities.

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NUTRIENT OVERLOAD

Michael Krebs

Nutrient excess is associated with reduced insulin sensitivity (insulin resistance) and plays a central role in the pathogenesis of type 2 diabetes. Recently, free fatty acids as well as amino acids were shown to induce insulin resistance by decreasing glucose transport/phosphorylation with subsequent impairment of glycogen synthesis in human skeletal muscle. These results do not support the traditional concept of direct substrate competition with glucose for mitochondrial oxidation but indicate that the cellular mechanisms of such lipotoxicity and "proteotoxicity" might primarily affect the insulin signaling cascade. Increased intracellular concentrations of lipid metabolites and/or increased plasma FFA will activate signal transduction pathways which will induce inflammation and impair insulin signaling. Chronic inflammation will further augment insulin resistance but also cause endothelial dysfunction ultimately leading to macro-vascular disease. Therefore, future therapeutic strategies should aim at (i) reduction of plasma FFA availability and lipid storage, (ii) normalization of lipid oxidation and (iii) inhibition of inflammatory pathways in insulin responsive tissues.

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HYPERGLYCEMIA HAMPERS NEUTROPHIL ACTIVATION AND EXAGERATES COAGULATION, WHEREAS HYPERINSULINEMIA IMPAIRS FIBRINOLYSIS DURING HUMAN ENDOTOXEMIA

Michiel Stegenga, Saskia Van der Crabben, Regje Bluemer, Marcel Levi, Hans Sauerwein, Tom Van der Poll

Objective: Type 2 diabetes patients have an increased risk for infections and a higher mortality due to infection. In addition, diabetes is associated with an imbalance between coagulation and fibrinolysis resulting in a tendency towards a procoagulant state. We recently showed that hyperglycemia stimulates coagulation, whereas hyperinsulinemia inhibits fibrinolysis in healthy volunteers. The current study investigated the selective effects of hyperglycemia and hyperinsulinemia on innate immune, coagulation and fibrinolytic responses in a human model of systemic inflammation.

Material and Methods: 24 healthy humans were studied for eight hours during one of the following experiments: (1) lower insulinemic euglycemic clamp, (2) lower insulinemic hyperglycemic clamp, (3) hyperinsulinemic euglycemic clamp and (4) hyperinsulinemic hyperglycemic clamp. In the hyperglycemic clamps target levels of plasma glucose were 12 mmol/l versus 5 mmol/l in the euglycemic clamps. In the hyperinsulinemic clamps target plasma insulin levels were 400 pmol/l versus 100 pmol/l in the lower insulinemic clamps. Three hours after initiation of clamping, 4 ng/kg of E.coli endotoxin was injected intravenously. Data are shown as means ± SEM.

Data: Neutrophil activation: hyperglycemia attenuated endotoxin-induced increases in plasma elastase levels (euglycemic clamps: 791.4 ± 99.3 ng/ml, hyperglycemia 502.8 ± 98.6 ng/ml).

Cytokine production: endotoxin induced plasma cytokine elevations. Hyperinsulinemia especially in the presence of euglycemia led to increased interleukin (IL)-10 levels. Between the four clamps we found no difference in plasma concentrations of tumor necrosis factor (TNF)-α, IL-6 and IL-8.

Endothelial activation: following endotoxin injection, soluble ICAM-1, soluble VCAM-1 and soluble E-selectin were equally increased in all clamps.

Coagulation: hyperglycemia led to potentiation of endotoxin-induced coagulation irrespective of insulin levels: peak thrombin-antithrombin complexes were 80.0 ± 1.7 ng/ml during euglycemia vs. 92.4 ± 1.6 ng/ml during hyperglycemia. Peak soluble tissue factor levels were 211.6 ± 3.5 (euglycemia) vs. 296.1 ± 3.7 pg/ml (hyperglycemia).

Fibrinolysis: regardless of glucose levels, hyperinsulinemia led to an exaggerated upregulation of plasminogen activator inhibitor-1 before and during endotoxemia compared to the lower insulinemic clamps (mean 9.3-fold rise vs. 5.1-fold rise, respectively). In line, endotoxin-induced upregulation of plasminogen activator activity was severely hampered by hyperinsulinemia (hyperinsulinemia: 2.9-fold rise, lower insulinemia: 2.0-fold rise), despite concurrent higher concentrations of tissue plasminogen activator (hyperinsulinemia: 8.9-fold rise, lower insulinemia: 5.7-fold rise).

Conclusion: These data suggest that during inflammation, innate immune functions are mostly affected by hyperglycemia due to impaired neutrophil activity. Furthermore, the net procoagulant state during systemic inflammation is even further imbalanced by differential effects of hyperglycemia (stronger coagulation) and hyperinsulinemia (impaired fibrinolysis). This may imply that type 2 diabetes patients may be especially vulnerable to thrombotic events during inflammatory states.

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EARLY AND SUSTAINED ACUTE FALLS IN TOTAL SERUM CHOLESTEROL ARE ASSOCIATED WITH CRITICAL ILLNESS MORTALITY IN THE SETTING OF TIGHT GLYCAEMIC CONTROL

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Objective: To determine if rapidly-falling and low total serum cholesterol are independent risk factors for death in critically ill patients with tight glycaemic control.

Methods: We performed a case cohort study of patients admitted to a 16 bed University Hospital intensive care between April and July 2006. Waived consent for anonymous data was approved by the NHS Central Office for Research Ethics Committees. Tight glycaemic control is the norm during critical care in our Unit. The testing of routine blood samples for lipid biochemistry was performed every morning on each critically ill patient admitted to our intenisve care. A random effects logistic regression model of mortality was used to accommodate the repeated biochemical measures - with the patient unique number as the panel variable. All statistical analysis was carried out using Sata version 9.2 (Stata Corp, College Station, Texas, US). Results are presented as the main effect with a 95% confidence interval unless stated otherwise.

Results: 223 patients were studied - 137 men (mean age 54 years) and 86 women (mean age 57 years). 163 patients survived past 28 days and 65 died, of which 60 died in the intensive care unit. Mean APACHE II score was 16.1. Total serum cholesterol levels were 0.53 (0.21 to 0.86) mmol/l lower, on average across the days of admission, in those who died within 28 days compared with survivors. Differences were not present on admission. Furthermore, a rapid early fall in cholesterol predicted death. Total cholesterol across the first two recordings after admission fell by 0.17 (0.12 to 022) mmol/l in those who survived compared with 0.37 (0.31 to 0.45) mmol/l in those who died. This difference remained statistically significant ($P < 0.001$) after adjustment for age and gender. In models of mortality, low cholesterol remained a consistent and significant ($P = 0.001$) predictor of death, irrespective of adding potential confounders or effect modifiers, including age, gender, time from admission to blood test, and proxy indicators of pre-morbid organ dysfunction. HDL-cholesterol predicted death only from the second day after admission, and then much weaker ($P = 0.04$) than total cholesterol.

Conclusions: Our study confirms that lower total serum cholesterol is associated with higher mortality in intensive care patients, and demonstrates this for the first time under strict euglycaemic conditions. Furthermore we have show that the early rate of decline in total serum cholesterol is the most discriminating factor. We can not assert whether or not the association between mortality and cholesterol decline is causal. There is, however, emerging evidence from animal studies that cross-talk between nuclear factors may induce a positive feedback state of increasing inflammation and lowering cholesterol. Whether the cholesterol level changes are causal, or result from inflammatory mediators, is difficult to determine from observational studies; therefore we are currently exploring interventional studies in animal models. This work could lead to novel therapeutics in critical illness and challenge the use of lipid lowering therapies in patients with life-threatening inflammatory states.

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DOES STRESS INDUCED INSULIN RESISTANCE AND HYPERGLYCEMIA LEAD TO CHRONIC GLUCOSE INTOLERANCE IN SEVERELY BURNED PEDIATRIC PATIENTS

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Background: Post receptor insulin resistance following burn leads to hyperglycemia which is associated with increased morbidity and mortality. The aim of the present study was to determine whether severely burned pediatric patients have pre-existing diabetic conditions and whether burn causes persistent glucose intolerance and leads to latent diabetes mellitus.

Patients and Methods: One hundred two severely burned pediatric patients were included in the study. Blood was obtained at admission and throughout hospital course, as well as 3, 6, 9, and 12 months postburn. Serum HbA1c, glucose, insulin and C-peptide were analyzed by standard laboratory techniques. Statistical analysis was performed by ANOVA with Bonferronis' correction with significance accepted at $p<0.05$.

Results: HbA1c was normal (<6mg/dl) in all patients at admit and no significant increase was found within 12 months postburn. Blood glucose increased significantly during the acute hospital course (Range: 120-220 mg/dl). Fasting glucose returned to normal values approximately 6 months postburn. Serum insulin increased significantly 5-7 fold during acute hospitalization indicating insulin resistance and returned to normal levels at 3-6 months postburn, $p<0.05$. Serum C-peptide was elevated within the first 6 months post burn and returned to normal values at 9-12 months postburn, $p<0.05$.

Conclusion: Severely burned pediatric patients exert a strong insulin resistant hyperglycemia that persists up to 6 months postburn. HbA1c levels indicate that none of the severely burned patients in this study developed diabetes mellitus.

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Baseline Characteristics and Outcomes of "Enteral Tolerant" Subgroup (n=249)

	EN + Early PN (n=32)	EN Only(n=217)	Adjusted HR (95% CI)
Age	40	42	-
Male	133(61%)	24 (75%)	-
ISS	36	33	-
APACHE II	30	30	-
Abdominal AIS	1.8	1.8	-
Any Nosocomial Infection (%)	22 (69)	92 (42)	1.7 (1.1-2.8)
Pneumonia	12 (38)	55 (25)	1.2 (0.6-2.3)
Blood Stream Infection (BSI)	10 (31)	24 (11)	2.9 (1.4-6.2)
Catheter Related BSI	2 (6)	2 (1)	4.8 (0.7-34)
Urinary Tract Infection	5 (16)	24 (11)	1.4(0.5-3.8)
Surgical Site Infection	6 (19)	31 (14)	1.1 (0.4-2.6)
Late Death (>7 days)	6 (19)	14 (6)	3.5 (1.3-9.6)

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EARLY PARENTERAL NUTRITION IS ASSOCIATED WITH INCREASED INFECTIOUS MORBIDITY AND MORTALITY IN CRITICALLY ILL TRAUMA PATIENTS

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Objective: Parenteral nutrition (PN) is frequently administered to severely injured patients when caloric goals cannot be achieved enterally. A clear benefit to this practice has not been established. The purpose of this study was to determine the impact of early parenteral nutrition on infectious morbidity in critically ill trauma patients.

Material and Methods: We analyzed patients enrolled under the auspices of the "Inflammation and the Host Response to Injury" study consortium. In the primary analysis, we included all adult patients who survived at least 72 hours post injury. Patients were divided into 2 groups: the "early PN" group received at least 750 kcals of parenteral nutrition during any day during the first post injury week. The remaining patients comprise the control group. In a separate analysis, we evaluated early PN use in a relatively homogeneous subgroup of patients receiving early enteral nutrition ("enteral tolerant") who were given additional PN during the first week. Cox proportional hazards regression was used to determine if PN was associated with an increased risk of nosocomial infection.

Data: A total of 567 patients were analyzed. 95 (17%) received early PN. Early PN use was associated with an increase in nosocomial infections (unadjusted hazard ratio 2.2, 95% CI 1.6-3.0, $p<0.001$). This association remained after adjustment for age, gender, injury severity, APACHE II, and abdominal procedure (HR 1.7, 95% CI 1.3-2.4, $p<0.001$). Early PN use was also associated with an increase in late mortality (>7 days; unadjusted HR 3.1, 95% CI 1.8-5.3, $p<0.001$). After adjustment for potential confouders including massive transfusion, this association was no longer significant (HR 1.6, 95% CI 0.74-3.5, $p=0.219$). In the "enteral tolerant" subgroup (n=249), early PN administration was associated with a similar risk of both nosocomial infection (unadjusted HR 1.7, 95% CI 1.1-2.8, $p=0.021$) and late mortality (unadjusted HR 3.0, 95% CI 1.1-7.7, $p=0.027$). In this subgroup, the association between early PN use and both nosocomial infection and mortality persisted after adjusting for confounding variables.

Conclusion: In critically ill trauma patients, administration of PN during the first 7 days post injury may contribute to increased infectious morbidity and a worse clinical outcome.

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THE PROTECTIVE EFFECTS OF HIGH-FAT FEEDING AFTER SHOCK

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Objective: Trauma patients are prone to develop a systemic inflammatory response that may evolve into multiple organ failure and death. Previously, our group has shown in a rat model of hemorrhagic shock that high-fat enteral feeding attenuates inflammation and preserves gut barrier function via activation of the autonomic nervous system through release of cholecystokinin (CCK). However, in trauma patients shock is already present before treatment can be initiated. The aim of the current study is to investigate the effects of a high-fat diet administered shortly after hemorrhagic shock.

Material and Methods: A model of nonlethal hemorrhagic shock was used in male Sprague Dawley rats in which 30-40% of blood volume was withdrawn. Rats were either fasted following hemorrhagic shock or fed with high-fat nutrition, containing high amounts of phospholipids, or an isocalorical low-fat feeding at 80, 180 and 360 minutes after hemorrhage. To investigate the role of CCK, antagonists against CCK-A and CCK-B receptors were administered at 60 and 160 minutes after shock. In this study gut barrier function was assessed at 24 hours

following shock by measuring permeability to horseradish peroxidase (HRP) in ileal segments and bacterial translocation to mesenteric lymph nodes, liver and spleen.

Data: High-fat feeding significantly reduced bacterial translocation (40.4 ± 4.0 CFU/g) at 24 hours after shock in comparison to low-fat (68.6 ± 4.9 CFU/g; $p<0.01$) and fasted groups (133.1 ± 16.8 CFU/g; $p<0.001$). Furthermore, gut permeability to HRP in high-fat animals was significantly lower compared to fasted animals (6.5 ± 0.2 vs. 9.1 ± 0.3 μg/ml; $p<0.001$). Translocation of HRP in the high-fat group was also reduced in comparison to the low-fat group. CCK-A and CCK-B receptor antagonists abrogated the protective effect of high-fat nutrition.

Conclusion: Administration of high-fat enteral nutrition after initiation of shock still preserved gut barrier function in a CCK-dependent manner. These data imply that even after trauma the inflammatory response can be dampened via nutritional stimulation of the autonomic nervous system. This study provides new nutritional therapeutic opportunities in trauma patients that are prone to inflammatory syndromes such as sepsis.

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PREVENTION OF SKIN SCARRING

Mark Ferguson

Scarring in the skin is a major clinical problem often resulting in adverse functional (e.g. restriction of movement or growth), aesthetic or psychological sequelae. Many years ago, we discovered that skin wounds on early mammalian embryos healed perfectly with no scars. We investigated the cellular and molecular mechanisms underlying scar free healing in the embryo compared to scar forming healing in the adult. Skin wounds on mouse embryos before Day 16 of gestation healed perfectly, whilst wounds on later embryos and adults scar. Of the many differences between scar free and scar forming healing, the TGFb family appear to be important controlling factors. Embryonic wounds, which heal with no scarring, have reduced/absent levels of $TGFb_1$ and $TGFb_2$, which by contrast are present at high levels in adult wounds which scar. In adults the high levels of $TGFb_1$ and $TGFb_2$ derive from degranulating platelets and inflammatory cells: embryos do not clot their blood and therefore show little platelet degranulation, whilst the inflammatory cell profile at the embryonic wound site is quantitatively and qualitatively different compared to that in the adult. By contrast, embryonic wounds, which heal with reduced or absent scarring show elevated levels of $TGFb_3$ compared to adult wounds which scar. $TGFb_3$ is a morphogenetic factor involved in the development and growth of the embryonic skin. $TGFb_3$ exerts isoform specific effects when binding at the TGFb receptor e.g. stimulation of filopodia formation on epithelial cells and fibroblasts and the stimulation of enhanced random cell migration. In experimental wounds in rats, mice and pigs, we have shown that reduction of the levels of $TGFb_1$ and $TGFb_2$ (by the intradermal injection of neutralising antibodies) or elevation of the levels of $TGFb_3$ (by intradermal injection of the recombinant protein) result in markedly improved or absent scars in the adult. These findings have now been translated into human clinical trials. To date, more than 1,000 human subjects have been safely exposed to varying doses of intradermal human recombinant $TGFb_3$. In a series of efficacy trials, full thickness wounds were made in anatomically matched sites, under the arms of human volunteers. In a prospective double blind randomised design, wounds were treated by intradermal injection of either human recombinant $TGFb_3$ or placebo (the vehicle in which the $TGFb_3$ was dissolved). All wounds received optimal care e.g. moist wound healing. Scars were followed-up monthly, typically for 7 - 12 months, assessed clinically and photographed under standardised conditions. In dose response clinical trials, it was demonstrated that injection of human recombinant $TGFb_3$ into the wound margins significantly reduces subsequent scarring in the dose range 50ng/100μl/Lcm of wound margin to 200ng/100μl/Lcm of wound margin. Importantly, frequency of administration clinical trials demonstrated that 200ng/100μl/Lcm of wound margin given once at the time of surgery, produced a clinically and statistically significant reduction in subsequent scarring. These trials are now being extended to a variety of clinical operations e.g. surgical revision of disfiguring scarring, breast augmentation/reduction, varicose vein removal surgery.

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VASCULAR ENDOTHELIAL GROWTH FACTOR: ITS ROLE IN TISSUE REPAIR, INFLAMMATION AND SEPSIS

Sabine Eming

Our recent data indicate that Vascular Endothelial Growth Factor-A (VEGF-A) is a key mediator in tissue repair. VEGF-A controls angiogenesis, vascular permeability and inflammation, all of which are central mechanisms of wound healing. Whereas VEGF-A levels are regulated through transcriptional control and mRNA stability, VEGF-A protein activity can be regulated by proteolytic mechanisms and interaction with other extracellular molecules. We hypothesize that VEGF-A protein activity is impaired in chronic non-healing wounds and is in part responsible for the reduced and disturbed angiogenic response in non-healing wounds. We analyzed VEGF-A expression, protein stability and the presence of the most potent endogenous VEGF-A inhibitor, the soluble form of the VEGF receptor VEGFR-1 (sVEGFR-1), in non-healing versus healing human wounds. VEGF-A mRNA expression was highly upregulated in non-healing wounds. Interestingly, the stability of VEGF165 protein was significantly reduced in wound fluid obtained from non-healing wounds. Protease-inhibitor studies, protein sequencing and MALDI-TOF mass

spectrometry of VEGF cleavage products, as well as a plamin resistant VEGF165 mutant indicated that plasmin is one of the serine proteinases critically involved in this degradation process. In addition, sVEGFR-1 mRNA expression and protein levels were highly upregulated in non-healing versus healing wounds. Only in those chronic wounds, which entered a phase of granulation tissue formation and finally wound closure, wound healing progression correlated significantly with a decline in sVEGFR-1 levels. Our studies provide novel mechanisms how VEGF-A protein activity might be inhibited in non-healing versus healing wounds and provide the basis for novel VEGF based therapies.

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STEM CELLS FOR BIOENGINEERING OF NERVE REGENERATION

Giorgio Terenghi

The current repair method to bridge nerve defects is to use autologous nerve grafts, which provide the regenerating axons with natural guidance channels, Schwann cells and extracellular matrix molecules. Because the harvesting of a nerve graft results in co-morbidity and the functional recovery following surgical nerve repair is poor, an alternative method of nerve reconstruction is needed. Biocompatible nerve conduits have been acknowledged as an alternative solution, as their surface micro-geometry may be combined with cultured cells, growth factors and extracellular matrix molecules to form a tissue engineered nerve graft.

Numerous biomaterials have been tested experimentally with different degrees of success. In our experience, bioresorbable mats formed by poly-3-hydroxybutyrate (PHB) fibres have proved to be the most suitable for nerve regeneration. The addition of cultured Schwann cells within these conduits has proved to have a crucial role, and by using a stable genetic labelling method for the identification of the transplanted cells we have been able to demonstrate their active participation in the regenerative process. More recently, interest has been directed towards the use of adult bone marrow mesenchymal stem cells because of their potential to differentiate towards glial cells type. The differentiated stem cells express phenotypic and molecular biological markers as Schwann cells, and in co-culture with neurons they also show similar functional characteristics. Furthermore, their transplantation in bioengineered nerve conduits has proved beneficial in promoting enhanced nerve regeneration.

There is a clear need for a tissue engineered approach which would enhance functional recovery after peripheral nerve surgery, and development of new biomaterials and cultured cells to create an artificial nerve is crucial in this achievement.

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3D CULTURE SYSTEMS TO STUDY OSTEOCHONDRAL TISSUE ENGINEERING POSSIBILITIES

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Current biomaterial design focusses on simulating the extracellular matrix (ECM) using a biodegradable matrix or scaffold which contains the essential bioactive signal molecules to elicit a physiological regenerative response. The life sciences have a major task in providing relevant model systems to test and hopefully eventually predict these biological responses in newly developed biomaterial strategies. In this presentation examples will be given of how 3D models using human cells can be employed to develop sophisticated systems to study cell-biomaterial interactions for osteochondral tissue engineering (TE). In bone regeneration vascularization plays a central role. We model this using human microvascular endothelial cells (EC) in the "healing matrix" of collagen type I and fibrin [1], as well as in interaction with 3D scaffolds developed for TE [2,3]. Examples will be given of studies with the silk protein, fibroin, and a blend of starch with poly(-epsilon-caprolactone). These are being investigated in the form of micro- and nanofibre meshes, in some cases with a combined micro- and nanofibre architecture. A variety of functional parameters at both protein and nucleic acid level are being employed, usually with the techniques of CLSM, ELISA and RT-PCR to study EC functionality on these biomaterials. In addition, examples will be given to illustrate how in vitro models can be adopted to understand the differentiation of human adult stem cells, in particular, the endothelial progenitor cell (EPC) as an essential precursor for vascularization. We have just recently been able to demonstrate that human peripheral blood EPCs in the form of so-called outgrowth EC (OEC) can maintain a stable endothelial phenotype over numerous passages [4] and colonize biomaterial scaffolds [5]. Furthermore, we are using in vitro methods to study how vascular cells, including OEC, interact in cocultures with other cell types, such as osteoblasts, in the context of vascularization in bone TE.

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Thanks are expressed to the European Commission for support of this research via the projects HIPPOCRATES and EXPERTISSUES

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BIOREACTORS FOR SKELETAL TISSUE ENGINEERING

Ivan Martin

In this presentation, the functions of bioreactors in the specific context of 3D scaffold-based skeletal tissue engineering will be reviewed. In particular, examples will be given to illustrate the following take-home messages:

1. Similar to bioreactors in classical fermentation processes, the key functions of bioreactors in tissue engineering are to provide control and standardization of physiochemical culture parameters during cell/tissue culture.

2. Bioreactors can improve the quality (i.e., cell distribution and cell utilization) and reproducibility of the process of seeding cells into three-dimensional porous scaffolds.

3. Mass transport of nutrients and waste products to and from cells within engineered constructs can be enhanced by convective bioreactor systems. Bioreactors which perfuse media directly through the scaffold have the greatest potential to eliminate mass transport limitations and maintain cell viability within large 3D constructs.

4. Mechanical conditioning within controlled bioreactor systems has the potential to improve the structural and functional properties of engineered tissues. However, optimizing the operating parameters (i.e., which specific mechanical force(s) and regimes of application) for a particular tissue will require significant quantitative analysis and computational modeling.

5. By recapitulating aspects of the actual cellular microenvironment that exists in vivo, bioreactors can provide in vitro model systems to investigate cell function and tissue development in 3D environments.

6. Bioreactor systems for 3D cell culture can be used to bypass the critical phase of monolayer cell expansion, thus obtaining cell populations with a higher regenerative capacity and at the same time streamlining the generation of a graft.

7. Innovative and low-cost bioreactor systems, which automate, standardize, and scale the production of a tissue engineered product will be central to future manufacturing strategies and will play a key role in the successful exploitation of an engineered product for widespread clinical use

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A 412

USE OF SURROGATE MEASURES AS OUTCOME VARIABLES

Beat Muller

As surrogate for an improved antibiotic stewardship and assessment of outcome in respiratory tract infections the use of "hormokines" have been proposed. The term "hormokine" encompasses the cytokine like behaviour of hormones during inflammation and infections. The concept is based on the finding of an ubiquitous expression of calcitonin peptides during sepsis. All these peptides are increased to variable extents during inflammation and infection. Most prominently, circulating procalcitonin (PCT) levels increase several-thousand fold during sepsis. Using a sensitive assay, a PCT-based therapeutic strategy can safely and markedly reduce antibiotic usage in those respiratory tract infections that are mostly viral, and in viral meningitis. Adrenomedullin, another member of the calcitonin peptide superfamily, was shown to complement and improve the current prognostic assessment in lower respiratory tract infections. Other peptides share features of hormokines, e.g., natriuretic peptide and copeptin. Hormokines are not only biomarkers of infection. Hormokines are also pivotal inflammatory mediators. Like all mediators, their role during systemic infections is basically beneficial, possibly to combat invading microbes. Yet, with increasing levels they can become harmful for their host. Multiple mechanisms of action were proposed. In several animal models the modulation and neutralization of hormokines during infection was shown to improve survival and thus might open new treatment options for severe infections, especially of the respiratory tract.

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A 413

INTRACELLULAR SENSORS OF VIRAL NUCLEIC ACIDS

Simon Rothenfusser

The cytoplasmic proteins retinoic acid-inducible gene I (RIG-I) and melanoma differentiation associated gene 5 (MDA-5) sense intracellular viral infection and trigger a signal for innate antiviral responses including the production of type I IFN. Both proteins contain a homology domain present in members of the DExD/H-box helicase family and exhibit N-terminal caspase recruitment domains (CARD). Both proteins sense viral RNA with their helicase domain and trigger downstream signals by interaction of their CARDs with the CARD domain containing adapter MAVS (mitochondrial antiviral signaling) localized in the outer membrane of mitochondria. RIG-I and MDA-5 were found to be essential and non redundant for the defense against a variety of viruses. Infections with paramyxoviruses like Sendai Virus and RSV as well as the Hepatitis C virus trigger RIG-I while picorna viruses like the encephalomyocarditis virus

(EMCV) trigger MDA-5. A third member of the RNA helicase family Lgp2 shares high homology with the helicase domain of RIG-I. In contrast to RIG-I or MDA-5 Lgp2 however lacks CARDs or any other known signaling domain. Overexpression of Lgp2 inhibits the triggering of IRF- and NF-κB-dependent pathways by SV and Newcastle disease virus. Like RIG-I and MDA-5 Lgp2 binds poly IC a synthetic mimic of double stranded RNA. Quantitative PCR analysis demonstrates that Lgp2 is present in unstimulated cells at a lower level than RIG-I, although both helicases are induced to similar levels after virus infection. Lgp2 therefore is supposed to act as an endogenous negative feedback regulator of antiviral signaling by sequestering viral RNA ligands away from RIG-I and MDA-5.

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A 414

WHEN IS RESISTANCE TOO MUCH FOR EMPIRIC THERAPY: IMPACT OF ANTIMICROBIAL RESISTANCE IN CLINICAL TRIALS

Carl Erik Nord

Antimicrobial resistant microorganisms have increased during the last 10 years and is now a threat to disease management. Meticillin-resistant staphylococci, penicillin-resistant pneumococci, vancomycin-resistant enterococci and multidrug-resistant Gram-negative rods are now isolated in increased frequencies. The lack of new antibiotics, especially against Gram-negative bacteria such as Pseudomonas, Acinetobacter, Enterobacteriaceae and Bacteroides, will be problematic. The clinical and economical impact of antimicrobial resistance is significant. The major costs in developing new agents arise during the clinical trial programme. Phase I studies in a small number of healthy subjects to document disposition of the agent and lack of toxic reactions are expensive. Phase II and III clinical trials for documentation of clinical efficacy and safety generate the main costs for developing new drugs. The usage of pharmacokinetic and pharmacodynamic documentation has increased and may reduce in the future the demands for standard clinical trials. The pharmacodynamics of antimicrobial agents describe the interaction between an agent and its target pathogen. For regulatory purposes there is enough information about the correlation between pharmacokinetics and pharmacodynamics for many antimicrobial agents. For cephalosporins and carbapenems the efficacy depends on the time-concentrations at the infection site exceeding the minimum inhibitory concentration of the antibiotic tested. These betalactam antibiotics should therefore be administered at short dose intervals but high doses are not necessary. For aminoglycosides and fluoroquinolones the area under the serum concentration curve is important for efficacy and therefore these drugs should be administered in high doses at long intervals. The classical phase III clinical trial of a new antimicrobial agent is a randomised controlled comparison with an active control agent. Most clinical trials are non-inferiority investigations, thus demonstrating that a new agent is not inferior to standard treatment. Apart from the use of pharmacodynamic and pharmacokinetic documentation in small trials, the possibility to extrapolate from results generated in one type of infection to another type of infection should be tested, for example treatment of lower intraabdominal infections and pelvic infections. The bacterial etiology is similar in both infections, i.e. mixed aerobic and anaerobic microflora in the gut or vagina.

Microbiologically inadequate antimicrobial treatment is an important determinant of outcome in patients with severe intraabdominal infections. Microorganisms that had been considered to have low virulence such as enterococci, coagulase-negative staphylococci, Acinetobacter baumannii and Candida species are now causing more severe infections. These microorganisms are often resistant to many antimicrobial agents and special attention should be drawn to these pathogens in compromised patients. Clinical failures are now reported in an increasing frequency. The role of enterococci as primary pathogens in intraabdominal infections is still controversial but the presence of enterococci increases the postoperative complications. The use of intravascular catheters contributes to the increased incidence of coagulase-negative staphylococci. A. baumannii infections are now endemic in many hospitals and are problematic since the strains are resistant to most antibiotics. The increased incidence of Candida glabrata and Candida krusei which express decreased susceptibility to several antifungal agents is alarming and should be monitored. The effect of antimicrobial resistance caused by new agents in phase II and III clinical trials should therefore be studied and analyzed.

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A 415

IMPROVING THE PROCESS OF CLINICAL TRIAL DEVELOPMENT OF ANTIBIOTICS: A PHARMACEUTICAL PHYSICIAN'S PERSPECTIVE

Gary Noel

Designing clinical trials that include the goal of supporting registration of an antibiotic for regulatory approval require addressing the voices of those defining medical need, regulatory guidance and potential commercial value. This process is often complicated by the need for critical components of trials to be revisited as new information becomes available. Complications of revisiting development programs are accepted as part of the risk of drug development. Antibiotic drug development has, until recently, enjoyed a relatively stable period where regulatory guidances and acceptance of well-worn paths of development have minimized risk of failure in phase 3 programs, in regulatory approval and in physician acceptance of the usefulness of new agents. In an era where there is uncertainty around the acceptance of

programs that have followed previous successful approaches, the importance of collaboration and transparency of parties involved in this process will be critical in keeping the antibiotic pipeline healthy. Physicians are the obliged to take the lead in ensuring that clinical research aimed at establishing the risk and benefits of new agents be based on scientifically sound principles and be conducted with the expectation of making meaningful advances in patient care and in improving human health. Success in improving the process of clinical trial development of antibiotics depends on physicians meeting this obligation.

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A 416

TARGETS FOR THERAPEUTIC INTERVENTION IN DEATH RECEPTOR SIGNALLING

Thomas Kaufmann, Marc Pellegrini, Erika Cretney, Razq Hakem, Vishva Dixit, Andreas Strasser

Hepatocyte apoptosis, a feature of many chronic and acute liver diseases, is often a consequence of over-activation of the immune system caused by the action of membrane-bound or secreted TNFa. Our analysis of gene-targeted mice showed that both forms of TNFa required caspase-8 for hepatocyte killing. Although Bid, a pro-apoptotic BH3-only protein activated by caspase-8, is essential for Fas ligand-induced hepatitis, its loss had no impact on membrane-bound TNF-mediated hepatocyte killing triggered by concanavalin A (ConA)-induced T cell activation. However, combined loss of Bid and another BH3-only protein, Bim, inhibited lipopolysaccaride (LPS) plus galactosamine-induced hepatitis and, unexpectedly, sole loss of Bim diminished ConA-induced hepatocyte destruction. Thus, secreted and membrane-bound TNFa kill hepatocytes via distinct mechanisms, both requiring caspase-8, but in addition also needing either Bim and Bid, or Bim alone. These observations identify caspase-8 and the BH3-only proteins Bid and Bim as potential therapeutic targets for treatment of liver diseases.

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MANIPULATING APOPTOSIS FOR THERAPEUTIC GAIN: NOVEL THERAPEUTIC APPROACH III, SIRNA

Alfred Ayala, Doreen Wesche-Soldato, Mario Perl, Joanne Lomas-Neira, Chun-Shiang Chung

Over the last 15 years our laboratory along with others have been attempting to assess the contribution of aberrant immune cell apoptosis to the pathology of sepsis induced multiple organ failure. In this respect, while it is clear that divergent mediators such as, LPS, NO, steroids, TNF, etc., contribute to the apoptotic changes seen in the critically ill septic animal/ patient, we have found that the inhibition of FasL-Fas signaling (a component of the extrinsic apoptotic pathway) is a particularly salutary target for not only protecting immune/non-immune cell populations from developing apoptosis but in reducing the morbidity and mortality encountered in the septic animal. In light of this, we hypothesized that up-stream as well as down-stream components of this extrinsic apoptotic death pathway should present themselves as logical targets for the initial application of in vivo inhibition using interference/silencing (si) RNA in an attempt to suppress FasL-Fas induced septic morbidity/mortality. Here, we overview our experiences with the application of naked siRNA constructs (against Fas, caspase-8, and various chemokines) using two modes of delivery (intravenous hydrodynamic & intra-tracheal pulmonary), in two models of critical illness, i.e., sepsis alone and shock/ sepsis-induced acute lung injury (Wesche-Soldato DE, et al [2005] Blood, 106:2295; Perl M, et al [2005] Amer. J. Pathol. 167:1545; Lomas-Neira JL, et al [2005] J. Leukocyte Biol. 77:846). In brief, what we show is not only that naked siRNA constructs can be utilized in vivo to attenuate the development of both systemic as well as local/organ specific indices of inflammation, tissue injury and apoptosis, but also, at least in a model of polymicrobial sepsis, RNA interference can improve survival. Intriguingly, as we have attempted to delineate the nature of the cellular targets of these forms of siRNA delivery we are learning novel information about the pathological processes that appear to underpin the shock and/or septic pathology induced through extrinsic FasL-Fas receptor pathway. (Supported by NIH GM-53209).

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A 418

THE DEVELOPMENT OF A PORCINE MODEL FOR BURNS AND RECONSTRUCTION

Ludwik Branski, Marc Jeschke, Rainer Mittermayr, Heinz Redl, Daniel Traber, David Herndon

The porcine wound model is extremely useful in the evaluation of therapeutic agents that are destined for use in human wounds. Long-term studies of burn, reconstruction and wound healing in the pig create multiple technical and handling challenges. The aim was to develop a reliable porcine wound healing model which mimics the situation after burn, subsequent fascial excision and autograft coverage.

Methods: Yorkshire Pigs (40-45kg) were used in our studies. Jugular venous lines were inserted for monitoring of hematologic parameters. After initial trials with various burn depths and excision time-points, contact burns with hot aluminum and fascial excision 24h post burn were chosen as standard burn and excision procedures, most closely mimicking the clinical situation.

Twelve to 16 burn wounds of app. 50cm^2 could be accommodated on each animal; after excision, wounds were grafted with 1:4 autograft skin from the hind of the animal. The optimal graft fixation method was part of one study (fibrin sealant versus standard fixation with staples). Wound coverage was also subject to studies; novel nanocellulose dressings were compared to standard wound dressing material (oil emulsion non-adhesive gauze). A three-layer outer wound dressing (Op-Site™, Vet-Wrap and Nylon animal stocking) was developed to shield the wounds from contamination and infection. Dressing changes were performed every 2 to 4 days; these procedures were done under light anesthesia and full analgesia to minimize stress. Wound healing progress was assessed using a standardized measurement scale for graft adherence, dislocation, reepitheliazation, hypergranulation, hematoma, and infection. Digital photography was used to trace and quantify the wound healing progress. Wound biopsies were taken for immunohistochemistry, histology and molecular biology. Animals received oral antibiotics and transcutaneous pain medication throughout the course of the experiments.

Results: The outer wound dressing successfully detained infection and contamination from the test fields. After an initial peak, no elevated White Blood Cells were observed. Handling of dressing changes was satisfactory and the animals tolerated the necessary manipulations well. The use of fibrin sealant vs. normal staples significantly improved graft adherence and graft dislocation, and accelerated wound healing. In wound fields treated with nanocellulose as primary dressing material, infections and hematoma occurred significantly less than in the controls, and graft adherence was significantly improved.

Summary: A reliable and reproducible model for wound healing after burn and autografting was developed.

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A 419

INHIBITORS OF GLYCOGEN-SYNTHASE KINASE 3BETA

Christoph Thiemermann

Glycogen synthase kinase (GSK)-3 was initially categorized as one of several protein kinases that phosphorylate glycogen synthase and, hence, regulate glucose metabolism. We know today that GSK-3 is an ubiquitous serine/threonine protein kinase that participates in a multitude of cellular processes, ranging from cell membrane-to-nucleus signalling, gene transcription, translation, and cytoskeletal organization to cell cycle progression and survival. Thus, GSK-3 may play a much greater role than previously anticipated in the pathophysiology of a number of diseases. Two closely related isoforms have been found in mammalians, GSK-3alpha and GSK-3beta. Unlike most kinases, GSK-3 is constitutively active in cells, and a wide range of extracellular stimuli, including insulin, epidermal growth factor, and fibroblast growth factor, exert their effects by inhibiting GSK-3 activity. Phosphorylation of Ser9 on GSK-3beta and Ser21 on GSK-3alpha inhibits GSK-3 activity and, hence, reduces its activity to alter cell function. GSK-3beta has been identified as a key regulatory switch in the modulation of the inflammatory response and the dysregulation of GSK-3beta has been implicated in the pathogenesis of several diseases, including shock.

We have shown that GSK-3beta inhibitors reduce the organ injury/dysfunction associated with systemic inflammation caused by severe endotoxemia or co-administration of lipopolysaccharide and peptidoglycan in the rat. The protective effects of GSK-3beta inhibitors were associated with the prevention of the activation of nuclear factor (NF)-kB and reduction in the expression of NF-kB-dependent pro-inflammatory genes. GSK-3beta inhibition has also been shown to exert anti-inflammatory effects and ameliorate the tissue damage associated with collagen induced-arthritis.

It is well known that insulin reduces morbidity and mortality among critically ill patients, but the molecular mechanisms of its effect remain unknown. Insulin is a well-known inhibitor of glycogen synthase kinase-3, which may play an important role in systemic inflammation and shock. We have shown that therapy with insulin or TDZD-8 (a specific inhibitor of GSK-3beta) reduces the organ injury/dysfunction caused by lipopolysaccharide and peptidoglycan in the rat. We propose that the inhibitory effect of insulin on the activity of GSK-3beta contributes to the protective effect of insulin against the organ injury/dysfunction caused by excessive systemic inflammation, independently of any effects on blood glucose. We have also demonstrated that the inhibition of GSK-3beta may represent a novel therapeutic approach in the therapy of hemorrhagic shock. GSK-3beta inhibition may be a new strategy for the prevention or therapy of the organ injury/dysfunction associated with shock and other conditions associated with local or systemic inflammation.

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17ß-ESTRADIOL FOR THE TREATMENT OF TRAUMA-HEMORRHAGE-INDUCED DEPRESSION OF CARDIOVASCULAR AND IMMUNE RESPONSES

Irshad Chaudry, Martin Angele, Michael Frink, Frank Hildebrand, Martin Schwacha, Mashkoor Choudhry

Studies have shown that gender dimorphism exist in response to trauma-hemorrhage. More specifically, proestrus female rodents have maintained cardiovascular and immunological functions following trauma-hemorrhage as opposed to markedly decreased functions in males under those conditions. This advantage that the young females have, however, disappears post menopausal, with

aging as well as following ovariectomy. Nonetheless, administration of a single dose of 17β-estradiol (estradiol) following trauma-hemorrhage prevents the depression in cardiovascular/immune responses under those conditions in ovariectomized females as well as males. Studies have also shown that the salutary effects of estradiol on cardiac functions are mediated via estrogen receptors (ER). Furthermore, the ER distribution was found to be organ specific in that heart and lung primarily have ER-β whereas ER-α is predominant in the liver. It also appears that ER-α and ER-β-induced upregulation of heat shock proteins (Hsp) plays a significant role in the estradiol-mediated cardioprotection under those conditions. In addition to estradiol, administration of ER-β agonist (DPN) appears to be a novel adjunct for restoring cardiac function and for normalizing pulmonary function following trauma-hemorrhage. Alternatively, ER-α agonist (PPT) improved hepatic and Kupffer cell functions after trauma-hemorrhage. The results also indicate that both genomic and non-genomic pathways are involved in producing the salutary effects of estradiol on cardiac functions following trauma-hemorrhage. Administration of estradiol following trauma-hemorrhage also decreases the mortality rates from subsequent sepsis. Thus, the adjunct use of estradiol which is readily available clinically and which does not produce adverse hemodynamic effects; appear to be safe and novel immunomodulating agents for the treatment of immune/cardiovascular depression following severe blood loss and for decreasing mortality from subsequent sepsis in females as well as males (supported by USPHS grants RO1 GM37127 and R37 GM39519).

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A 421

HYDROGEN SULFIDE IN DISEASE

Ahila Sivarajah, Massimo Collino, Mohammed Yasin, Salvatore Cuzzocrea, Christoph Thiemermann

Gaseous transmitters are endogenous gases of small molecular weight, which exert important physiological functions. Hydrogen sulfide (H_2S) is formed enzymically in the metabolic pathway that regulates tissue turnover of the sulphur-containing amino acids. Cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE) are the key enzymes responsible for the endogenous synthesis of H_2S in mammalian cells and their expression is tissue-specific. Although other enzymes can catalyse the production of H_2S, CBS appears to be the main H_2S-forming enzyme in the central nervous system whereas CSE is the principle H_2S-forming enzyme in the vasculature and heart. There is evidence that alterations in the synthesis of H_2S play a role in the physiology and pathophysiology of the cardiovascular system, including vasodilatation, which is attenuated by a specific covalent inhibitor DL-propargylglycine. Emerging evidence suggests that H_2S also plays an important anti-inflammatory, including in animal models of endotoxemia, colitis, colorectal distension and carrageenan-induced inflammation. Conversely, in a rat model of hypoxic pulmonary vasoconstriction parenteral administration of H_2S attenuated the rise in pulmonary arterial pressure.

Interestingly, in models of ischaemia and reperfusion, H_2S has been reported to exert beneficial effects. Studies in the heart have also shown, that NaHS produces negative inotropic effects, both in vitro and in vivo, and that this effect was partially inhibited by the K_{ATP} channel blocker glibenclamide, indicating that H_2S maybe be endogenously produced by heart tissues, as a regulator of physiological cardiac function. Furthermore, we have reported that (i) endogenous H_2S is produced during regional myocardial ischaemia and reperfusion in amounts that can limit myocardial injury and (ii) the synthesis of H_2S, by CSE may contribute to the delayed protection caused by endotoxin. Also, in this model of myocardial ischaemia and reperfusion, we have shown that NaHS i) attenuates markers of apoptosis, including alterations in both caspase-9 activity and Bcl-2 protein expression, ii) increases mitogen activated protein kinases phosphorylation/activation and iii) decreases activation of NF-kB and markers of inflammation.

In conclusion, H_2S, exhibits both beneficial and detrimental effects and, therefore, H_2S and inhibitors of its synthesis provide novel therapeutic opportunities in a variety of pathophysiological conditions.

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A 422

TARGETING ENDOGENOUS DANGER SIGNALS AND THE CHOLINERGIC ANTI-INFLAMMATORY PATHWAY IN ACUTE AND CHRONIC DISEASE

Gregory LaRosa, Lyn Libertine, Roger Clark, Diana Lamppu, Christopher Tunkey, Patricia Rao

There are endogenous pro-inflammatory and anti-inflammatory pathways that ideally work in balance to protect the body from both dangerous exogenous assaults as well as from unchecked pathologic inflammation. Two such opposing pathways are the system of danger signals, or Alarmins, and the "cholinergic anti-inflammatory pathway". The former can signal a need for a vigorous protective immune response, while the latter can dampen innate and adaptive immune responses, providing protection form a "runaway" inflammatory response. When these systems become unbalanced as a result of many mediators produced during disease, destructive inflammatory cascades can occur, leading to pathologic tissue damage.

High Mobility Group Protein 1 (HMGB1) normally functions as a nuclear protein, but when released to the extracellular space by necrotic cells or through secretion by certain activated cells, it acts as an Alarmin, and assists in stimulating a vigorous inflammatory response. We have found that HMGB1 is a heterogeneous factor when

present in biological fluids from disease tissue, and that it acts in concert with ligands of the Toll-like Receptor system to greatly enhance the inflammatory response. Despite this heterogeneity, it has been possible to identify monoclonal antibodies with therapeutic potential for sepsis and autoimmune disease.

As an opposing pathway, the central nervous system has the capacity to monitor and regulate immunological responses. The cholinergic anti-inflammatory pathway can act to reduce an inflammatory response via the vagal nerve, through a mechanism mediated at the cellular level by the alpha 7 nicotinic acetylcholine receptor (α7nAChR). Using selective α7nAChR agonist compounds, we have shown that pharmacologic modulation of this pathway is possible, and can provide significant protection in murine models of acute, severe inflammation, as well as in allergic lung disease. Several different chemotypes of α7nAChR agonists were tested in the ovalbumin (Ova) sensitization and challenge mouse model of allergic lung inflammation. After the sensitization period, oral administration of 1 to 10 mg/kg α7nAChR agonists just prior to each of the 3 Ova challenges, resulted in significant inhibition of both the eosinophilic and neutrophilic infiltrates seen 8 hr after the third Ova challenge, in both the airway lumen and lung interstitium. Additionally, α7nAChR agonist treatment significantly attenuated the bronchial hyperreactivity to methacholine challenge, resulting from the airway inflammation.

Through the exploration of the functions of both of these opposing systems, we hope to discover novel modulators, that either block the HMGB1 pro-inflammatory pathway, or exploit the cholinergic anti-inflammatory pathway, for the treatment of inflammatory disease. An overview of our recent work in both of these areas will be presented.

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ANTIBODY AGAINST THE RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS (RAGE) AS A TREATMENT STRATEGY FOR SEPSIS

Steven Opal, Emily Lutterloh, Debra Pittman, Xiang-Yang Tan, James Keith

(RAGE) is a member of the immunoglobulin superfamily that is widely expressed on the membrane surface of multiple cell types. While its pathophysiologic role in sepsis remains incompletely defined, RAGE contributes to the acute response. Using a standard model of polymicrobial sepsis, cecal ligation and puncture (CLP), we evaluated survival of RAGE knock-out/129 mice, heterozygotes (C57BL/6-SvEv129, wild-type/129 mice and in BALB/c mice given an anti-RAGE antibody. The survival rate for RAGE knock-out animals (n=15) was 80%; ($p<0.01$); the survival rate in heterozygotes (n=23) was 69%; and survival was 37% in wild-type mice (n=27) ($p<0.01$). Survival benefits were evident in wild-type mice (n=15) with an IgG2b anti-RAGE monoclonal antibody (15 mg/kg) compared with serum-treated control mice (n=15), $p<0.05$. Significant survival advantage was found with delayed anti-RAGE antibody treatment given as long as 24 hours after CLP. There was a non-significant increase in organ tissue colony counts of aerobic bacteria in animals given higher doses of anti-RAGE antibody.

Anti-RAGE antibody salvages animals and improves survival even in overtly ill mice with polymicrobial sepsis with delayed antibody treatment. Further studies are needed to determine the potential clinical utility of anti-RAGE antibody as a novel treatment strategy for human sepsis.

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ESTROGEN RECEPTOR BETA (ERB) AGONISM INCREASES SURVIVAL IN EXPERIMENTALLY INDUCED SEPSIS

James Keith, Jr., Steven Opal, Patricia Cristofaro, Emily Lutterloh, Edward LaVallie

Estrogen receptor beta (ERb) is expressed in multiple tissues including lung and gastrointestinal epithelial membranes at similar levels in both males and females. Upon binding to its ligand, ERb mediates a number of cytosolic and transcriptional effects that may protect the host in pro-inflammatory conditions.

An ERb-selective agonist (WAY-202196) was studied in the murine cecal ligation and puncture (CLP) model of polymicrobial sepsis. WAY-202196 was given at a range of doses either orally (po) or intravenously (iv) at time 0, 24 and 48 hours following CLP in male and female BALB/c mice. Survival, inflammatory markers, histopathology and microbiologic parameters were assessed.

WAY-202196 (1-50 mg/kg po and 1 & 3mg/kg iv) provided a significant survival advantage ($p<0.01$) following CLP. There was no difference in effectiveness between males and females (studied at 50mg/kg po or 1 & 3mg/kg iv). The ERb agonist lowered levels of bacteremia and decreased injury in the gut. Lower levels of peritoneal cytokines were also seen in WAY-202196-treated animals.

The administration of this ERb-selective agonist provided a significant survival advantage during experimental polymicrobial, intra-abdominal sepsis in mice. This new treatment modality may be useful prophylactically in patients at risk for severe sepsis and septic shock.

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PRECLINICAL TRIALS OF BLOCKADE OF C5A AND C5AR IN SEPSIS

Peter Ward

Objective: To determine therapeutic effects of blockade of C5a or C5aR in rodents with experimental sepsis (cecal ligation and puncture, CLP).

Materials and Methods: Rats and mice were subjected to CLP, a commonly used model of experimental polymicrobial sepsis. Endpoints included survival and attenuation of defects in innate immune functions (chemotaxis, phagocytosis, H_2O_2 production) of blood PMNs. C5a or C5aR blockade was induced by the use of neutralizing antibodies to C5a or C5aR. The C5aR antagonist (C5aRa) was also used. Details are included in an extensive series of publications.

Data: Using the CLP model of experimental sepsis in rodents, extensive activation of complement occurred, as defined by anaphylatoxins (C3a, C5a) present in plasma. This led to interaction of C5a with the C5a receptor (C5aR) on blood PMNs as well as on a variety of different cell types. Such interactions caused paralysis of MAPK signaling pathways in blood PMNs. In vivo interception of C5a or C5aR with blocking antibody or use of C5aRa had a variety of protective effects: 1.) Survival of rodents was dramatically improved. 2.) Paralysis of MAPK signaling pathways in PMNs was reversed, preserving innate immune functions such as phagocytosis, chemotaxis and the respiratory burst (H_2O_2 production). 3.) The numbers of colony forming units of bacteria in blood, liver, lungs and spleen after CLP were greatly reduced. 4.) The "cardiomyopathy of sepsis" was greatly attenuated, resulting in preservation of left ventricular pressures as well as preservation of in vitro contractility of cardiomyocytes. 5.) Reversal of the consumptive coagulopathy of sepsis. These data suggest that excessive generation of C5a and its interaction with C5aR during experimental sepsis results in a variety of detrimental outcomes, all of which were remarkedly attenuated when C5a or C5aR was blocked by the presence of a neutralizing antibody or use of C5aRa. In humans with septic shock, C5a was present in plasma, and blood PMNs showed reduced surface expression of C5aR and defective innate immune functions.

Conclusions: Sepsis in the CLP model was associated with unregulated activation of the complement system, in which excessive amounts of C5a were very harmful, causing loss of innate immune functions of PMNs. There was also extensive upregulation of C5aR in a variety of organs. In vivo interception of either C5a or C5aR provided dramatically protective outcomes with greatly improved survival, preservation of MAPK signaling pathways and intact innate immune functions in blood PMNs, reversal of the consumptive coagulopathy of sepsis, and preservation of left ventricular functions. In humans with septic shock, many of the changes in blood PMNs were similar to those changes found in the CLP model. These data suggest that interception of either C5a or C5aR in septic humans may represent a promising therapeutic strategy in the setting of sepsis.

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PRE-CLINICAL AND CLINICAL TRIALS: TLR INHIBITORS

Jean-Louis Vincent

Toll-like receptors (TLRs) are a type of pattern recognition receptor and recognize pathogen-associated molecular patters (PAMPs). They are present on a large number of immune cells as well as epithelial cells and play an essential role in the immune response to microbial pathogens. Following pathogen recognition, TLRs initiate intracellular signal transduction, resulting in the expression and activation of genes involved in inflammation. Individual TLRs activate distinct transcription factors, such as NF-κB, to drive specific pro-inflammatory responses. The development of TLR inhibitors may serve to counteract the harmful pro-inflammatory response that complicates infection. Several issues need to be considered when contemplating TLR antagonism as a potential therapeutic agent in patients with sepsis. First, not all mammals have the same TLRs and translating data from animal models to the human situation may be difficult. Second, bacteria often activated several TLRs and several TLR antagonists may, therefore, need to be administered at the same time. Third, activation of TLRs is one of the initiating events of sepsis and timing of TLR antagonism may be crucial; many patients may present too late for TLR antagonists to be effective. TLR antagonists have shown some promising effects in animal models of sepsis and preclinical studies. TLR4 is recognized as a key factor in the recognition and signaling of lipopolysaccharide with resultant cytokine production. TAK-242, a cyclohexine derivative, is a selective inhibitor of TLR4 intracellular signaling and, in vitro, suppresses the production of various cytokines, including tumor necrosis factor (TNF)-a and interleukin (IL)-6, as well as nitric oxide (NO). A phase III randomized clinical trial of TAK-242 in patients with severe sepsis is currently ongoing.

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EFFECTS OF POLYCLONAL ANTI-TNF IN SEVERE SEPSIS

Gordon Bernard

Objective: To evaluate whether administration of CytoTAb®, an affinity purified, anti-tumor necrosis factor (TNF) alpha, ovine Fab fragment, is safe and effective in decreasing plasma levels of TNFα, IL-6 and IL-8 and

increasing the number of shock-free and ventilator-free days in patients with severe sepsis

Design: Prospective, randomized, double-blind, placebo-controlled, multicenter, phase IIb clinical trial.

Setting: Nineteen intensive care units in the United States and Canada.

Patients: Eighty-one patients aged 12 or older were enrolled if they had documented or presumed site of infection, presence of at least three abnormal vital sign criteria and newly developed shock or two other organ failures.

Interventions: Patients were randomly assigned 1:1 to receive either CytoTAb®, infused intravenously over 30 minutes in a loading dose of 250 units / kg followed by nine doses of 50 units/kg every 12 hours or 5 mg / kg human albumin as placebo.

Measurements and Main Results: CytoTAb® promptly reduced plasma TNFα concentrations during the 5-day dosing period compared to placebo ($P = 0.001$), and also decreased plasma IL-6 concentrations ($P = 0.002$). CytoTAb® also significantly decreased the concentration of TNFα in bronchoalveolar lavage fluid ($P < 0.001$). Several clinical outcome variables were examined in this trial and results will be presented in detail

Conclusions: CytoTAb® is safe and well tolerated in patients with severe sepsis, effectively reducing serum and BAL TNFα and serum IL-6 concentrations, and improving clinical outcome.

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INVESTIGATOR VERSUS INDUSTRY-INITIATED RESEARCH IN SEPSIS

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The ultimate aim of clinical research in patients with sepsis is to improve our understanding of how this common and still frequently fatal disease process can be prevented, diagnosed, and treated. All research is associated with considerable costs, whether they be for equipment, personnel, administration, pharmaceutical or laboratory products, technology, time, or a combination of all or some of these. Funding can be provided either from public or private, generally industry-derived, resources. A characteristic of industry funded research is that it is almost always profit-oriented and thus tends to be more lucrative. A drug company will only invest in research into something that will ultimately provide it with sufficient profit to surpass its original input. The industry tends to invest little on research in fields that are unlikely to be profitable in the near future, even if such research could lead to highly beneficial results in terms of, for example, better disease understanding or improved outcomes for a very small subgroup of patients. Industry-driven studies often, therefore, try to focus on broad groups of patients with the hope that the new drug will be usable in as many patients as possible, and to encourage more rapid patient enrollment. Clinician-initiated research, on the other hand, can concentrate on more specifically defined patient groups but can then be hampered by long enrollment times and smaller groups of patients. Industry-funded research has come under considerable criticism in recent years, with several widely publicized cases in which potentially harmful data were held back from the public domain. Several studies have also suggested that publications of studies supported by the industry are biased and that some authors even allow sponsoring pharmaceutical companies to alter manuscripts to their interests before publication. These are important considerations, but, realistically, new treatments of sepsis will never be developed without industry-initiated funding. Funding from public sources is in short supply and when available is often used for other diseases considered to be of greater priority than sepsis, e.g. cancer. Hence, industry-initiated funding must be accepted, but collaborations between the industry and clinician must be direct and honest. Clinicians must be involved at all points of the development, design, conduct, and analysis process and must have free and open access to all data at all times throughout the study. Clinician-initiated research is is limited in what it can achieve in terms of development of new therapies; industry-funded studies can benefit patients but must be conducted with honesty and integrity.

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PATHOGENESIS OF SURGICAL SITE INFECTIONS

E. Patchen Dellinger

Surgical wound infections are caused by bacteria, and will not occur in the absence of bacteria. However, surgeons have known for many years that many other factors also influence the risk of infection. John Burke demonstrated in 1963 that all (50 of 50) clean surgical incisions contain bacteria at the end of an operation, but only a small number (4% in that report) become infected. An animal study of the relationship between bacterial inoculum and infection risk demonstrated an increasing risk of infection with increasing numbers of bacteria. This was described by a typical sigmoid, biological curve when inoculum size was graphed against infection incidence. However, there was no inoculum in that study of 1028 incisions that resulted either in a zero or in a 100% risk of infection. The authors concluded that the development of infection in a surgical incision is "dependent on many factors other than the presence of bacteria". They further predicted that reductions in the incidence of postoperative infection could be achieved by techniques both to reduce the numbers of bacteria that gain access to surgical wounds and by focusing on methods to increase the efficiency of

host defenses in resisting those bacteria that do gain access to the wound. While some bacteria (S. aureus, E. coli) must be present in large numbers to cause a surgical site infection, others (beta-hemolytic streptococci, C. perfringens) can do so in very small numbers. Many commonly assumed surgical practices are not supported by evidence. In animal models of infection placing sutures to reduce "dead space" or placing drains in the wound increases the infection rate and severity. Clinical trials in humans have demonstrated the value of keeping the patient warm, giving high percentages of inspired oxygen, and achieving tight glucose control. Surveys of large numbers of patients demonstrate higher SSI rates when patients have more co-morbidities, operations are longer, or transfusions are required. However, no prospective trials compare short operations with long ones or patients who bleed and are then randomized to transfusion or no transfusion. It is self evident that operations that take longer or require transfusions are not the same as those completed quickly without transfusion. In the end it is a complex balance of the numbers of bacteria, the virulence of those bacteria, the intrinsic immune status of the patient, and the local conditions in the surgical wound that determine whether a surgical site infection will occur. The surgeon has the greatest influence on the numbers of bacteria that gain access to the wound, on the nature of the wound, and on factors that influence immediate antibacterial defenses such as temperature, oxygenation, glucose levels, and perioperative antibiotics.

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WOUND INFECTIONS SEEN IN THE TRAUMA PATIENT

Christian Schinkel

An accident, unlike an elective preparation, causes the transmission of force of an undetermined magnitude to human tissue. In the immediate hours or days areas of skin demarcation, skin slough, bruising, or thrombosed vessels appear. In addition to the initial trauma, after operation, there is additional opportunity for accumulation of hematoma.
Two major groups of wound infection can be distinguished: primary infections resulting from penetrating trauma or secondary infections caused by hematogenous or intraoperative contamination.
Three factors are crucial: the pathogenic germ, the non-perfused dead space or tissue and the susceptibility to infection.Typical bacteria found are Staphylococcus spec. and Streptococci.
Potential risk factors and therapeutic approaches for wound infections after trauma are similar to other surgical site infections (SSI). Special attention must be paid to prophylaxis that includes proper surgical excision or debridement, second look operations if indicated, 48h antibiotic coverage for open fractures, shock prophylaxis, maintainance of normothermia and euglycemia, and avoidance of immunosuppression.

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PREVENTION OF SURGICAL WOUND INFECTION VIA MICROBIAL SEALING

William Cheadle

Microbial contamination of the surgical site is a necessary precursor of a SSI. Studies have shown the rate of SSIs can be associated with the amount of bacteria intra-operatively; 1-5% of clean surgeries performed will result in an infection and 10-20% of clean-contaminated surgeries will develop a SSI. While a minority of the contamination sources are exogenous (surgical personnel, operating room environment, and tools, instruments and materials brought into the operating room), the source of pathogens for most SSIs, in the absence of damage to hollow viscera, is the endogenous flora of the patient's skin. When skin is incised, the exposed tissues are at risk for contamination by endogenous skin flora. A number of preventive measures have been proposed to reduce the risk of SSIs, including patient and skin preparation, surgical team hand/forearm antisepsis, antimicrobial prophylaxis, operative room management, asepsis and surgical technique, and postoperative incision care. While all of these applications act as broad spectrum topical antiseptics, each has its disadvantages and, despite rigorous disinfection, ultimately some skin bacteria continue to survive. These surviving endogenous pathogens can be transferred into the surgical incision by irrigation fluids, gloves, instruments, sponges, or implants, and could cause a surgical site infection. A surgical sealant has been developed to bond to the skin surface, immobilizing the bacteria which survive the application of conventional surgical skin preparation products. Microbes are trapped in the skin epidermis and hair follicles and coating is complete by electron microscopy. Data from a recently completed clinical trial will be presented which will compare microbial recovery from hernia incisions.

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INTERNATIONAL GUIDELINES FOR THE MANAGEMENT OF SEVERE SEPSIS AND SEPTIC SHOCK: THE FIRST REVISION

Phillip Dellinger

The first revision of the International Guidelines for the Management of Severe Sepsis and Septic Shock will be published in 2007. The first revision will update the guidelines in line with recent publications concerning importance of prompt administration of appropriate antibiotics, role of steroids in septic shock, role of rhAPC, glycemic control, choice among vasopressors/ fluids and mechanical ventilation of acute respiratory

distress syndrome (ARDS). The guidelines are being revised in collaboration with the GRADE group, international leaders in evidence-based medicine, and scores recommendations both as to quality of evidence (A to E) and strength of recommendation (weak or strong).

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INFECTIONS OF PARTICULAR IMPORTANCE IN LUNG TRANSPLANT RECIPIENTS

Jens Gottlieb

Infections are the leading cause of death (28% of all cases) and increase morbidity substantially after lung transplantation (LTx). The risk of infections increases with the intensity of immunosuppression. Beside immunospuppression several factors contribute to increased infection rates in LTx-recipients: enviromental exposure of the graft, airway ischemia and stenosis, decreased cough reflex and muciliary clearance. Also the underlying disease, age and comorbid conditions such as diabetes, renal dysfunction, metabolic and nutritional disturbances, breakdown of mucosal barriers and leukopenia may predispose to infections. Some infections derive from preexisting colonization in the donor or recipient; some are acquired through nosocomial or environmental exposures and some relate to mechanical complications of the transplant.There are three major time periods after transplant. During the first month, nosocomial and postoperative infections are most commonly seen. In the second period (month 2 to 6 posttransplant) opportunistic infections including CMV, Epstein-Barr virus-related lymphoma (EBV-PTLD), fungal infections and nocardiosis occur commonly. In the late period from 6 months on infections depend on the intensity of immunosuppression and presence of chronic organ dysfunction (bronchiolitis obliterans syndrome (BOS)); common respiratory viruses and bacterial pathogens and still opportunistic infections occur. CMV is the most important single pathogen following LTx. Aggressive prophylaxis for some infections (e.g., cytomegalovirus, CMV) has reduced the prevalence of serious CMV-infections. Aspergillis spp. are the dominant fungal pathogens, tracheobronchial aspergillosis is a nearly unique clinical entity seen in LTx-recipients. Fungal prophylaxis is performed aggressively in most centers. Nocardia spp. may cause severe infections in patients following LTx. Infections may cause damage to allograft function by triggering acute rejections and BOS as well. Prophylaxis and preemptive therapy of infections are therefore crucial.

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VIRAL TRANSMISSION BY ORGAN TRANSPLANTATION

Pascal Meylan

Various types of viral infections entail a risk of transmission through organ transplantation.

First, chronic systemic infection with viremia, including HIV, HBV and HCV. As a general tenet, the likelihood of infection is related to the inoculum size, accounting for instance for the graded risk associated with the transplantation of organs from HBV-infected donors with different infectivity serologic markers. This risk is further compounded by the infectious status of the transplant organ: passively infected by blood contamination, or active virus replication by organ tissue (e.g. liver transplantation and hepatitis viruses).

Second, viruses that establish latent infection, especially in circulating leukocytes, but also in resident cells in the transplant organ are known to transmit infection during transplantation (CMV, EBV, HHV-6, -7 and -8). Of note, not all (about 80%) of recipients with the CMV donor positive/recipient negative serostatus pattern do convert after follow-up. The risk factors for CMV transmission in this instance are poorly known, although their identification may lead to preventative approaches against transmission. We have observed an EBV seronegative kidney recipient from a donor with active infectious mononucleosis, who has a persistently elevated EBV DNA blood three years after transplantation, suggesting that not only likelihood of infection, but also post-transplant infection course can be influenced by inoculum size.

Third, acute viral infections can transmit to organ recipient. On one hand, sytemic infections such as rabies, West Nile virus and LCMV have led recently to clusters of recipient infection. The recent increase in Western Europe of Tick-borne encephalitis incidence raises the possibility of transmission if organ procurement were to occur during the initial viremic phase of the disease. On the other hand, acute infection of the transplant will also transmit infection. Thus, we recently described the first instance of influenza occurring in the immediate postoperative period in a lung transplant from a donor with influenza virus infection.

Appropriate targeted screening for viral infection and exclusion of donors with clinical syndromes compatible with viral infection should reduce the risk for transmission.

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FUNGAL INFECTIONS IN SOLID AND NON-SOLID ORGAN TRANSPLANT RECIPIENTS

Herbert Hof

Yeasts and hyalohyphomycetes represent the most common causes of invasive fungal infections. Recently, emerging pathogens such as zygomycetes and phaeohyphomycetes ("dematiaceous fungi"), have attracted publicity.

The so-called group of yeasts consists of a very heterogenous population of fungi, presenting the same microscopic characteristics, namely blastopsores which replicate by budding. But in reality - according to the botanical classification - the genetic relatedness is not very close. Candida albicans, which is a normal member of the human flora, is the predominant yeast infecting transplant recipients. Whereas in most cases this is an endogenous infection, occasionally this pathogen as well as other yeasts are nosocomially transmitted. Besides local infections of various mucosal sites blood stream infections as well as localized manifestations in practically all organs may occur. Diagnosis is made primarily by histology and culture; fluconazole remains the corner stone in therapy; the newer azoles, such as voriconazole and posaconazole, can replace it in few instances. Echinocandins, which are well tolerated, dispose of a broad spectrum of activity, even against azole resistant yeasts, and are fungicidal.

Among the hyalohyphomycetes Aspergillus spp., and in particular A. fumigatus, play a major role. In certain hospitals local outbreaks with A. terreus largely resistant to most of the antimycotics available have been reported. The spores of these fungi reside in the environment and are transported via the air. After inhalation of these fungal elements, in most cases a lung infection develops at first. Secondary, continous or hematogenous dissemination to other organs, in particular to the brain, have to be considered depending on the degree of immunosuppression which favors spreading. In solid organ transplantation, occasionally the transplanted organ itself or the direct surroundigs can be the site of fungal multiplication. After heart transplantion the sutures of the large vessels represent a locus minoris resistentiae. The time to manifestation may vary considerably from a few days to several months. Histology and culture are able to prove infections. Imaging techniques, PCR and antigen or antibody detection can further support the clinical suspicion; in many cases, however, the diagnosis cannot be proven but remains propable or even possible. Since the outcome highly depends on the early begin of a specific antimycotic treatment, in many cases a preemptive therapy is justified. Even prevention with suitable drugs, such as posaconazole, may be indicated even though this implicates a risk of overtreatment. Besides liposomal amphotericin B, which can be nephrotoxic when given in high doses and to patients with reduced kidney function, the newer triazoles, such as voriconazole and posaconazole, and the echinocandins, such as caspofungin, are indicated for therapy or salvage therapy, respectively. The benefit of combinations is not yet completely approved.

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IS THERE A SEX DIFFERENCE IN HYPOXIC PULMONARY VASOCONSTRICTION?

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Objective: Hypoxic pulmonary vasoconstriction (HPV) is an adaptive mechanism to decrease ventilation-perfusion mismatch in the setting of acute hypoxia. However, if hypoxia becomes global and prolonged, HPV can lead to pulmonary hypertension (PH) and cor pulmonale. It is well recognized that sex differences exist in the inflammatory response to sepsis and ischemia. Estrogens possess known vasodilatory properties in the systemic circulation. It is also known that there are gender differences in the in vitro vasomotor effects of different steroid hormones on isolated rat pulmonary arteries. Under normoxic conditions, pulmonary arteries from male animals are more sensitive to the vasodilatory effects of exogenous testosterone than those from female animals. In humans, idiopathic pulmonary arterial hypertension (iPAH) is much more prevalent in females. However, it is not known if there are any gender differences in the response to hypoxia. We hypothesized that the degree of HPV differs between males and females. To test the hypothesis, isolated pulmonary arteries from male and female rats were exposed to hypoxia and the degree of HPV was measured.

Material and Methods: Pulmonary artery rings (n=7-8/group) from adult male and female Sprague-Dawley rats (weights=250-350g) were isolated and suspended in physiologic organ baths at 37°C. To induce hypoxia (pO_2= 0-35 mmHg), the organ bath was bubbled with 95% nitrogen/5% carbon dioxide for 60 min. Force displacement was continuously recorded. Data (mean±SEM) were analyzed with two-way analysis of variance with post-hoc Bonferroni test.

Data: There was no significant difference in HPV between males and females. Maximum phase II vasoconstriction was 89.78 ± 7.24% for males and 95.89 ± 14.23% for females (p=0.9342).

Conclusion: We conclude that there are no gender differences in the response of isolated pulmonary artery rings to acute hypoxia. This is in contrast to the difference in vasomotor effects of sex hormones observed under non-hypoxic conditions. The data may explain why there is a female predominance in iPAH but not in secondary, hypoxic PH. Whether exogenous sex hormones have a different effect on HPV, and whether there is a dose-response curve, is currently under investigation.

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DEHYDROEPIANDROSTERONE (DHEA) DECREASES T-CELL RESPONSES IN SURGICAL PATIENTS

Johannes Dietz, Siegfried Zedler, Eugen Faist, Florian Loehe, Martin K. Angele

Purpose of the study: Clinical studies indicate suppressed proinflammatory cytokine release by peripheral mononuclear blood cells (PBMC) following major abdominal surgery. Treatment of PBMC in vitro with DHEA restored the suppressed LPS induced cytokine release by antigen presenting cells following abdominal surgery in patients. Nonetheless, it remains unknown whether DHEA also affects lymphocyte responses in surgical patients.

Methods: To study this blood samples were obtained from 10 patients undergoing major abdominal surgery preoperatively and 2 hours postoperatively. Wells were coated with anti-CD3 for 90 minutes and subsequently PBMCs cultured in the presence or absence of 10^{-5} M DHEA for 24 hrs. The release of IL-2, IFN-γ, IL-10 and the expression of CD4, CD8 and CD69 were measured.

Results:

	IL-2	IFN-γ	IL-10
Preop. without DHEA	121±37	786±40	950±212
Preop. + 10^{-5}M DHEA	24±7*	408±53*	266±58*
Postop. without DHEA	75±26	542±61	505±102
Postop. + 10^{-5}M DHEA	36±12*#	264±68*#	111±27*#

N=11 patients, paired t-test, *$p<0.05$ vs. preop. without DHEA, #$p<0.05$ vs. postop. without DHEA

Anti-CD3 induced release of IL-2 and IL-10 was not affected by abdominal surgery, however, significantly decreased by DHEA. The release of IFN-γ was significantly suppressed following surgery and further decreased by DHEA. The distribution of CD4/CD8 was not significantly different. The activation marker CD69 on CD4 positive T-cells was significantly suppressed by DHEA and unaltered on CD8 positive T-cells.

Conclusions: In contrast to LPS stimulated PBMC, anti-CD-3 induced T-cell activation and cytokine release capacities are decreased by DHEA. Thus, DHEA differently modulates PBMC responses depending on the stimulation pattern in vitro. Further studies, however, are required to understand the underlying mechanisms for those controversial results before using DHEA in surgical patients.

(Supported by DFG AN 357/1-1)

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ORGAN BLOOD FLOW IS BETTER PRESERVED IN FEMALE RATS FOLLOWING TRAUMA/HEMORRHAGIC SHOCK

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Objective: Our studies have focused on the role of injury leading to gut ischemia and the associated release of pro-inflammatory and tissue injurious factors that lead to the development of systemic inflammatory response (SIRS) and multiple organ dysfunction syndrome (MODS). Female rats, especially those during the proestrus stage of their menstrual cycle, have been implicated to be more resistant to SIRS and MODS following trauma hemorrhagic shock (T/HS) when compared to male rats. In this set of experiments, we test our hypotheses that following shock leading to intestinal ischemia-reperfusion, there is preservation of end-organ blood flow in proestrus female rats versus male rats.

Material and Methods: Sprague-Dawley male and proestrus female rats were subjected to trauma-hemorrhagic shock (laparotomy and 90 min of shock at a mean arterial pressure (MAP) of 35-40 mmHg) or trauma-sham shock (T/SS, laparotomy alone). MAP, cardiac index (CI) and organ microcirculatory blood flow were measured at four time points post reperfusion (0, 15min, 1hr, 3hr) using microsphere technique with Sc^{46} and Nb^{95}.

Data: Although, there was no difference in MAP between males and females at the conclusion of shock, later reperfusion time points show superior MAP maintenance in females (1hr: male 95±6mmHg vs. female 109±3mmHg; 3hr: male 80±3mmHg vs. female 105±3mmHg; $p<0.05$). CI was significantly worse in both male and female animals during shock, but was better preserved in female rats ($p<0.05$ vs. male groups). A similar response was found after reperfusion at all time points (males 62-82% and females 80-96% of T/SS controls; $p<0.05$). T/HS was associated with decreased blood flow to the heart, lungs, kidneys, spleen, liver and pancreas of male and female rats, but the decrease was significantly greater in male rats, especially during the post-reperfusion period ($p<0.05$ vs. female groups). Similarly, male rats had a more profound decrease in blood flow to the stomach, proximal small bowel, terminal ileum and colon than female rats at all time points (i.e. proximal small bowel: males 34-50% and females 50-76% of T/SS controls; $p<0.05$).

Conclusion: The hemodynamic response to T/HS was better preserved in female than male rats as shown by better maintenance of MAP, CI and organ microcirculatory blood flow. These results support the hypothesis that proestrus female rats have increased resistance to intestinal ischemia-reperfusion associated injury, which is in part due to the maintenance of blood flow to the end organs.

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SYSTEMIC ADMINISTRATION OF FUNCTIONALLY MODIFIED DENDRITIC CELLS IMPROVES OUTCOME TO SEPSIS

Kerri OMalley, Claudia Moreno, Ricardo Ungaro, Phillip Scumpia, Matthew Delano, Lyle Moldawer

Objective: Dendritic cells (DCs) link innate and adaptive immunity and are essential to an effective septic response. Systemic loss of dendritic cells in septic mice results in decreased survival, suggesting that strategies to maintain DC numbers or function may improve outcome. Our lab has previously demonstrated that recombinant adenovirus (Adv) readily transduces DCs in vitro allowing directed delivery of transgenes that modify DC function; localized administration of these dendritic cells can improve survival. Our objective was to determine whether transduction of murine DCs with adenoviral vectors and subsequent systemic administration of these DCs would improve outcome to sepsis.

Material and Methods: Bone marrow cells were harvested from the tibia and fibula of Ly5.1 mice and cultured for 10 days in the presence of GM-CSF. On day ten bone marrow-derived DCs were incubated with 10^4 particles per cell of recombinant adenovirus (Adv IL-10 or Adv GFP) for two hours or overnight with 1 ug/ml of LPS. Twenty four hours post transfection 10^6 Adv-dendritic cells were harvested and injected into the retro-orbital sinus of C57 Bl/6 female mice (8 weeks of age) immediately prior to cecal ligation and puncture (CLP). Control CLP mice received administration of saline only. The cultured dendritic cells were analyzed via flow cytometry and the cell supernatants were analyzed using a multiplex bead array. Mice were observed for seven days to determine survival outcome and then sacrificed; spleen, liver, and lung were harvested upon sacrifice and where possible, upon expiration from CLP.

Data: Survival was improved in the adv IL-10 CLP group (80%, n=10) and advGFP CLP group (60%, n=10) as compared to CLP alone (30%, n=10). The CD11c+ adIL-10 DCs had decreased expression of both MHC II (79.5%± 2.4) and CD86 (75.5%±3.3) when compared to adGFP (MHCII 91.1%± 0.8 and CD86 81.7% ±2.0) and LPS (MHCII 90.4% ± 0.6 and CD86 92.2% ± 0.4) stimulated cells. Surprisingly, the adIL-10 DC supernatants had higher levels of IL-6 concentrations (2490 pg/ml) when than those in the unstimulated (84.9 pg/ml) and adGFP (767 pg/ml) groups. The spleen, lung and liver of surviving animals had similar numbers of donor derived DCs at day 7.

Conclusion: These current studies extend our previous findings by demonstrating that improvements in survival can be obtained when targeted IL-10 expressing DC populations are administered systemically, a more viable option for potential therapy. Our data suggests that these improvements are not attributeable to differences in homing but are due to the altered phenotype of the IL-10 transduced cells. Our current investigation into this altered phenotype could provide new insight into the potential for dendritic cell therapy.

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TREM-1 POLYMORPHISMS AFFECTS IMMUNE RESPONSES AFTER BURN TRAUMA

Fernando Rivera, Jureta Horton, Agnes Burris, Ming-Mei Liu, Sue Vaneck, Joseph Minei

Background: The triggering receptor expressed on myeloid cells (TREM-1) plays an important role in the modulation of the innate immune and inflammatory responses. We previously demonstrated that the carriage of a single nucleotide polymorphisms (-25 A→T) in the TREM-1 gene was strongly associated with decreased risk of complicated sepsis (adjusted odds ratio = 0.36, 95% CI = 0.13-0.94) as well as death (adjusted odds ratio = 0.23, 95% CI = 0.03-0.86). However, it is not know how this SNP affects the immune responses. Aim: to further characterize the effect of this SNP in the immune responses.

Methods: patients admitted to the Burn Unit at Parkland Hospital were enrolled. Blood was taken at 1, 3,7, 10, 14 days.HLA-DR and TREM-1 expression was measured by Flowcytometry. Plasma IL-10 and s TREM-1 by ELISA. Clinical data was collected daily.

Results: 44 patients enrolled of those 7 died, and 15 developed sepsis. Carriage of the T-allele was associated with increased expression of HLA-DR, ($p < 0.001$) and Il-10 ($p = 0.03$) levels. AA homozygotes were associated with higher increased risk for sepsis and mortality as well as lower HLA-DR and increased TREM-1 concentrations.

Conclusions: polymorphism (-25 A→T) in the TREM-1 gene is associated with different immune response and clinical outcomes.

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CD4 EXPRESSING CELLS ARE EARLY MEDIATORS OF THE INNATE IMMUNE SYSTEM DURING SEPSIS

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Objective: It is well established that the immune response to infectious agents during sepsis is mediated by leukocytes associated with the innate immune system. However, there is an emerging view that T lymphocytes can also mediate this response. In this study, we assessed the function of CD4 expressing within the first 30 hours of sepsis.

Materials and Methods: CD4 deficient ($CD4^{-/-}$) and wild type (WT) mice underwent cecal ligation and puncture (CLP). Here, 80% of the cecum was ligated and punctured once with a 23 gauge needle. This CLP produced a less severe model of sepsis with a mean survival time of 110 hours and a 50% mortality.

Data: Splenocytes from $CD4^{-/-}$ mice (Table 1) have no CD4 T cells. However, there is a compensatory increase in CD8 T cells in $CD4^{-/-}$ mice as compared to WT mice. Next, we inflicted CLP in $CD4^{-/-}$, CD8 deficient and WT mice. In Figure 1, we show that $CD4^{-/-}$ mice were more susceptible to CLP as compared to CD8 deficient mice. Interestingly, within the first 30 hours following CLP, we found a significantly higher mortality in the CD4 (-/-) as compared to WT mice. Next, we measured IL-6 and IL-10 concentrations in serum and peritoneum 18 hours following CLP. In the $CD4^{-/-}$ mice, serum and peritoneum IL-6 concentrations as well as the clinically relevant IL-6 / IL-10 ratio were higher as compared to WT mice (Fig 2). Next, we determined there was increased bacteremia in $CD4^{-/-}$ mice as compared to WT mice (Fig 3) and that treatment with the antibiotic, Primaxin, led to a significant decrease in the mortality of the $CD4^{-/-}$, but not WT mice (Fig 4). As neutrophils are key cells in eliminating bacteria, we examined whether CD4 cells act as early mediators upon these cells. Isolated neutrophils from $CD4^{-/-}$ had decreased spontaneous oxidative burst as compared to neutrophils taken from WT mice (Fig 5). However, stimulation with fMLP showed a similar increase of the oxidative burst as the WT, indicating that there is no loss in the capacity of oxidative burst but a change in activation. Interferon gamma is well established to have potent anti-microbial activity. We found that IFN-gamma KO mice had a higher susceptibility to CLP than the WT mice (Fig 6). Further, our studies have shown that there is an inverse correlation between the percentage of CD11b cells that produce IFN-gamma and IL-6 concentrations at 6 hours. Here, we found that in the peritoneum of WT mice, there is a two-fold increase in these cells (Fig 7).

Conclusion: The actions of CD4 expressing cells are beneficial during sepsis. The data suggest that CD4 cells facilitate the early clearance of bacteria by acting on innate immune cells, possibly through an IFN-gamma dependent mechanism.

Support: SHC Project 8560 and NIH R01-GM072760

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WOUND HEALING- LOCAL LYMPHATIC SYSTEM DOWN-REGULATES REACTION TO MICROBES AND OWN ANTIGENS?

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Introduction: Open and closed wounds cause response of the regional lymphoid tissue. Open injuries of soft tissues and bone cause damage to tissues and contamination with microorganisms. Closed injuries are not connected with infection, however, the cellular debris freed from the damaged cells also evokes response of the local lymphoid tissue. In our previous studies we documented on lymphoscintigraphies that fractures of lower limb cause dilatation of lymphatics draining the site of injury and enlargement of inguinal lymph nodes. The question arises what kind of reaction proceeds in the lymph nodes and whether it reflects the cellular and molecular events in the healing wound.

Aim: To study the phenotypes of cells in the wound and draining lymph nodes after primary and secondary injury.

Material and Methods: Studies were carried out in 3 groups in WIS rats. Group 1a. Incisional wound of dorsum of paw and stiching. Group 2a. Subdermal injection into paw of 10^8 Staph.epidermidis for 6 days. Group 3a. Closed fracture of tibia. Follow-up period lasted in all groups for 7 days. Group 1b. Incisional wound followed after 7 days by another incision. Group 2b. Re-injection of 10^8 Staph.epidermidis. Group 3b. Re-fracture of tibia. Specimens from the site of injury or bacterial injection and popliteal and iliac lymph nodes were taken on day 7 in groups 1a, 2a and 3a, and on day 14 in groups 1b, 2b and 3b. The infiltrating and lymph node cell phenotypes were identified with moAbs against W3/13 (T cells, leukocytes), W3/25 (helper cells), OX8 (suppressor cells), OX6 (class II), OX7 (stem and thymocytes), OX12 (B cells), ED1 (macrophages) and HIS48 (granulocytes). Cells were also isolated for FACS analysis.

Results: Groups 1a, 2a, 3a.

Paw skin: In all groups, 7 days after the primary injury, multiple (++) MHCII+, ED1+ and CD54+ cells were found in the subepidermal region or fracture gap.

Lymph nodes: In all groups an increase in lymph node weight and cell concentration was observed. The W3/25+, MHC II+, ED1+, HIS48+, OX7+ and OX62+ and CD54+ cells accumulated in the follicles, paracortex and medulla. The percentage of W3/25+CD25+ (T regulatory) was not increased. High frequency of B cells was seen in follicles and medulla. The medulla extracellular matrix was stained OX12+. Endothelial cells stained strongly for OX6 and OX7. Interestingly, there was not more HIS48 granulocytes compared with controls. FACS analysis of node cells did not reveal changes in the frequency of individual cell populations compared to controls. Groups 1b,2b,3b. In all groups evidently less of infiltrates in the paw and positively stained cells in lymph nodes were seen.

Conclusions: There were no qualitative changes in infiltrates and lymph node cell subtypes depending on the type of primary injury both in wounds and fracture gaps. Surprisingly, secondary injury caused less inflammatory reaction. The mechanism of the developing local suppression is studied.

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THE ROLE OF PROMOGRAN PRISMA* ON THE EXCESSIVE INFLAMMATORY RESPONSE IN CHRONIC WOUNDS

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Inflammation is an important part of the wound healing process, which begins within hours of injury and provides host defense, by removing invading microorganisms, and devitalized tissue. It is characterized by the infiltration of neutrophils/ monocytes to the wound site, which initiates wound debridment in a controlled manner. An alteration in this process can lead to a persistent state of inflammation, and delay wound repair. The biochemical changes associated with uncontrolled inflammation includes excess pro-inflammatory cytokines, proteolytic activity and reactive oxygen species (ROS); all of which cause significant damage, and further perpetuate the inflammatory state.

In this study we investigated the impact of PROMOGRAN PRISMA* matrix (an ORC/collagen matrix containing 1% silver-ORC) on the ability of endotoxins to stimulate a pro-inflammatory response in an in vitro model of inflammation. Lipopolysaccarhide (LPS) was used to stimulate monocyte /macrophages (THP-1 cells) to produce pro-inflammatory cytokines. The ability of PROMOGRAN PRISMA* to reduce the level of these cytokines was measured using ELISAs. While previous studies have reported the ability of PROMOGRAN PRISMA* matrix to affect protease activity, in this investigation we examined the affect on pro-inflammatory cytokine production and free radicals.

Results show that PROMOGRAN PRISMA* matrix is capable of reducing the levels of pro-inflammatory cytokines. This dressing also has antioxidant activity, thereby reducing the levels of free radical species. We hypothesis that these effects should result in an overall 'anti-inflammatory' action, limiting the destruction of new tissue and promoting wound closure.

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LOW RECRUITMENT OF IMMUNE CELLS AND PRESERVED EXPRESSION OF CYTOKINES AND GROWTH FACTORS IN THE MARGIN OF DIABETIC FOOT ULCERS

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Diabetes is characterized by thickened basement membrane of the blood arterioles and capillaries. This may affect the transcapillary transport of immune humoral factors and cells to the extravascular space. Keratinocytes (KC) and dermal endothelial cells (EC) beside of leukocytes infiltrating wound are the main source of factors regulating healing of skin ulcers. We examined by immunohistochemistry the infiltrating cell phenotype and expression pattern of adhesion molecule on leukocytes, dermal fibroblasts and endothelial cells as well as the expression of various cytokines, chemotactic and growth factors and their receptors in the margin of diabetic foot ulcers and in normal nondiabetic foot skin. Twelve patients with type 2 diabetes, mean age 61.8 years, with foot ulcers of grade 2-4 by Wagner classification and without acute dermatitis or tissue edema around the ulcers were randomly selected. The biopsy material from the border area of ulcer was compared with that obtained from five nondiabetic orthopedic patients, mean age 62.0 years, undergoing elective surgery of the foot. Although there was accumulation of granulocytes on the surface and superficial layers of the granulation tissue, rare perivascular granulocyte infiltrates in the dermis were seen. Moreover, lack of macrophage and CD3+ T cell infiltrates was observed. In contrast, there was increased intensity of CD1a staining of Langerhans' cells in the epidermis and papillary dermis ($P<0.05$). Fibroblasts revealed increased presence in the ulcer margins compared with normal skin ($P<0.05$). Skin endothelial cells expressed stronger the von Willebrant factor (f.VIII) and E-selectin compared with normal skin ($P<0.05$). Our study found significantly elevated expression of transforming growth factor -β1 (TGF-β1) and type I TGF-β receptors (TGFβ RI), granulocyte macrophage colony stimulating factor (GM-CSF), and epidermal growth factor (EGF) in KC in the ulcer margin ($p<0.05$). Significantly increased expression of monocyte chemotactic protein-1 (MCP1), GM-CSF, CXCR1, and TGFβRI and decreased expression of interleukin (IL) -10, IL-15 and TGFβ1 were observed in ulcer dermal EC ($p<0.05$). There was a lack of up-regulation of IL8, CCR2A, IL10 receptor, GM-CSF receptor, platelet- derived growth factors and their receptors, vascular endothelial growth factor and its type II receptor, EGF receptor, insulin-like growth factor-1, and nitric oxide synthase (NOS)-2 in both KC and EC cells in the ulcer. Finally, there was a lack of upregulation of IL10, IL15 in KC and of EGF, basic fibroblast growth factor and NOS-3 in EC in the ulcer margins. In conclusion, our study provides evidence that increased expression of endothelial cell adhesion molecules responsible for immunocyte extravasation is not associated with increased inflammatory cell infiltration of the ulcerated diabetic foot tissue. The enhanced expression of some factors responsible for keratinocyte behaviour could suggest unimpaired capacity of KC to reepithelialize the margin of diabetic foot ulcers. However, lack of upregulation of some angiogenic and leukocyte chemotactic factors may account for a poor formation of granulation tissue and chronicity of ulcer epithelialization. Therefore, we suggest that the healing process of diabetic foot ulcers may be hampered by mechanisms decreasing accumulation of leukocytes. This implies that pharmacological or biological stimulation of leukocyte extravasation into the ulcer tissue should be tried.

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HIGH DENSITY EXPRESSION PROFILING REVEALS PRONOUNCED MATRIX AND SIGNAL TRANSDUCTION GENE EXPRESSION INDUCED BY FIBRIN GLUE TREATMENT IN HUMAN GASTRIC ULCERS

Thorsten Pohle, Jan Becker, Wolfram Domschke

Background: Fibrin glue is used in the endoscopic therapy of bleeding ulcerations. Accelerated closure of ulcers has been attributed to this treatment; underlying molecular mechanisms, however, remain unclear.

Methods: Two artificial gastric lesions were induced in two healthy, Helicobacter pylori negative volunteers and treated by injection of either saline solution or fibrin glue. After 72 hours, biopsies were taken from the resulting ulcers for whole human genome analysis (Applied Biosystems). Only genes with a more than 5-fold difference in their cDNA expression following injection therapy of either saline solution or fibrin glue were regarded as significantly regulated. Subsequently those genes were grouped according to their protein function for further analysis.

Results: Treatment with fibrin glue led to a significant induction of 20 genes when compared to normal healthy gastric tissue (=control) and of 142 genes in comparison to an injection therapy with saline solution. Notably fewer genes were downregulated (8 vs. control, 56 vs. saline). Classification of genes according to their protein function revealed a marked induction of genes encoding for proteins involved in cell-cell and cell-matrix interactions (13 genes induced, 2 repressed) as well as in signal transduction (22 up-, 2 downregulated) when compared to effects of saline.

Conclusions: Our experiments demonstrate that fibrin glue is a biologically active substance inducing genes encoding for proteins involved in cell-cell and cell-matrix interactions as well as signal transduction. Therefore its impact in ulcer healing is more substantial and does not only depend on activation of the coagulation cascade and local compression.

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FAST TRACK SURGERY OF RECURRENT PNEUMOTHRACES IN PATIENTS WITH CYSTIC FIBROSIS - SUPERIORITY OF MINIMALLY INVASIVE TISSUE MANAGEMENT

Roman Carbon, Carbon Roman, Reingruber Bertram, Huemmer Hans Peter, Kriegelstein Stefanie

Objective: The options for operative tissue management can be greatly expanded by the use of a collagen bandage dry coated with components of tissue glue. This biodegradable tool has proven to be of particular value especially in minimally invasive surgery (MIS) and video-asisted thoracic surgery (VATS), and even leads to interesting extensions of indications. Pneumothorax in cystic fibrosis (cf) is a complex problem demanding emergency management and tissue saving strategy. There are a lot of options for treatment: Therapeutic (thoracic drainage, abrasio, pleurodesis, resection) and tactical (conventionally / VATS). MIS treatment in recurrent pneumothorax can be an advantage to be protective to tissue and adhesions with special value in pre-Lx situation.

Methods: a: In a biosimulator model (CCP) the different methods of gluing 1.liquid, 2. impregnation of biodegradable fleeces with fibrin glue by hand (**prepare-to-use**), 3. Collagen fleece **ready-to-use** impregnated with components of tissue glue (TachoSil) were evaluated for adhesive strength on porcine pleura. The pressure at the upper limit was measured in hPa. b: An adjustable MIS applicator (AMISA) for fleece bound sealing was developed which simplified the introduction and placing of the approx. 10x5 cm TachoSil bandage (ATCS=AMISA-TachoSil-System). So not only hemostasis, but also rapid sealing of larger tissue defects in the pleural cavity was made possible. c: Selective leak closure (SLC) with the ATCS was administered in pediatric surgery with recurrent pneumothorax in cystic fibrosis (cF).

Results: a: Adhesive strength (CCP) showed highly significant differences ($p<.0001$) in different sealing techniques: Liquid: 4.2 hPa, prepare-to-use: 23.6 hPa, ready-to-use: 50.6 hPa. b: AMISA and ATCS showed a high grade of feasibility, flexibility and practicability. c: Clinical data: Control group: 284 pat. (1985-96, mean age 13.2 yrs., cf, drainage) vs 122 pat. (1994-2005, mean age 14.3 yrs., cF, ATCS). Drainage: Demand (%): 100,0 vs 48,0(***), time (d): 17,2 vs 0,5 (***). Hospital stay (d): ICU: 3,7 vs 1,8 (**), ward: 34,3 vs 5,8 (***). Drugs (%): antibiotics: 43,1 vs 57,4, analgetics: 78,5 vs 7,2(***). Complications (%): Drainage: 34,9 vs 4,1 (***), recurrence: 45,1 « 12,3 (***). [* $p<.05$ **$p<.01$ ***$p<.001$, corr. accord. to Bonferroni]

Conclusions: Fleece bound ready-to-use sealing is significantly more resistant and has a higher practicability than any other sealing (liquid, prepare-to-use). The innovative VATS application of fleece bound sealing increased and expanded indications, especially in pneumothorax management. Endpoints: Increase in the effectiveness of the selected procedure (reduction of drainage time), increase in the efficiency (reduction of complications), decrease in intensive care, improvement in psychosocial structure (decrease in hospitalization, return to school/work, improved family-relationships). ATCS shows a high pharmacoeconomic and socioeconomic potency and is a reliable tool for processing tast-track-surgery in that complex disease management.

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ASSESSMENT OF HEMOSTATIC AGENTS FOR TRAUMATIC ARTERIAL HEMORRHAGE FOR THE BATTLEFIELD USE

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Background: Traumatic arterial hemorrhage is the leading cause of death in conventional warfare. Early and effective hemorrhage control is crucial to reduce combat mortality. This has led to the development of hemostatic agents specifically for use in the pre-hospital setting.

Objective: This study was to evaluate the efficacy of hemostatic products in a swine model of uncontrolled arterial hemorrhage and to recommend the most effective dressing suitable for military use in the pre-hospital setting.

Material and Methods: Uncontrolled hemorrhage was initiated by lacerating the femoral artery. After 5 minutes of bleeding, the animals were randomly assigned to receive: 1) QuikClot (QC), 2) TraumaDEX (TD), 3) Surgicel (SC), and 4) TachoComb (TC). The efficacy of hemostatic agents was assessed by animal survivability, hemostatic efficiency, and physiological parameters. Coagulation parameters and microscopic and gross examination of organs were also evaluated.

Results: SC showed the best outcome overall, reflected by the highest animal survival rates (Table 1). This was followed by TD, QC and TC, respectively (Table 1). SC also achieved the fastest hemostatic effect and the lowest rate of re-bleeding at the laceration site (Table 2-4). TD took a longer time for hemostasis (Table 2) and had higher rates of re-bleeding (Table 4). QC was able to stop bleeding as fast as Surgicel (Table 2-3), but animal survivability in this group was poor (Table 1). This may due to the relatively high rates of re-bleeding (Table 4). A disadvantage of QC is the exothermic reaction that occurs after application, leading to secondary burn injuries to the skin and adjoining soft tissues. TC was not effective in arresting the hemorrhage (Table 2-4), and demonstrated the poorest survival rate (Table 1). There were no significant differences in selected parameters of blood coagulation, namely prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen (Fbg), between groups. This implies that the topical application of hemostatic agents may not interfere with the systemic blood coagulation profile.

Conclusion: Surgicel (SC) significantly improved the overall outcome in the swine model of lethal arterial hemorrhage. Since SC is FDA-approved, relatively cheap, and can be stored at room temperature, we recommend that SC be deployed for military use.

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Table 1. Survival Rate

Group	N	Survival Rate (%)		
		Before hemorrhage	2 hr after hemorrhage	24 hr after hemorrhage
QuikClot	10	100	90	40
TraumaDEX	7	100	71	57
Surgicel	10	100	90	90
TachoComb	10	100	70	20

Table 2. Average Bleeding Stop Time

Group	N	Average Bleeding Stop Time (min)
QuikClot	10	2.40 ± 0.79
TraumaDEX	7	7.29 ± 2.32
Surgicel	10	2.40 ± 1.03
TachoComb	10	15.00 ± 4.02

Table 3. Bleeding Stop Rate

Group	N	Bleeding Stop Rate (%)		
		< 1min	< 5min	< 30 min
QuikClot	10	50.0	90.0	100.0
TraumaDEX	7	14.3	58.0	100.0
Surgicel	10	80.0	90.0	100.0
TachoComb	10	0.0	20.0	90.0

Table 4. Re-Bleeding Rate

Group	N	Re-bleeding Rate (%)
QuikClot	9	55.5
TraumaDEX	5	60.0
Surgicel	9	22.2
TachoComb	7	85.7

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CONTINUOUS VERSUS BOLUS INFUSION OF TERLIPRESSIN IN OVINE ENDOTOXEMIA

Matthias Lange, Christian Ertmer, Katrin Broeking, Daniel Traber, Hugo Van Aken, Martin Westphal

Objective: In advanced sepsis, hemodynamic support is often complicated by a tachyphylaxis against exogenous catecholamines. Bolus infusion of terlipressin, a vasopressin analog, has been reported to increase mean arterial pressure in patients with catecholamine-resistant septic shock. However, bolus infusion of terlipressin may be associated with severe side effects, including pulmonary vasoconstriction and impairment in oxygen delivery. We hypothesized that continuous low-dose infusion of terlipressin may reverse sepsis-related systemic arterial hypotension with reduced side effects as compared to the traditional concept of bolus administration.

Material and Methods: Twenty-seven adult sheep were chronically instrumented to measure hemodynamics of the systemic and pulmonary circulation. After 24 hrs of recovery, a baseline measurement was performed in the healthy state. Thereafter, a continuous infusion of S. typhosa endotoxin (10 ng/kg/min) was administered for the following 40 hrs. After 16 hrs of endotoxemia, the surviving sheep (n=24) were randomly assigned to be treated with either a continuous infusion of terlipressin (2 mg over 24 hrs), bolus injection of terlipressin (1 mg every 6 hrs), or placebo (normal saline; each n=8).

Data: Continuous infusion of terlipressin permanently reversed endotoxin-induced systemic arterial hypotension ($p<0.001$ vs. baseline in endotoxemia) and improved left ventricular stroke work index ($p<0.05$ vs. baseline in endotoxemia) in all sheep. Intermittent bolus injections of terlipressin were linked to decreases in heart rate and cardiac index, as well as increases in pulmonary vascular resistance index (each $p<0.001$ vs. baseline in endotoxemia). These unwanted side effects were prevented by continuous low-dose infusion of the drug.

Conclusion: In ovine endotoxemia, continuous infusion of low-dose terlipressin stabilized hemodynamics and improved myocardial performance without obvious side effects. Low-dose terlipressin infusion appears to be superior to terlipressin bolus injection in the treatment of arterial hypotension related to sepsis and systemic inflammatory response syndrome.

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CORTICOSTEROIDS IN THE COMPLEX MANAGEMENT OF NECROTIZING PANCREATITIS

Andre Perejaslov, Serge Chooklin

Objective. Despite the potent anti-inflammatory and immunosuppressive properties of glucocorticoids its applying in the management of severe necrotizing pancreatitis is still controversial.

Materials and Methods. The plasma levels of interleukins (IL-6, IL-8, and IL-18) and adhesion molecules (E-selectin and ICAM-1) were measured in 48 patients with necrotizing pancreatitis who admitted in clinic within 48 hours after disease onset. The measurement was performed immediately after admission, at the 3, 7, and 14 day. All patients were divided on two groups: first group compiled 26 patients, in which dexamethasone (24 mg/day during 4-6 days) was applied in the complex management of acute pancreatitis, and control group - 22 patients that did not receive corticosteroids. All patients received the initial therapy, which included adequate fluid replacement, pain medication, proteases inhibitors, pentoxifylline, as well as the administration of antibiotics.

Results. The increased levels of IL-6, IL-8, IL-18, ICAM-1, and E-selectin were noted in both groups of patients at the time of admission. The signs of MODS were present in the 10 patients of the first group and in the 9 patients of the control group. The gradually increase of all proinflammatory mediators plasma levels up to seventh day was noted in patients of the control group. Its levels clear correlated with the severity of MODS and spreading of necrotic processes confirmed by CT. Starting from the third day the gradually decrease of mediators levels were noted in the patients of the first group. The incidence of contamination of necrotic foci had no difference in both groups of patients (23.1% in the first and 22.7% - in the control group).

Conclusion. The ability of glucocorticoids to inhibit expression of proinflammatory mediators due to the glucocorticoids-mediated repression of NF-kappa B pathway provide the pathogenetic substantiation for the applying of glucocorticoids in the complex management of necrotizing pancreatitis.

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BENEFICIAL EFFECTS OF LONG TERM PROPRANOLOL THERAPY IN PEDIATRIC BURNS

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Background: The hypermetabolic response caused by increased catecholamine levels following burn injury is associated with changes in infection rates during the acute hospital admission as well as changes in heart rate, cardiac output and stroke volume that can be seen for 2 years after injury. Propranolol has been shown to reduce hypermetabolism and improve cardiac function during the acute hospital course; however, its use in long-term burns care has yet to be evaluated fully. This study investigates the changes seen in resting energy expenditure (REE), cardiac function and body composition when using Propranolol long-term following burn injury.

Methods: Forty six patients (23 Propranolol, 23 Control) with burns greater than 40% total body surface area (TBSA) burns were enrolled into the study. Each treatment patient was placed on Propranolol for up to 1 year following closure of the burn wound. Cardiac Output, Stroke Volume and Heart Rate were collected on each patient at 6, 9, 12, 18 and 24 months under controlled conditions. Patients' cardiac results were calculated as a percentage of predicted value, taken from normograms for age matched non-burned individuals. Statistical analysis was performed using ANOVA with Bonferoni's correction and Student's t-test where applicable. Body weights and body composition (lean body mass, LBM, total body fat, TBF, and total body bone mineral content, BMC, measured with dual energy x-ray absorptiometry) were determined at discharge (baseline), 6, 9, and 12 months post burn. Results were calculated as a percentage of baseline values. Statistical analysis was performed using two-way ANOVA with post-hoc Tukey's test for inter-group comparisons and one-way repeated measures ANOVA with post-hoc Bonferroni's t-test for intra-group comparisons to baseline values.

Results: Cardiac Output and Heart rate were reduced by approximately 20% in the treatment group up to 1 year following burn ($p<0.05$) compared to controls. Following cessation of Propranolol, the treatment group increased Heart rate and Cardiac Output to match the control group. A significant reduction in Cardiac Index was seen at 12 months following injury ($p<0.05$). However, no significant differences in Stroke Volume were seen at any point. A significant reduction in REE was seen up to 12 months following injury ($p<0.05$); this was then lost after cessation of Propranolol. Propranolol significantly increased LBM ($p<0.02$), BMC ($p<0.01$), and body weight ($p<0.02$) vs. control at 12 months post burn. Within the Propranolol group, LBM and weight significantly increased at 9 and 12 months ($p<0.001$), and TBF and BMC at 12 months ($p<0.02$) compared to baseline. Within the control group, patients only showed increase in LBM and weight at 12 months ($p<0.05$) compared to baseline.

Conclusions: Long-term administration of Propranolol for 12 months following injury significantly improves REE and cardiac function in pediatric patients. The beneficial effect on REE and cardiac function is lost following cessation of treatment. Body composition is significantly improved at 12 months following burn injury in those receiving Propranolol. The beneficial effects of Propranolol administration on body composition, once the drug is stopped, have yet to be evaluated. Administration of Propranolol for up to 2 years may further improve outcome in this patient group.

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PERSISTING EFFECTS OF EXTENDED GROWTH HORMONE THERAPY IN SEVERELY BURNED CHILDREN ARE DOSE-RELATED

Rene Przkora, Marc Jeschke, Arthur Sanford, David Chinkes, Ronald Mlcak, David Herndon

Objective: Study the efficacy of high-dose growth hormone given to severely burned children for an extended time after discharge.

Summary Background Data: Hypermetabolism and catabolism continue for up to two years after burn, resulting in a significant delay in recovery. Low-dose recombinant human growth hormone (rhGH), given to children after a thermal injury improved growth, however, effects on lean mass were observed only during treatment. The purpose of this study was to investigate if a high-dose rhGH therapy will show more and continuing effects in severely burned children.

Methods: Sixty-six patients with over 40% total body surface area burns were studied for 24 months after burn. Patients were randomized to receive either 0.05 mg/kg (low-dose), 0.1 mg/kg (high-dose) rhGH, or placebo for the first year. Body composition, serum hormones and proteins, resting energy expenditure were measured. Statistical analysis used a two-way ANOVA, followed by Tukey's tes. Significance was accepted at $p<0.05$.

Results: Lean body mass was significantly improved with rhGH, effects with high-dose rhGH continued to improve and were significantly better at 24 months compared to low-dose rhGH and placebo. Increases in height were observed with both doses of rhGH. Resting energy expenditure was significantly lower with high-dose rhGH. Serum growth hormone and IGF-1 were significantly increased with high-dose therapy when compared to low-dose rhGH and placebo. Cortisol was significantly lower in the rhGH groups. No adverse effects were noticed.

Conclusions: High-dose rhGH demonstrated more and continuing effects on body composition during and after treatment in severely burned children.

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THERAPEUTIC EFFECT OF AN ATIII PREPARATION ON PATIENTS WITH DIC AND A HIGH RISK FOR DEVELOPING ARDS

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Objective: E-selectin, an adhesion molecule expressed in the vascular endothelial cells by the inflammatory cytokine, has been noted as a marker of injuries to these vascular endothelial cells. We have reported its usefulness as a marker to determine the severity of diseases, such as acute lung injuries and ARDS. Recently, we investigated the therapeutic effects of an ATIII preparation on patients with SIRS who showed a high blood level of soluble E-selectin, indicated a high risk for developing a lung injury and had experienced the onset of DIC. The details are reported below.

Material and Methods: The subjects were 23 patients who were brought to our emergency center between February and December 2005 and were given a diagnosis of DIC, according to the Matsuda Schedule and based on the high SES concentrations (normal: 32.09 ng/ml) at the initial examination. To the ATIII group (n=10), 1,500 units of the ATIII preparation was given for 5 days; and to the other, non-ATIII group (n=13), no ATIII preparation was given. For 7 days, starting on the day when admitted to the emergency department, a comparative evaluation was conducted on these two groups based on data such as DIC scores (diagnostic criteria set by the Ministry of Health and Welfare) and P/F ratios.

Data: The SES values at the initial examination were 59.49±15.11 and 77.53±15.11 ng/ml, respectively, for the ATIII and non-ATIII groups. There was no significant difference between the ages of the two groups. There was no significant difference in the ATIII concentrations of these groups but the value tended to increase later in the ATIII group. There was no significant difference in the DIC scores of the two groups; however, the non-ATIII group showed a tendency to increase whereas the ATIII group exhibited a decreasing tendency. The P/F ratios of the two groups did not differ significantly at the initial examination; but after the initial examination the ATIII group showed a significant increase ($P = 0.0016$).

Conclusion: It was suggested that administering the ATIII preparation to patients with SIRS complicated with DIC (and with an exaggerated SES concentration exhibiting a high risk for lung injuries) is effective not only for the treatment of DIC but also for the subsequent lung injury.

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KETOCONAZOLE REDUCES HYPERCORTISOLEMIA FOLLOWING SEVERE BURN

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Introduction: Significant increases in cortisol are seen following a severe burn and are thought to initiate the cascade of events leading to the hypermetabolic response with its ensuing catabolic state. It has been proposed that this large initial rise in cortisol leads to increased resting energy expenditure and bone loss, reduce T helper lymphocyte proliferation, with a subsequent reduction in the patients ability to fight infection. Large increases in cortisol levels have also been shown to increase net protein breakdown of skeletal muscle and increase efflux of intracellular amino acids. Ketoconazole is a first generation antimycotic agent that reduces cortisol production by inhibition of 11β-hydroxlation in the final step in the synthetic pathway. This study investigates the safety and use of Ketoconazole to reduce synthesis of cortisol in burns patients.

Methods: In a prospective clinical study ten male patients (5 Ketoconazole, 5 Control) admitted to our unit between December 2005 and February 2006 with burns greater than 40% total body surface area were enrolled into the study. Each patient had 24hr urine collections at regular intervals throughout the acute admission. Each treatment patient received 5mg/kg Ketoconazole PO/NGT BD. Urine cortisol, serum cortisol and cardiac function (CO, SV and HR) were measured throughout acute hospital stay. Infection data was collected prospectively. Results were divided into three time periods (0-10, 11-20 and 21-40 days post burn). Statistical analysis was performed using ANOVA with Bonferoni's correction ($p<0.05$).

Results: Serum and urine cortisol were reduced by 90 and 85% respectively in the treatment group across all time periods ($p<0.05$). Patients were able to transiently raise cortisol production in response to additional stressful events such as surgery. We observed an 84% reduction in overall number of infections per patient in the treatment group when compared to controls which did not reach statistical significance. No episodes of hypocortisolemia or deleterious effects in cardiac function were seen in either control or treatment groups.

Conclusions: Ketoconazole can be safely used in burns patients to reduce cortisol production. A larger prospective study is now required to elicit the changes seen in protein kinetics and infection rate. If proved successful this would make a welcome addition to the small number of pharmacotherapeutic options available to support the severely burned patient.

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ISOFLURANE MODULATES THE IMMUNOLOGICAL AND PHYSIOLOGICAL RESPONSES TO ENDOTOXEMIA IN SHEEP

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Objective: Volatile anaesthetics are known to have anti-inflammatory qualities in-vitro and have therefore been suggested to have beneficial effects in septic patients. Isoflurane, a halogenated volatile anaesthetic, has been shown to reduce neutrophil recruitment, lung injury, cytokine release and increase survival in septicemic rodents. However, the long-term consequences of isoflurane anaestheisa during endotoxemia have not been explored. The objective of the current study was to investigate the effects of isoflurane on immunological and physiological function in a 48 hour model of sheep endotoxemia.

Material and Methods: Eight ewes (Texel cross-bred) were chronically prepared with exteriorized carotid arteries and ultrasonic flowprobes around the femoral, renal and superior mesenteric arteries, respectively. The sheep were randomly allocated to either receive isoflurane inhalation (mechanical ventilation, 1.5 % end-tidal concentration) or to remain conscious throughout the experiment. After a 60 min baseline period endotoxemia was induced and maintained by a continuous intravenous infusion of Escherichia coli lipopolysaccharide (serotype 0111:B4), 25 ng/kg/min together with 3 ml/kg/h 0,9% NaCl for 48 hours. Hemodynamics were monitored continuously. Urinary output was followed and urine samples collected for analyses every 120 min. Arterial blood samples were obtained for blood-gas analyses and lactate concentration during the baseline period, 4 and 8 hours after start of endotoxin infusion and thereafter every eight hours. At the same time points venous blood was drawn and later analysed regarding haematological variables, plasma-protein, osmolality and electrolytes. At baseline, 4, 24, and 48 hours after commencement of endotoxemia venous blood was obtained for determination of neutrophil transmigration in response to IL-8. Bronchoalveolar lavage was performed after the endotoxin infusion had been discontinued and the fluid was investigated for neutrophil recruitment and protein leakage.

Data: E. coli endotoxin induced a hyperdynamic sepsis with increased cardiac output and decreased vascular resistance in all sheep. In the conscious group urinary production and arterial blood pressure was initially decreased but recovered fully during the last 24 hours. Isoflurane anaesthesia prevented the recovery and resulted in sustained oliguria and hypotension. Furthermore, the isoflurane anesthetized sheep developed acidosis with decrease in base excess and hyperlactemia. Neutrophil recruitment into lung interstitium and alveoli was increased in the isoflurane group. Endotoxemia decreased in vitro neutrophil transmigration in response to IL-8 in both groups. The maximum decrease was 4 hours after initiation of endotoxin infusion, thereafter the ability of neutrophils to transmigrate slowly recovered. The decrease in transmigration was less in the isoflurane group and the recovery rate faster in comparison to the conscious animals.

Conclusion: These findings suggest that isoflurane anaesthiesa improves neutrophil function and aggravates detrimental effects on circulation and renal performance in response to endotoxin in sheep.

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EFFECTS OF IGM ENRICHED SOLUTION ON PMN FUNCTION, BACTERIAL CLEARANCE AND LUNG HISTOLOGY IN ENDOTOXEMIA

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Objective: Immunological interventions in endotoxemia and sepsis have been tested in experimental [1] and clinical studies [2]. Our group evaluated the effects of an IgM-enriched solution in an established model of gram negative bacteraemia.

Materials and Methods: 10 New Zealand White rabbits (2-3 kg) were randomized to a treatment or control group. In the intervention group IgM-enriched solution (Pentaglobin®: 2ml/kg/h) was applied. In addition, LPS was infused in both groups at a rate of 40 µg/kg/h. After intravenously bolus injection of $1*10^8$ colony forming units of E. coli bacterial clearance was determined. Baseline hemodynamic + respiratory parameters, blood E. coli concentration (30 min prior to and 1, 15, 30, 60, 90, 120 and 180 min after E. coli injection), PMN oxidative burst and phagocytosis activity (both 30 min before and 1, 15, 60, 120 and 180 min post injection). Ex vivo phagocytosis activity was measured in a separate experiment and evaluated by electron microscopy. Diffuse alveolar damage (DAD) was measured, characterized by alveolar oedema, interstitial oedema, haemorrhage, inflammatory infiltration, epithelial destruction, microatelectasis and over-distension. Organ colonization (kidney, lung, liver, spleen) was assessed in aseptic organ samples.

Data: Hemodynamic parameters did not differ between the two groups. Bacterial clearance was not influenced by IgM application. Liver and spleen colonization was significantly reduced in the IgM group. IgM reduced phagocytosis at 30, 90 and 180 min and improved burst at 180 min. Ex vivo phagocytosis activity as documented by electron microscopy was increased in the IgM group.The sum of all weighted DAD scores (except over-distension) was significantly better in the IgM group (23±5 vs. 30±8).

Conclusion: IgM significantly improved 6 of 7 DAD score parameters and reduced liver and spleen E. coli count. Although ex vivo phagocytosis activity was significantly decreased in the IgM group, this can be explained by increased in vivo phagocytosis. Short term IgM intervention had an especially beneficial effect on LPS-induced pulmonary histological changes.

References: [1] Koch T. et al. Anaesthesiologie 1997;32: 420-5. [2] Tugrul S. et al. Crit Care 2002; 6: 357-62

Acknowledgements: MedDrive Fund, Medical Faculty Carl Gustav Carus Dresden; Biotest AG, Germany

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THE ANTI-INFLAMMATORY EFFECTS OF ULINASTATIN IN TRAUMATIC PATIENTS WITH A HEMORRHAGIC SHOCK

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Objective: The aim of this study was to investigate whether the use of ulinastatin is associated with the suppression of plasma proinflammatory cytokine and PMNE and the good prognosis in the patients with traumatic hemorrhagic shock.

Material and Methods: We enrolled traumatic hemorrhagic shock patients without severe brain injury(GCS<9) or renal failure, heart failure, COPD, or liver failure. Prospective, randomized, and controlled clinical analysis of 19 patients confirming to the enrolled standard was carried out. They were divided into two groups at random. One was control group (n=8) with regular treatment, and the treatment group (n=11) ulinastatin 300,000 IU for 1 day. The inflammatory indexes were determined before and after therapy on the 1 st, 2nd, 3rd, 7th day including TNF alpha, IL-6, Polymorphonuclear leukocyte elastase (PMNE), SIRS score, MODS score and APACHE III and clinical data.

Data: There were no significant differences baseline demographic data and baseline serum TNF alpha, IL-6 and PMNE between two groups. The serum level of PMNE in ulinastatin treatment group (4.48 ng/ml) was decreased (P=0.019) than control group (11.58 ng/dl) at the 2 day of therapy. But the SIRS score, MODS score and APACHE III score were not significantly differences between both groups at the 2 day, 3 day and 7 day after trauma (P>0.05).

Conclusion: Serum concentration of PMNE at the second hospital day after ulinastatin treatment was significantly lower than the control group, but there were no significant improvement of SIRS score and MODS score.

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INHIBITION OF PLATELET ACTIVATION REDUCES ORGAN FAILURE IN PATIENTS WITH COMMUNITY ACQUIRED PNEUMONIA AND IN ENDOXIN SHOCK IN MICE

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Background and Aims: Systemic inflammation and sepsis are associated with blood platelet activation which in turn may contribute to the development of organ failure. The aim of our study was to test whether anti-platelet drugs (APD) may have a benefit in such patients and/or in a mouse model of endotoxin shock.

Methods: Data obtained from 224 patients with community acquired pneumonia (CAP) were retrospectively analysed for an association between pre-hospital treatment with APD (acetyl salicylic acid, clopidogrel or ticlopidin) and clinical outcome. Use of statins was an exclusion criterion. BALB/c mice were pre-treated with clopidogrel (added to the drinking water) for 4 days prior to an intraperitoneal injection of LPS (E. coli 0111:B4). For platelet counts and blood gas analysis automated standard procedures were used. Lung tissues were stained with HE or a FITC-labelled anti-fibrin(ogen) antibody.

Data: When compared to controls (n=180), APD-patients (n=44) did not differ at day of admission in platelet and leukocyte counts, CRP and signs of organ failure. Although they were older (69±7 vs 58±13 y, p<0.00001) APD-patients developed less frequently organ failures (admission to intensive care unit: 9.1% vs. 26.1%; p<0.02). In the mouse model clopidogrel reduced the LPS-induced drop in platelet count and the degree of lung injury. Compared to controls we found 20 h after LPS injection in the clopidogrel animals a significant lower number of thrombi in the lung vasculature (6.1±2.3 vs 11.5±4.4 per screen, p<0.025) as well as higher blood pH and bicarbonate levels (7.01±0.01 vs. 6.93±0.04, p<0.04 and 10.2±0.14 vs. 7.3±0.14 mmoles/l, p<0.03, respectively).

Conclusions: Anti-platelet drugs may have a beneficial effect in systemic inflammation and sepsis, and could be a novel therapy option, at least in patients on low bleeding risk. One mechanism of the clopidogrel effect could be a reduction in the inflammation-induced thrombus formation within the microcirculation.

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INTERLEUKIN-1 LOCAL APPLICATION FOR THE THERAPY OF PATIENTS WITH CHRONIC BACTERIAL RHINOSINUSITIS

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Chronic bacterial purulent rhinosinusitis is a disease developing as a complication of various acute respiratory infections. Chronic rhinosinusitis has low response to antibiotic therapy probably due to disregulation in normal immune function. Cytokines of interleukin-1 (IL-1) family are one of the main mediators involved in the control of inflammation, local defense reactions and tissue repair. The aim of this study was to investigate the associations of chronic rhinosinusitis with IL-1 beta and IL-1 receptor antagonist (IL1RA) functional gene polymorphism and to study the possibility of local IL-1 therapy in patients with rhinosinusitis. IL-1beta and IL-1Ra gene polymorphism was analyzed by polymerase chain reaction (PCR) amplification of the polymorphic site, followed by site-specific restriction digestion. Two sites were examined: IL-1b (+3953 C>T, intron 5) and IL-1RA (86 bp variable tandem repeat, intron 2). Normal allele was designated as "1", polymorphic - as "2". Leukocyte functional activity was studied using phagocytosis, chemiluminescence and NBT reduction tests. Cytokine levels in supernatants of LPS-stimulated peripheral blood leukocytes were measured using cytokine-specific ELISA. Patients with more rare IL-1b $(+3953)^+$ allele had elevated IL-1 beta production and patients with IL-1RA 2*$(VNTR)^+$ allele had higher antagonist production compared with patients bearing normal alleles. These abnormal alleles were found in 30% and 90% of patients respectively that was much more frequently compared to healthy subjects suggesting for genetic predisposition to chronic rhinosinusitis probably due to the disregulation in the IL-1 family cytokines production. Human recombinant IL-1 beta has been used for local therapy in patients with chronic bacterial purulent rhinosinusitis after failure of routine antibiotic therapy. IL-1 beta solution (10 ng/ml) was applied directly to the inflammatory site daily for 3-5 days. We have found that recombinant IL-1 therapy was highly effective in patients carrying IL-1RA 2*$(VNTR)^+$ allele with increased endogenous IL-1RA levels. In these patients IL-1 beta local application significantly increased functional activity of leukocytes isolated from the inflammatory sites tested in the assays of neutrophil migration to fMLP, superoxide production, adhesion and phagocytosis. Negative results of IL-1 beta therapy was obtained in patient who had very rare combination of homozygous IL-1b $(+3953)^+$ and normal IL-1RA 2*$(VNTR)^-$ allelic variant that led to elevated IL-1 beta production and normal IL-1RA levels. According to obtained results the IL-1 family gene cluster polymorphism may play a significant role in the pathogenesis of chronic rhinosinusitis probably due to the disregulation in the IL-1 family cytokines production. Topical IL-1 application led to clinical improvement and did not induce any systemic adverse effects that usually limit cytokine therapy.

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RESUSCITATION WITH FRESH WHOLE BLOOD REDUCES COMPLEMENT ACTIVATION ASSOCIATED WITH HEMORRHAGE IN SWINE

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Objective: Traumatic hemorrhage is associated with an acute inflammatory response. However, the role of complement activation in this process, and in response to subsequent fluid resuscitation is not well understood. The present study investigated indices of complement activation after severe hemorrhage in swine and after infusion of different resuscitation fluids.

Materials and Methods: Anesthetized, splenectomized and instrumented 40 kg swine (n=5-7/gp) were subjected to a controlled hemorrhage of 20 ml/kg over 5 min that duplicated the blood loss profile of an uncontrolled hemorrhage. After 30 min, fluid resuscitation was initiated with lactated Ringer's (LR), normal saline (NS), hextend (HX) or the animal's shed blood (FWB) along with a second hemorrhage of 8 ml/kg. All fluids were infused warm and infusion was controlled to return systolic blood pressure (SBP) to 80 mmHg, as necessary throughout the experiment. Hemodynamic and metabolic variables were monitored continuously and blood samples were drawn at baseline (BL) and at select times throughout the 3.5 hr experiment or until death. Indices of complement activation were determined by CH50 levels and measurement of thromboxane B2 (TXB2), a stable metabolite of TXA2.

Data: Hemorrhage reduced mean arterial pressure to about 36 mmHg and lowered cardiac output (CO) to 36% of BL in all groups. All fluids were capable to return and maintain SBP at 80 mmHg, but the volumes of LR and NS required were about 4-fold higher than those required for HX or FWB. CO improved the most in the HX group, but differences were not statistically significant among groups. Plasma lactate increased during the 3.5 hr experiment in all groups, but levels were lower in the FWB group than in the other groups ($p<0.05$). Arterial base deficit levels followed the trend seen with plasma lactate. Hemorrhage activated complement as indicated by CH50 falling to about 75% of baseline at 30 min. In addition TXB2 levels rose about 20% during this time. Infusion of LR, NS or HX resulted in further reductions in CH50 values and sustained elevations in TXB2. In contrast, infusion of FWB raised CH50 levels to 85 to 90% of BL and reduced TXB2 levels to baseline.

Conclusions: All fluids improved hemodynamics in this model of severe hemorrhage, but only fresh whole blood improved the metabolic alterations (base excess, lactate, pH, O2 delivery or consumption, etc) that occurred during the 3.5 hr experimental period. In addition, fresh

whole blood also reduced complement activation associated with hemorrhage and fluid resuscitation.

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IMPROVED SURVIVAL IN NEONATAL MICE WITH SEPSIS FOLLOWING TLR AGONIST TREATMENT

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Objective: Neonatal mice are more susceptible to sepsis, and often exhibit an attenuated inflammatory response, when compared to adult mice. Toll like receptor (TLR) agonists have been shown to increase cytokine expression and augment innate immune responses. We hypothesized that systemic administration of a TLR agonist would improve survival to sepsis, in part by serving as an adjuvant for the inflammatory response.

Materials and Methods: Neonatal (5-7 day old) C57BL/6 (B6) mice were administered a single TLR agonist (either LPS-1 μg/g (TLR4), Poly I:C 10 μg/g (TLR3), and resiquimod-15 μg/g (TLR7/8)) or saline via an intraperitoneal (IP) injection 24 hours prior to creating a septic state (LD70) via generalized peritonitis using the cecal slurry (CS) method. Briefly, the cecal contents of an adult donor mouse were suspended in dextrose (80 mg cecal contents/1ml 5% dextrose). Neonatal mice received 50-75 μL (1.3mg/g) of the CS suspension IP and were returned to their mother. Survival was monitored for 5 days ($n \geq 27$ per group). Alternatively, plasma cytokines were determined (n=5/group/time point) and splenic leukocyte phenotypes were analyzed (n=5/group/time point) at 4 and 24 hours after TLR or sham injection, and at 12 and 24 hours following sepsis (n=5/group/time point), and were compared to sham.

Data: Survival was significantly improved in the LPS (71%, n=28, p=0.001) and resiquimod (56%, n=55, $p<0.02$) treated groups as compared to sham (35%, n=75). Poly I:C did not have any impact on survival (33%, n=27, p=1). Concentrations of multiple pro and anti-inflammatory cytokines were elevated in TLR-agonist treated animals compared to sham at 4 hours after TLR administration, and had returned to near baseline (sham) values at the time the cecal slurry was administered (24 hours after TLR treatment). Levels of IL-1a, IL-6, IL-10, IL-12p40/70, IL-13, IL-17, KC, MCP-1, MIP-1a and IFN-γ were all significantly elevated at 12 hours post-sepsis in the LPS pre-treated group as compared to other TLR agonists and sham animals ($p<0.05$). Flow cytometry revealed increases in plasmacytoid dendritic cells (resiquimod), natural killer (NK), and activated NK cells (Poly I:C and resiquimod) at 12 and 24 hours post-sepsis ($p<0.05$). Increased PMN cells were noted at 12 (Poly I:C) and 24 hours post-sepsis (LPS, Poly I:C, and resiquimod). Decreased $CD4^+$ and $CD8^+$ T cells at 12 (resiquimod and LPS) and 24 hours (resiquimod) were observed as compared to other TLR agonist treated groups and sham ($p<0.05$).

Conclusion: Neonatal mice pretreated with the TLR4 agonist, LPS, and the TLR7/8 agonist, resiquimod, exhibited significantly increased survival with an increased early inflammatory response. Although both adjuvants increased survival to subsequent sepsis, the differential alterations in cytokine responses and splenic leukocyte populations may suggest that the improvements in survival occur by fundamentally different mechanisms. Whereas LPS increased the global innate immune response, the improvements in outcome with resiquimod were associated with expansion of the plasmacytoid dendritic cell population and increased NK cell activity.

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TREATMENT WITH ECTO-5′-NUCLEOTIDASE INHIBITS AGGREGATION

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Objective: It is well known that adenosine inhibits platelet aggregation. Although it has been demonstrated that ecto-5′-nucleotidase (ecto-5′-nt, CD73) found on the surface of endothelial cells can convert AMP to adenosine and inhibit platelet aggregation in vitro and targeted disruption of ecto-5′-nt inhibits ferric chloride-induced carotid artery occlusion, the therapeutic effects of treatment with ecto-5′-nt on inhibition of platelet aggregation in vivo have not been investigated. We investigated the direct effects of ecto-5′-nt treatment on platelet aggregation using a whole blood aggregation method.

Material and Methods: Blood from healthy human volunteers was drawn into a 0.1 volume of sodium citrate (3.8%). Blood was diluted 1:2 with saline and preincubated at 37°C for 5 min in an aggregometer cuvette. Platelet activation was started by the addition of 10 μM/mL ADP or 5 μg/mL collagen and percent aggregation was measured for 6 minutes. In some experiments, whole blood was treated with ecto-5′-nt purified from Crotalus atrox venom with and without a specific inhibitor of ecto-5′-nt, 5′-[αβ-methylene] diphospate (APCP), immediately prior to addition of ADP or collagen. To further investigate the role of ecto-5′-nt in vivo, mice deficient in ecto-5′-nt (CD73 -/-) or littermate wildtype control mice were treated intraperitoneally (i.p.) with 500U/kg ecto-5′-nt or an equivalent volume of saline. After 30 minutes, blood was drawn via cardiac puncture into a 0.1 volume of sodium citrate (3.8%). Aggregation was measured as described above.

Data: Platelet aggregation in whole human blood was completely inhibited by ecto-5′-nt. Pretreament of ecto-5′-nt with APCP, an inhibitor of CD73, fully restored aggregation. CD73 -/- mice exhibited significantly higher aggregatory responses to ADP but not collagen when compared to littermate control mice. Administration of

ecto-5′-nt (500U/kg in saline, i.p.) to wildtype mice in vivo abolished ADP- and collagen-induced platelet aggregation. Furthermore, restoration of CD73 -/- with ecto-5′-nt in vivo significantly decreased aggregation ex vivo.

Conclusions: Using a whole blood aggregation method, we demonstrate that direct treatment of human blood with ecto-5′-nt inhibits aggregation. Furthermore, we demonstrate that treatment with ecto-5′-nt in vivo inhibits aggregation. Thus, ecto-5′-nt may represent a potential therapeutic treatment for prevention of thrombosis.

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ANTI-INFLAMMATORY ACTIVITY OF STEROIDAL SAPONINS (IND 002) ISOLATED FROM METHANOLIC EXTRACT OF FRUITS OF TRIBULUS TERRESTRIS

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Methanolic extract of fruits of tribulus terrestris was subjected to chromatographic separation was collected and labeled as IND 002 and evaluated in acute, sub-acute and chronic model of inflammation. The objective of present study was to evaluate the potential of IND 002 as anti-inflammatory compound and its ulcerogenic properties in rat models of inflammation. IND 002 at doses (50, 100 and 200 mg/kg) reduced carrageenan induced rat paw edema, granuloma tissue formation in cotton pellet induced granuloma model and paw edema in Freund's complete adjuvant induced arthritis model. Acute oral toxicity studies revealed that IND 002 was safe up to a dose level of 2000 mg/kg in mice. The anti-inflammatory effect was less compared to celecoxib (10 mg/kg). IND 002 and leflunamide did not induce ulcers in rats.However celecoxib did show mild ulcers.

Key words: IND 002; Tribulus terrestris; Anti-inflammatory

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IMPACT OF PERIOPERATIVE IMMUNONUTRITION AND STANDARD ENTERAL FEEDING ON IMMUNOLOGIC RESPONSE AFTER MAJOR PANCREATIC SURGERY

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The objective of the study was to investigate the alterations in peripheral blood mononuclear subsets (CD3+, CD4+, CD4+/CD25+, CD4+/CD38+, CD8+, CD8+/CD38+, CD8+/CD25+, CD19+, CD19+/CD95+, CD19+/CD38+, CD19+/CD34+, CD14+, CD14+/HLADR+, CD14+/CD68+) and systemic concentrations of cytokines (IL-1b, IL-1ra, IL-6, IL-8, IL-10, TNFa, sTNFRI) before and after pancreaticoduodenectomy in patients receiving enteral immunonutrition vs. standard enteral feeding. The prospective randomized studies included 41 patients with pancreatic cancer who had undergone pancreatic resection. In the routine evaluation of nutritional status a weight loss, BMI, albumin concentration and lymphocyte count were taken into account. Mononuclear cell subsets and serum cytokines levels were measured before and after operation on day 1,3,7 and 10 by flow cytometry and ELISA. In 22 patients standard pre- and postoperative enteral nutrition (Nutrison Standard, Nutricia) and in 19 patients immunonutrition (Stresson, Nutritia) were applied. Sixteen of the 41 patients developed postoperative complications (included 14 patients requiring reoperations). This study provided the following information: a) higher postoperative percentages of T, B cell subsets and monocytes (CD4+, CD4+/CD25+, CD4+/CD38+, CD19+, CD14+, CD14+/CD68+),(for CD19+ 17,53±14,1%, vs. 5,38±4,2%, p=0.001 on day 3) and higher serum IL-1ra concentrations in patients receiving enteral immunonutrition (3909,2±1398pg/ml vs. 2221,37±994 pg/ml, p=0.01 on day 7), b) lack of significant changes in T cell subsets and monocytes with higher serum IL-6 and IL-1ra concentration on day 1 after major surgery in patients receiving standard enteral nutrition and c) no significant difference between complication rate in both (immunonutritio vs. standard feeding) groups were found. We suggest that perioperative enteral immunonutrition with arginine, glutamine and fatty acids (PUFAs) improved rapidly cellular and humoral immunity in malnourished patients after pancreatic cancer surgery.

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THE ALTERED PROTEOME OF MESENTERIC LYMPH FOLLOWING HEMORRHAGIC SHOCK AND RESUSCITATION

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Background: Mesenteric lymph is the mechanistic link between splanchnic ischemia and subsequent remote organ dysfunction. Splanchnic hypoperfusion that occurs during hemorrhagic shock is a primary pro-inflammatory event using the lymphatics as a conduit for toxic mediators. Inflammatory states are profiled by a number of specific proteins (Acute phase, cytokines, cell lysate). We used MS-proteomics to analyze components of bioactive lymph produced after shock and resuscitation.

Methods: Hemorrhagic shock was induced in SD rats by controlled hemorrhage to a MAP of 30mmHg and sustained for 40min. Midline laparotomy was performed

with cannulation of the mesenteric duct for lymph collection. Resuscitation was performed by infusing 2x SB volume in NS over 30min, followed by $\frac{1}{2}$ SB volume returned over 30min, then completed with 2x SB volume in NS over 60min. Lymph was obtained in $\frac{1}{2}$ hr intervals. The fractions collected between 2-3hrs following resuscitation were consistently bioactive by a number of priming, signaling and physiological tests. Pre and post shock lymph, were labeled with fluorophores (Cy-3 and Alexa 488) and loaded at equal protein concentrations for separation on 2D-gels. In this initial survey, robotic spot picking focused on changes that were at least 2 fold and reproduced in all samples (n=4). Following trypsin digestion and identification of peptide fragments by mass spectrometry (MS-MS), a list of proteins with the highest degree of reproducibility and least unambiguous protein identification scores (numbers of peptide fragments identified X sequence assignment confidence) was prepared.

Results: During the preshock hour, a 300-350gram rat produced approximately 1-1.5mL of lymph. This decreased to approximately 100-200μL during shock; but upon resuscitation, the lymph flow increased to 2-3ml. The protein concentration in preshock lymph resembled plasma, but became dilute approximately 10 fold following resuscitation. Twelve proteins in post shock lymph had the most reproducible change from preshock lymph and highest identification confidence. Nine were extracellular in origin and several of these could be identified ontologically as Acute Phase Reaction proteins. Surprisingly, contrary to inflammation theory, these were decreased 50% or more (correcting for dilution) including several isoforms of fibrinogen, complement C3 and haptoglobin. Two intracellular proteins, carbonic anhydrase (involved in rectification of base deficit) and malate dehydrogenase (involved in lipid synthesis) were increased (>5 fold). A lipid carrier, a2 euglobulin (binds pheromones), increased 17 fold.

Conclusions: Following hemorrhagic shock and resuscitation, a number of prominent Acute Phase Reactant proteins (APRP) decrease abnormally in the lymph (after correcting for dilution) possibly due to a decoupling from the plasma. Two intracellular proteins are increased but it is unclear whether these are secreted or lost from lysed cells. The decreases in effective concentration of APRPs may reflect detrimental changes that occur prior to the onset of clinically recognizable inflammation thereby altering the clotting cascade, complement and overall immune function. Unbiased proteomic analyses may dramatically expand our understanding of pathological alterations following hemorrhagic shock with splanchnic hypoperfusion and guide careful examination of the mechanisms that predispose towards systemic inflammation.

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ASSOCIATION OF AORTA INTIMA PERMEABILITY WITH MYOSIN LIGHT CHAIN KINASE EXPRESSION IN HYPERCHOLESTEROLEMIC RABBITS

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The development of hypercholesterolemia is a multifactorial process in which elevated plasma cholesterol levels play a central role. This study analyzed the variability of the expression and activity of myosin light chain kinase (MLCK) and endothelial permeability in the artery wall of rabbits after feeding the animals with a normal or a high-cholesterol diet. Hypercholesterolemia was induced by a high-cholesterol diet for 4 weeks. Aortas were removed and analyzed for endothelial permeability and MLCK expression. Samples of the arterial media were analyzed for MLCK activity and expression. The aortas of high-cholesterol diet rabbits were showed an increase in MLCK expression and activity as well as endothelial permeability. These results indicate for the first time that hypercholesterolemia may be associated with MLCK expression and activity through which endothelial permeability is increased.

Keywords: high-cholesterol diet; hypercholesterolemia; permeability; myosin light chain kinase; rabbit; aorta; Intima

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PREOPERATIVE SERUM MMP-9 AND TIMP-1 LEVELS AND THEIR RELATIONS TO BREAST CARCINOMA METASTASIS

Qiang Wu

Objective: To detect the concentrations of MMP-9 and TIMP-1 in patient's preoperative serum with breast neoplasm, and to investigate their clinicopathologic significances and their relations to breast carcinoma invasion and metastasis.

Methods: The patients' preoperative serum concentrations of MMP-9 and TIMP-1 were detected by quantitive sandwich enzyme-linked immunoassays technique in 9 cases of breast benign neoplasm and 41 cases of breast infiltrating carcinoma.

Results: The patients' preoperative serum concentrations of MMP-9 and TIMP-1 in breast carcinoma were both significantly higher than those in breast benign neoplasm (83.2±58.0 ng/ml versus 21.3±13.0 ng/ml, $P<0.01$; 252.0±52.1 ng/ml versus 163.2±30.2 ng/ml, $P<0.01$). The concentration of MMP-9 was positively correlated to breast carcinoma TNM staging and axillary lymph node metastasis ($P<0.05$ and $P<0.01$, respectively). The

concentration of TIMP-1 was positively correlated to axillary lymph node metastasis ($P<0.05$). The concentration of MMP-9 was significantly higher in the group of tumor diameter more than 5cm than those in the group of between 2 and 5cm ($P<0.05$).

Conclusions: It might be valuable for differential diagnosis to detect the patient's preoperative serum concentrations of MMP-9 and TIMP-1 with breast neoplasm. The preoperative serum concentrations of MMP-9, TIMP-1 might reflect the status or potential of breast carcinoma invasion and metastasis.

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FUNCTIONAL POLYMORPHISM OF THE ANTI-OXIDANT ENZYME NQO1*2 IS MORE FREQUENT IN PROSTATE CARCINOMA AND LEADS TO LOWER LEVELS OF ANTI-OXIDANT IL-10 AND IL-13

Marion Schneider, Weidong Du, Eva Barthelmann, Bjoern Volkmer, Wolfgang Koenig, Wulf Dieter Seeling

Background: Functional polymorphisms of anti-oxidant enzymes may play a role in the manifestation of metabolic syndromes as well as malignancies. We asked for a role of the NAD(P)H:quinone oxidoreductase 1 C>T609 polymorphism (NQO1*2). The mutated gene displays a significantly reduced catalytic activity and is more rapidly degraded in cellular systems. NQO1*2 plays a major role in detoxifying quinone metabolites and cooperates with a number of cytochrome p450 oxido reductases.

Methods: A total of 509 healthy individuals of the local area was genotyped for the C609T single nucleotide polymorphism (SNP) by pyrosequencing. In addition, n=95 patients with prostate carcinoma were tested. Plasma cytokines were tested by Luminex based bead technologies and Immulite assisted determinations (DPC).

Results: For NQO1*2 SNP, we found the following frequencies in the healthy population: 358 individuals were wild type (C/C, 70.4%), 135 individuals were heterozygous (T/C, 26,5 %) and 16 individuals were homozygous for the mutation (T/T, 3.1%). In the group of prostate carcinoma patients we found 69.5 % wild type genotypes, 25.3% heterozygous and 5.2% with the homozygous mutation of NQO1*2. We further studied pro-and anti-inflammatory cytokines in patients with prostate carcinoma. A significantly lower concentration of the inflammatory cytokines IL-10 and IL-13 was found in patients with one or two mutated NQO1*2 alleles ($p=0.04$ and $p=0.03$, respectively). By contrast, concentrations of pro-inflammatory cytokines such as IFN-gamma were not found to be significantly different ($p>0.07$) in wild type versus mutated individuals.

Conclusions: We provide evidence that a widely expressed anti-oxidant enzyme NQO1*2 may play a role in chronic inflammation supporting the manifestation of prostate carcinoma which often presents as a secondary tumor. In tumor patients the mutated NQO1*2 allele is associated with a trend to a more pro-inflammatory phenotype and lower concentrations of the anti-inflammatory cytokines IL-10 and IL-13.

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STUDY OF A NEW TUMOR MARKER EPCAM EXPRESSED AND CLINICAL VALUE IN GASTRIC CANCER

Yi Liu

Background: The mortality of gastric carcinoma, one of the familiar malignant tumor, stands first on the list. It is important to detect and diagnose in nonage in order to prevent and cure gastric carcinoma. Study of tumor marker will improve it. EpCAM, a surface adhesion molecule expressed on epithelial cells and overexpressed on malignant epithelial cells such as lung cancer, colon cancer, rectum cancer, pancreas cancer, is a new pan-carcinoma antigen. Tissue microarray(TMA) are a potential technology for high throughput analysis of biomarker expression in a large number of tissue samples. Tissue microarrays have numerous advantages over general pathological sections: speed, throughput, standardization, ease-of-use, conservation of valuable tissue.

Objective: To study a new tumor marker EpCAM expressed in 91 cases of gastric cancer and clinical value by tissue microarray, and explore the feasibility of EpCAM as a tumor maker of gastric cancer. This study was to help diagnosing gastric cancer.

Methods: A total of 91 primary gastric cancer and 79 normal tissue specimens were obtained from gastric cancer patients undergoing surgical therapy for the treatment of gastric cancer. At least two core tissue biopsies 1mm in diameter were taken from selected morphologically representative regions of each paraffin-embedded gastric cancer and precisely arrayed using a custom-built instrument. Additional core tissue biopsies were taken from morphologically normal tissue. All we got three three- um-thick sections of the tissue microarrays block. EpCAM protein expression in tumors and matched morphologically normal gastric tissues was evaluated using anti- EpCAM immunohistochemistry. The relationship of EpCAM protein expression in tumors tissue and normal tissue was analyzed. And the difference of EpCAM protein expression in tumors tissue and tumor markers in -C12 protin microarray was compared to elucidate prognostic role in gastric cancer.

Results: (1) There only two patches of tissues were absent from Tissue Microarray, and the signals of the others were in focus. Tissue Microarray is a powerful tool for rapid

identification of the molecular alterations in gastric cancer and other pathological types. (2) EpCAM was expressed in 84 cases of 91 gastric cancers which positive expression rates were 92.3%. There were 11 cases of 79 normal tissues which positive expression rates were 13.9%. EpCAM in gastric carcinoma is significantly higher than that in control ($P<0.010$). EpCAM is weak or negative expressed in normal tissue but overexpressed in gastric cancer tissue. (3) The over-expression of EpCAM in gastric cancer was not related to the sex , age, location, the depth of invasion, the histological, differention, Borrmann types, pTNM types, et al. (4) The masculine rate of gastric cancer team is 44.0% of tumor markers in -C12 protein microarray. The positive EpCAM expression rate is significantly higher than that of C12($P<0.010$).

Conclusions: (1) Tissue Microarray is a powerful tool for rapid identification of the molecular alterations in gastric cancer and other pathological types. It has a lot of merits such as saving time, economies, reality, and so on. (2) The expression of EpCAM play an important role in gastric carcinogenesis. It may serve as a marker in the diagnosis of gastric cancer which wasn't influenced by the sex, age, location, the depth of invasion, the histological, differention, Borrmann types, pTNM types, and so on. (3) The role of EpCAM in gastric cancer about invasion and metastasis and prognosis need to be further investigated. (4) EpCAM protein expression in tumors tissue has a more useful than Tumor markers in -C12 protein microarray in blood serum in the diagnosis of gastric cancer.

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A STUDY ON THE TUMOR MARKER FOR DIAGMOSING OVARIAN CANCERS BY TISSUE MICROARRAY AND PREPARATION OF MONOCLONAL ANTIBODY AGAINST ANTIGEN ASSOSIATED WITH OVARIAN CANCER

Zhenshan Xu

Objective: To assess the value of CA125 and EPCAM for diagnosing and differentiating ovarian neoplasms by constructing and applying high-throughput tissue microarrays. To establish the monoclonal antibodys against Antigen Associated With Ovarian Cancer, study their characterization.

Methods: The ovarian tissue chip was prepared, CA125 and EPCAM were detected with immunohistochemical staining methods. The results were analyzed by referring to the pathology of the specimens to calculate the sensitivity, specificity, accuracy and expected positivity of the single monoclonial antibodies (MoAb) tests and the combinations of the tests. Using hybridoma techique, we prepare SK-OV-3. The hybridoma cells are obtained by fusing spleen cells of Balb/c mice to myeloma cells SP2/0 and culured in the HAT selective medium. The positive clones are screened by indirect ELISA and the limited dilution methods are used to select anti- Antigen Assosiated With Ovarian Cancer McAbs. McAb class and subclass are identified by Immunotype™ kit. The Western blotting method is analysed the specificity of McAb.

Results: The specificity of CA125 test was 86.67%, the value would be increased to 93.33% when the positive result of EPCAM was considered together, with the rate of false positivity being 3%. One of hybridoma cells secreting Antigen Assosiated With Ovarian Cancer mAbs is obtained. its subclass is IgM.The antigen recognized by 3c6 was a protein with a molecular weight of 82 KD .

Conclusion: Joint detection of CA125 and EPCAM will increase the specificity of the immunoassay and lower its rate of false positivity. The combination of CA125 and EPCAM is of high practical value for diagnosing ovarian cancer. One hybridoma cell line, which secretes monoclonal antibody against ovarian cancer, was established.

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EXPRESSION OF ENDOTHELIAL CELL SPECIFIC MOLECULE-1 IN LUNG CARCINOMA

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Objective: To investigate the relationship between the expression of endothelial cell specific molecule-1(ESM-1) and clinicopathological characteristics and the possible mechanism of ESM-1 in the development, progression and metastasis of lung cancer.

Methods: The expression of ESM-1 in tumoral and non non-tumoral lung tissues was analyzed by immunohistochemistry (IHC) (labeled streptavidin-biotin method).

Results: The ESM-1 was detected in the endothelium of tomural and non-tumoral lung tissues, in the cytoplasm of non-small cell lung cancer cell, while no in the cytoplasm of small cell lung cancer cell. The expression of ESM-1 in the endothelium of tomural tissue was significantly higher than that in the endothelium of non-tumoral tissues. There was a significant difference among lymph node involvements for ESM-1 expression in the endothelium of NSCLC. There was a significant difference among the different pathologic differentiated grades for ESM-1 expression in the cytoplasm of NSCLC.

Conclusions: It can be concluded that the over-expressed ESM-1 in patients with lung cancer both reflects the activation of tumor vascular bed and the tumoral proliferation. The over-expressed ESM-1 plays an important role in the development, progression and metastsis of lung cancer.

Key words: Lung neoplasms; endothelial cellular-specific molecule-1 (ESM-1); Immunohistochemistry

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ELEVATION OF THE IMMUNOMODULATORY ENZYME INDOLEAMINE 2,3-DIOXYGENASE IN TUMOR CELLS FACILITATES IMMUNE ESCAPE AND LEADS TO ENHANCED METASTASES

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Purpose: The immunomodulatory enzyme indoleamine 2,3-dioxygenase (IDO) is activated by interferon-γ and via local tryptophan depletion and the production of proapoptotic metabolites modulates T-cell function and promotes immune tolerance. IDO has been demonstrated to be critically involved in maternal tolerance and recent attention has turned towards its role in immune evasion of certain tumors. However, whether IDO expression is involved in tumorigenesis and tumor growth control still remains controversial. In this study we therefore addressed the question if IDO overexpression in tumor cells contributes to their metastatic properties.

Material and Methods: The human IDO gene was cloned into the pLIB vector containing the IRES-puromycin cassette driven by the CMV promoter. Murine CT26 colon adenocarcinoma cells were transfected with this gene construct and selected with puromycin to obtain cells stably expressing IDO. In vivo, CT26-IDO+ and CT26-vector control cells were injected subcutaneously into BALB/c mice and macroscopic tumor growth was assessed after 12 days. Concentrations of kynurenine and tryptophan in culture supernatants and serum were analyzed by HPLC. Kynurenine to tryptophan ratio (kyn/trp) was calculated to estimate IDO-activity. IDO gene expression in cell lines and tumor tissue was assessed by quantitative PCR and Western blot analysis.

Results: In vitro, IDO expression and functional enzyme activity in colorectal cancer cells was found to be strictly dependent on IFN-gamma stimulation. CT26-IDO+ cells exhibit high IDO enzyme activity as determined by HPLC and expressed as the kynurenine to tryptophan ratio (mmol/mmol) after 24 hours. In vivo, IDO activity (kyn/trp) determined in serum of tumor bearing mice was significantly higher (75.25 ± 10.21 μmol/mmol) in animals with CT26-IDO+ tumors as compared to 37.29 ± 7.85μmol/mmol in animals with IDO negative tumors. All mice developed clinically evident tumors after six days, as confirmed by histology. Tumor size of CT26-IDO+ tumors was significantly smaller than that of CT26 vector controls (136.2 ± 28.46 mm^3 vs. 237.0 ± 63.02 mm^3). However, only mice with CT26-IDO+ tumors developed peritoneal carcinosis, malignant aszites and distant metastases, whereas mice with CT26-IDO negative tumors showed locally restricted tumor growth.

Conclusion: This is the first preclinical model to demonstrate that IDO expression by colorectal tumor cells significantly contributes to disease progression, frequency of metastases and overall survival. Interfering with the IDO pathway by the use of IDO inhibitors might add a novel tool in the panel of cancer therapeutics and may enhance T cell-dependent antitumor immunitiy.

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THIOREDOXIN, MIF AND REDOX OF THE MICROENVIRONMENT AFFECT GROWTH AND INVASIVENESS OF HUMAN LUNG CARCINOMA CELLS

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Objective: Goals of this study are: 1. the elucidation of the involvement of thioredoxin (TRX) and MIF, two proteins able to exert both oxide-reductase and cytokine activities, in the phenotype and biological profiles of human lung carcinomas; 2. the definition of the molecular mechanism(s) linking MIF and TRX expression to tumor progression.

Materials and Methods: a. 10 cases of non small cell lung cancer were analyzed by immunohistochemistry with anti MIF and TRX antibodies and compared with the adjacent non neoplastic tissue. Non-protein thiols in both normal and neoplastic tissues were quantified and their spatial distribution was investigated by histochemical stain with the fluorescent dye Mercury Orange. b. A lung carcinoma cell line expressing high level of MIF and TRX (SK-MES) was selected and clones displaying low expression of the two proteins were generated by limiting dilution. Clones displaying stable inhibition of MIF were also achieved using siRNA technique. The phenotype and biological properties of the high expressing vs low expressing clones and the effects of exogenous reductants (DTT) were analyzed in vitro and in vivo in SCID mice.

Data: a. All the primary lung cancers analyzed stained strongly but not homogenously for MIF and TRX. Strong positivity was observed both in groups of tumor cells and in infiltrating inflammatory cells. The neoplastic tissues also contained non-protein thiols up to 10-fold more than adjacent normal tissues. b. SK-MES cells overexpress MIF and TRX and release abundant non-protein thiols in vitro. Conversely, cell clones expressing low levels of the two proteins release little thiols. In addition, low-expressing clones display retarded growth and reduced invasion properties both in vitro and in vivo. Exposure of SK-MES cells to DTT resulted in increased secretion of MIF and TRX and in downregulation of surface adhesion molecules (alpha-1 and alpha-3 integrins, and e-cadherin), with loss of adherence to the relevant substrates.

Conclusion: TRX and MIF contribution to tumor progression is mediated not only by intracellular enzymatic events but also by the generation of a reducing microenvironment sustained by non-protein thiols and

secreted TRX and MIF. This altered microenvironment concurs to increase proliferation and invasiveness of the tumor cells, mediated in part by altered expression of adhesion molecules. MIF and TRX expression in all the lung cancers examined was considerably higher in nests or group of the neoplastic cells, and in infiltrating inflammatory cells. Therefore, it is tempting to speculate that progression of a tumor toward a more aggressive/metastatic phenotype is mediated by those neoplastic cell nests that express more MIF and TRX or that are highly infiltrated by inflammatory cells producing these cytokines.

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HEAT SHOCK TREATMENT OF SARCOMA CELLS: KINETICS OF HEAT SHOCK PROTEIN EXPRESSION

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Objective: The overall benefit of hyperthermia in combination with chemotherapy has been demonstrated in several clinical trials enrolling patients with soft-tissue sarcoma and other solid tumors showing prolonged disease free survival and local tumor control with tolerable toxicity. However, molecular mechanisms underlying anti-tumoral effects of hyperthermia are not entirely understood. The in vivo studies in mice described mechanism of increased sensitivity of tumor cells against effector cells and enhanced ability of T cells to become activated upon heat exposure is currently a focus of further investigations. Here, we aimed at acquiring new insights into altered heat-induced gene expression over time in tumor cells by use of real time RT-PCR and oligonucleotide microarray technology.

Material and Methods: Human Ewing's sarcoma cells (RD-ES) were exposed to 41.8°C for 2 hours as well as 44°C for 30 minutes. Treatment modalities were chosen in order to achieve an equal survival fraction of cells. Control cells were incubated at 37°C. Kinetics of protein expression of hsp70, as a thermo sensitive marker for the heat shock response, was assessed by western blot analysis. The constitutively expressed hsc70 served as internal control. For four heat shock proteins kinetics of mRNA levels were monitored by real time RT-PCR. Subsequently cRNA of cells 6 hours after treatment with 44°C and control cells kept at 37°C was synthesized using total RNA of lysed cells and hybridised onto oligonucleotide microarrays (HG-U95Av2; Affymetrix) to assess expression levels of more than 3.000 genes.

Data: Using human Ewing's sarcoma cell line RD-ES we could show increased mRNA levels for hsp70, 110 and 40 despite unaltered hsp60 levels after treatment with two different temperatures. Hsp70 and 40 showed a peak expression as early as 1 hour after exposure to 41.8°C and 4 hours after 44°C treatment. Whereas maximal hsp110 RNA levels were detected 4 hours after 41,8°C and 6 hours after 44°C treatment. This was followed by a peak protein expression of hsp70 in 41°C-treated cells after 8 hours and 44°C-treated cells after 15 hours of recovery.

Next, for 44°C- and 37°C-treated cells the gene expression profile was analysed 6 hours after treatment (HG-U95Av2 microarrays, Affymetrix). In total, we identified 315 heat-induced transcripts and 101 down-regulated genes in the heat-treated versus untreated cell population. Data confirmed the increased RNA levels of heat shock proteins as assessed by real time RT-PCR.

Conclusion: Our data are giving new insights into heat shock-induced protein expression in sarcoma cells. Besides the relevance of the kinetics of heat shock protein expression in designing further clinical trials combining hyperthermia and chemotherapy, the up-regulated expression of transcriptional factors, IL-6, IL-2-inducible T cell kinase and minor histocompatibility antigen HA-1 might support the development of an anti-tumor response.

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EXPRESSION OF OCCLUDIN IN HUMAN HEPATOCELLULAR CARCINOMA

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Occludin has been suggested to be associated with carcinogenesis and metastasis of cancer. However, the relationship between the expression of occludin in human hepatocellular carcinoma(HCC) and the genesis and development of HCC has not been reported. In this study, we detected the expression pattern of occludin in HCC tissues. The expression and cellular distribution of occludin were detected by immunohistochemistry on 43 HCC tissues. The cellular structure of HCC was observed by hematoxylin-eosin (HE) staining. Immunohistochemistry indicated that occludin was mainly located in the cell junction area and cytoplasm. The positive rate of normal liver tissue was higher than that of tumor tissue ($P<0.01$). The ++~+++ grade intensities of non-tumor liver tissue were stronger than those of the tumor tissue ($P<0.005$). There was no significant difference between the normal tissue and the cancer with better differentiation (I,II grade) in 10 cases($P>0.05$),while the difference was significant between the normal liver tissue and the corresponding HCC tissue with worse differentiation (III,IV grade) in 33 cases($P<0.05$). In conclusions, the expression of occludin in hepatocellular carcinoma decreases significantly in both positive rate and intensity compared with that in normal tissue. The decreased expression of occludin was been well correlated with the differentiation grade of HCC, and may play an important role in the genesis and development of HCC.

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EXPRESSION AND THEIR CLINICAL SIGNIFICANCE OF OSTEOPONTIN AND OSTEOPONTIN MRNA IN LUNG CANCER

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Objective: To investigate the expression of osteopontin(OPN) in lung cancer tissues and explore the role of OPN in the development and progression of lung cancer.

Methods: The expression of OPN and its mRNA was detected by S-P immunohistochemistry and situ hybridization to evaluate their effects on 57 lung cancer tissues, 30 inflammatory pseudotumor and 20 pulmornary bullae.

Results: The positive rate of OPN protein expression in lung cancers and inflammatory pseudotumor were 57.9%, 16.7% , respectively. The positive rate of OPN mRNA expression in lung cancers and inflammatory pseudotumor were 71.9%, 30%, respectively. The expression in all 20 cases of pulmonary bullae was negative. Statistic analysis showed that there was a significant difference between lung cancer group and benign disease groups($p<0.05$). The rates of OPN protein and OPN mRNA expression in 38 lung cancer tissues with lymph node metastasis were 71.1%, 86.8% respectively, while in 19 lung cancers without lymph node metastasis were 31.6%, 42.1%, respectively($p<0.05$). The expression levels of OPN protein and OPN mRNA were closely associated with tumor metastasis. The positive rates of OPN protein and OPN mRNA expression in 47 non-small lung cancers were 68.1% and 78.7%, respectively, compared with small lung cancer(10% and 25%, respectively), there being statistically significant difference($p<0.05$). The expression of OPN protein had positive correlation with that of OPN mRNA in cancer tissue samples. The 57 patients were followed up for 12-30 months (mean 18 months). The OPN and OPN mRNA expression positive group(32 cases) had recurrence in 8 patients and distant metastasis in 13 patients, while the negative group (16 cases) had only 1 case with recurrence($P<0.05$). 12 patients died in OPN and OPN mRNA expression positive group but no patient died in negative group($P<0.05$).

Conclusion: OPN protein and its mRNA are overexpressed in lung cancers compared with non tumoral lung tissue samples. Their high expression reflects the progression of disease and association with poor prognosis and metastasis of lung cancer.

Key words: Osteopontin mRNA; Lung neoplasms/pathology; Neoplasm metastasis

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THORACIC AORTIC OCCLUSION AND REPERFUSION: EFFECTS OF SEVOFLURANE AND PROPOFOL ON EXPERIMENTAL ISCHEMIA-REPERFUSION INJURY

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Halogenated anesthetics protect the ischemic myocardium by preconditioning like effects. K_{ATP} channel activation and mitigated leukocyte-endothelium interactions have been shown to protect the heart during the reperfusion period. It is reasonable to assume that such potentially injury limiting actions are not restricted to the heart, but may be effective in other organs, as well. In this study, we compared sevoflurane versus propofol in a porcine model of severe abdominal-visceral ischemia induced by occlusion and reperfusion of the descending aorta. This experimental model can be considered to mimic the situation of proximal vascular surgery requiring temporary occlusion of the thoracic aorta. Material and Methods: Experiments had been approved by local animal care committees. In 18 pigs (25-30 Kg) a thoracotomie and a laparotomie were performed during midazolam and fentanyl anesthesia. Invasive hemodynamic monitoring including arterial, central venous, pulmonary artery and portal venous catheterisation was established. Aortic, pulmonary artery and portal vein ultrasonic flow-probes were used to monitor cardiac output and regional blood flow in the splanchnic system. After stabilisation, 9 animals were randomised to each group to receive either sevoflurane or propofol in a investigator blinded fashion. Lower body ischemia was induced by inflating a balloon-tipped catheter in the descending aorta. The aorta was occluded for 90min. After 120min of reperfusion the study anesthetic was stopped and midazolam re-established for additional 180 min. Fentanyl was given throughout the experiment. A goal directed therapy (intravenous fluid, vasopressors, buffer solution) to maintain hemodynamic stability was performed throughout the reperfusion period. Number and intensity of interventions, lactate concentrations and intestinal tissue specimens were obtained to assess injury severity. 10 additional animals without aortic occlusion served as time-controls. Results: Severe declamping shock occurred in both groups following reperfusion. Net fluid intake to achieve predefined cardiac filling pressures did not differ between groups. However, norepinephrine requirement decreased over time only in the sevoflurane group ($*p<0.05$). This was accompanied by cessation of vasopressor treatment in 4/9 animals, whereas all 9/9 animals in the propofol group were still vasopressor dependent at the end of the experiments. This was paralleled by significant reduced lactate concentrations over time with sevoflurane ($*p<0.05$). Scores of intestinal tissue injury severity and tissue granulocyte count were not different. Conclusion: Sevoflurane is superior to propofol in reducing the consequences of lower body ischemia and reperfusion injury. This evidences from both a better recovery of hemodynamic stability and by reduced lactate concentrations.

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ATTENUATION OF MYOCARDIAL ISCHEMIA REPERFUSION INJURY BY ECTO-APYRASE (CD39)

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Objective: Previous studies have demonstrated that extracellular adenosine signaling contributes to cardioprotection from ischemia. As extracellular nucleotide levels are increased during myocardial ischemia and represent the main source for extracellular adenosine generation, we hypothesized that enzymatic control of ATP/ADP phosphohydrolysis via ecto-apyrase (E-NTPDase 1, CD39) contributes to cardioprotection. Therefore, we pursued a functional role of CD39 in cardioprotection using a previously described model of murine myocardial ischemia and in situ preconditioning.

Material and Methods: Following left parasternal thoracotomy the left coronary artery (LCA) was visually identified and an 8.0 nylon suture was placed around the vessel. Atraumatic LCA occlusion was performed using our previously described hanging weight system model. For ischemic preconditioning (IP) mice were subjected to 4 cycles (5 min occlusion, 5 min perfusion) of IP followed by an ischemia time of 60 min and reperfusion time of 120 min. Infarct sizes were determined by calculating the percentage of myocardial infarction compared to the area at risk (AAR) using a double staining technique with Evan's blue and triphenyltetrazolium chloride. **Data**: Transcriptional studies of ischemic preconditioned myocardial tissues demonstrated prominent induction of CD39 transcript and protein. Histological analysis of preconditioned myocardium localized CD39-induction to endothelia and myocytes. Pharmacological studies using a newly designed NTPDase inhibitor (polyoxometalate, POM) revealed a functional role of CD39 in cardioprotection, as infarct sizes were increased and cardioprotection by IP was completely abolished. Similarly, $cd39^{-/-}$ mice showed significantly larger infarct sizes with ischemia and no cardioprotection by IP. Infusion of the metabolic product AMP or treatment with apyrase recovered cardioprotection by IP in $cd39^{-/-}$ mice. Moreover, apyrase treatment of wildtype mice resulted in over 50% reduction of infarct size.

Conclusions: Taken together, these studies reveal a critical role of extracellular nucleotide phosphohydrolysis via CD39 in cardioprotection and suggest apyrase-treatment as therapeutic approach in myocardial ischemia.

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PROTECTIVE ROLE OF ECTO-5′-NUCLEOTIDASE (CD73) IN RENAL ISCHEMIA

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Objective: Acute renal failure from ischemia significantly contributes to cardiovascular morbidity and mortality. Extracellular adenosine has been implicated as an anti-inflammatory metabolite particularly during conditions of limited oxygen availability (e.g. ischemia).

Material and Methods: We used age and gender matched mice to perform a right nephrectomy, followed by temporary ligation of the left renal artery. We performed four IP cycles (4 min ischemia, 4 min reperfusion) prior to 30 minutes of ischemia. As first step, renal CD73 mRNA content and activity was measured. In addition, mice with and without IP prior to ischemia were placed in metabolic cages for 24 hours and serum creatinine was measured. Finally, the left kidney was isolated for histological analysis and measurement of myeloperoxidase activity (MPO).

Data: Since ecto-5′-nucleotidase (CD73) is rate limiting for extracellular adenosine generation, we examined the contribution of CD73-dependent adenosine production to ischemic preconditioning (IP) of the kidneys. Following our initial observation that murine CD73 transcript, protein and function are induced by renal IP, we next studied its role in IP-mediated kidney protection. In fact, increases in renal adenosine concentration with IP are attenuated in $cd73^{-/-}$-mice. Moreover, pharmacological inhibition of CD73 or its targeted gene deletion abolished renal protection by IP as measured by clearance studies, plasma electrolytes and renal tubular destruction. Moreover, reconstitution of $cd73^{-/-}$-mice with soluble 5′-nucleotidase resulted in complete restoration of renal protection by IP. Finally, renal injury following ischemia was attenuated by i.p. treatment of wildtype mice with soluble 5′-nucleotidase to a similar degree as by IP.

Conclusion: Taken together, these data reveal what we believe to be a previously unrecognized role of CD73 in renal protection from ischemia and suggest treatment with soluble 5′-nucleotidase as a novel therapeutic approach in the treatment of renal diseases precipitated by limited oxygen availability.

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GENTAMICIN AND ANTI-IL-10 ANTI-BODIES PROLONG SURVIVAL IN A CHRONIC INTRA-ABDOMINIAL INFECTION MODEL

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We previously developed an infection model by intraperitoneal (IP) injection of **Klebsiella pneumoniae**. Its high mortality prompted studying the effect of gentamicin on the disease course and investigation into the role of interleukin-10 (IL-10) in the survival of our chronic peritonitis model. Methods: Infection was induced in Balb/c mice by IP inoculation of 103 colony forming units (CFU) Kpn serotype 2. Mice received either gentamicin (5mg/kg/d) or saline twice daily. Survival was determined over 14 days. From the liver homogenate, myeloperoxidase was used as a measure of neutrophil accumulation. Bacterial counts in blood, liver homogenate, and peritoneal lavage were determined by culture. The role of IL-10 was investigated in gentamicin treated mice by administering either anti-IL-10 antibody or control IgG. IL-10 levels were measured in the liver homogenates, blood, and peritoneal lavage using enzyme-linked immunosorbent assay. Results: Significant survival benefit was seen in the gentamicin group by 48 hours ($p<0.05$), 50% of gentamicin treated mice survived two weeks while none of the saline control mice survived past the third day. Gentamicin did not clear peritoneal bacteria and levels remained at inoculum concentration (103 CFU), but elicited no significant neutrophil influx. While there was a progressive increase in total lavage leukocytes over time, the gentamicin group showed no significant neutrophil influx in the peritoneum until bacterial counts were over 105 CFU. Mice treated with anti-IL-10 antibody showed increased survival over mice treated with control IgG antibody. Conclusions: Despite persistent intraabdominal infection, gentamicin and anti-IL-10 treatment significantly prolonged survival in this uniformly lethal model. Currently, we are investigating the intracellular mechanisms of IL-10 and its possible effect on the mechanism of neutrophil influx to the site of infection.

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EXPRESSION PROFILING OF HEAT SHOCK PROTEINS IN A PRIMATE NON-HUMAN BABOON MODEL OF HEATSTROKE

Mohammed Dehbi, Maqbool Ahmed, Aaron Kwaasi, Arslan Loualich, Mohammed Chishti

Background and Objectives: Classic heatstroke is a leading cause of mortality and neurologic morbidity during heat waves. However, the mechanism of tissue injury and the subsequent death are not known and therefore, no specific therapy is available. Induction of Hsp-72 has been proposed as a marker of organ susceptibility to heat injury. Moreover, a circulating form of Hsp-72 has been documented in human heatstroke and was associated with outcome. Using a baboon model of heatstroke, we examined the expression profile of Hsp-60, Hsp-70 and Hsp-72 in various organs. We also investigated whether the proteins are released in the circulation and if so, the possible relationship with outcome.

Methods: Anesthetized Papio hamadyras were randomly assigned to control or heatstroke group. Heatstroke was induced by placing the animals in an incubator set at 44 ± 1.5°C, until the rectal temperature reached 42.5°C (moderate heatstroke group, n=3) or until systolic blood pressure fell below 90 mm Hg, which occurred at 43.3 ± 0.1°C (severe heatstroke group, n=6) and then moved to room temperature for recovery. The sham-heated group (n=3) were handled in an identical manner except that the incubator was not warmed and acted as control. Plasma Hsp-72 was assayed by ELISA at baseline (pre-heat stress), onset of HS and during cooling and recovery using ELISA kit (Stressgen). Tissue samples were obtained at immediate autopsy (non-survivors) and euthanasia at 72-h (survivors). Expression of Hsp-60, the constitutive Hsp-70 and the inducible Hsp-72 was monitored in various organs by Western blot using specific antibodies (Stressgen).

Results: Only 3 baboons survived severe heatstroke. Western blotting analyses indicated a marked increase of Hsp-72 expression in all organs of heatstroke animals, but more in severe than moderate heatstroke. Hsp-72 expression appeared to be tissue-specific with higher level in heart and lung compared to liver and kidney. In contrast, Hsp-60 and Hsp-70 showed no significant change between the 3 groups. ELISA tests performed on plasma samples indicated that, compared to baseline (4±3 ng/ml), a significant increase of Hsp-72 release was noted at onset of severe heatstroke (31±0.5 ng/ml), peaked at T+18h (141±11 ng/ml), and declined at T+26 (100±1 ng/ml) and 70h (52±11 ng/ml). Circulating Hsp-72 was not detected in moderate and sham animals. Non-survivors had a higher level of circulating Hsp-72, but this was not statistically significant. The fact that under the same conditions, Hsp-60 and Hsp-90 were not detected in the circulation suggests that the observed release was specific to Hsp-72 rather than a leakage from tissues.

Conclusion: This study shows a differential expression of Hsp-72 in various organs subjected to severe heatstroke and this might be indicative of organ susceptibility to early heat stress-induced injury. It also demonstrates that circulating Hsp-72 is an early and long lasting marker of severity of heatstroke.

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ADHESION MOLECULES IN PANCREATITIS-ASSOCIATED LUNG INJURY

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Objective: Acute lung injury or acute respiratory distress syndrome is a common early complication of acute pancreatitis (AP) that leads to significant complications or death of patients with severe forms. Adhesion molecules and oxidative stress are important factors in the lung injury associated with severe acute pancreatitis.

Materials and methods: In experimental model of acute pancreatitis we studied expression of P- and E-selectin molecules, ICAM-1, xanthine oxidase (XOD) and myeloperoxidase activity, neutrophil sequestration in lung tissue.

Results: The increased expression of P-selectin appears to be triggered in model of pancreatitis by a mechanism dependent on free radicals generated by circulating XOD released into the bloodstream by the damaged pancreas. The upregulation of P-selectin in the lung is dependent on free radicals generated by xanthine oxidase. P-selectin expression in the lung was seen with peaks at 24 and 48 hours; E-selectin expression peaked at 48 hours. Histological scoring of lung tissue demonstrated progressive injury with the increased infiltration of CD18-positive cells and increased myeloperoxidase activity in the lung. Constitutively expressed ICAM-1 is also involved in the process of cell infiltration into the lung.

Conclusion: These changes suggest that pulmonary injury in AP is mediated by the upregulation of adhesion molecules.

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CPG-DNA ACTIVATES TOLL-LIKE RECEPTOR 9 AND CAUSES LUNG INFLAMMATION

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Objective: Bacterial DNA (CpG-DNA) initiates an innate immune response mediated by the pattern recognition receptor Toll-like Receptor 9 (TLR9). This leads in particular to the expression of proinflammatory mediators. Previously, the induction of proinflammatory mediators has been linked to the development of acute lung injury. This is of major interest because TLR9 is expressed in human and murine pulmonary tissue. Therefore, the hypothesis was tested whether CpG-DNA administration induces an inflammatory response in the lung via TLR9 in vivo.

Materials and Methods: Wild-type (WT) and TLR9-deficient (TLR9-D) mice were handled according to the principles of laboratory animal care. Experimental procedures were approved by the German government ethical and research boards. Mice received 1 nmol/g CpG-DNA (Thioat 1668; TibMolBiol, Berlin, Germany) intraperitoneally (i.p.). The activation of NFkB in the lung was determined by electromobility shift assay (EMSA) The pulmonary cytokine mRNA and protein expression was analyzed by RNAse protection assay and ELISA. Cytokine plasma levels were determined using the microsphere array technique (Luminex) Content of lung myeloperoxidase (MPO) activity, an indicator of polymorphonuclear cell (PMNs) accumulation, was documented following application of CpG-DNA. Finally, subpopulations of leukocytes in the BAL fluid were determined by hemocytometer.

Data: WT mice developed shock-like symptoms 2h after CpG-DNA challenge while TLR9-D mice were not affected. CpG-DNA treatment led to a time-dependent activation of pulmonary NFkB in WT mice. In contrast, this effect was not detectable in TLR9-D mice. Furthermore, the application of CpG-DNA led to a robust increase of pulmonary cytokine mRNA expression of tumor necrosis factor (TNF-a), interleukin-1 (IL-1β) and interleukin-6 (IL-6). In addition, a significant increase in cytokine protein production was observed 1h after injection of CpG-DNA with a peak protein expression at 2h. To exclude extrapulmonary effects of CpG-DNA on the lung, WT- and TLR9-D mice received CpG-DNA intratracheally. This route of administration also resulted in lung inflammation, e.g. demonstrated by a significant cytokine response in WT animals. 2 hrs after CpG-DNA challenge, pulmonary TNF-a tissue levels were significantly increased in WT mice (7.0±0.6 pg/mg tissue) when compared to TLR9-D animals (0.6±0.2 pg/mg tissue; $p < 0.05$). Also IL-1b levels were significantly raised in WT mice (62±12 pg/mg tissue) when compared to TLR9-D animals (16±1 pg/mg tissue; $p < 0.05$). WT animals showed significantly elevated plasma levels of TNF-a, IL-1b and IL-6 after 2h as well. In contrast, the inflammatory response was abolished in the TLR9-D mice after CpG-DNA challenge. Increased pulmonary content of lung myeloperoxidase (MPO) was documented in WT mice following application of CpG-DNA. Bronchoalveolar Lavage (BAL) revealed that CpG-DNA stimulation significantly increased total cell number as well as neutrophil count in WT animals. BALs demonstrated a significant increase in total cell number as well as the number of recruited neutrophils after CpG-stimulation in WT animals, which were abolished in TLR9-D mice.

Conclusion: Taken together, this study suggests that bacterial CpG-DNA causes lung inflammation via TLR9.

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IDENTIFICATION OF ECTO-NUCLEOTIDASES CD39 AND CD73 IN INNATE PROTECTION DURING ACUTE LUNG INJURY

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Acute lung injury (ALI), such as occurs with mechanical ventilation, contributes to morbidity and mortality of critical illness. Nonetheless, in many instances, ALI resolves spontaneously through unknown mechanisms. Here, we used ventilator-induced lung injury as model to identify endogenous mechanisms of lung protection. Initial in vitro studies revealed that supernatants from stretch-induced injury contained a stable factor which diminished endothelial leakage. This factor was subsequently identified as adenosine. Further studies in vivo demonstrated prominent increases in pulmonary adenosine levels with ALI. Since ecto-apyrase (CD39) and ecto-5′-nucleotidase (CD73) are rate limiting for extracellular adenosine generation, we examined their contribution to ALI. In fact, both CD39 and CD73 are induced by mechanical ventilation. Moreover, we observed pressure- and time-dependent increases in pulmonary edema and inflammation in ventilated cd39$^{-/-}$-mice. Similarly, pharmacological inhibition or targeted gene-deletion of cd73 was associated with increased symptom severity of ventilator-induced ALI. Reconstitution of cd39$^{-/-}$/cd73$^{-/-}$-mice with soluble apyrase/5′-nucleotidase reversed such increases. In addition, ALI was significantly attenuated and survival improved after i.p.-treatment of wildtype-mice with soluble apyrase or 5′-nucleotidase. Taken together, these data reveal a previously unrecognized role for CD39/73 in lung protection and suggest treatment with their soluble compounds as novel therapeutic strategy for non-infectious ALI.

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LEGIONELLA PNEUMOPHILA INDUCED IFNBETA IN LUNG EPITHELIAL CELLS VIA IPS-1 AND IRF3 WHICH ALSO CONTROL BACTERIAL REPLICATION

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Objectives: Legionella pneumophila, a Gram-negative facultative intracellular bacterium, causes severe pneumonia (Legionnaires disease). Type I IFNs were so far associated with antiviral immunity, but recent studies also indicated a role of these cytokines in immune responses against (intracellular) bacteria.

Material and Methods: A549 cells were infected with wild-type L. pneumophila 130b and JR32, L.p ΔdotA and L.p. ΔflaA. RNAi, ELISA, RT-PCR, Q-PCR, Immunoblot, ChIP and bacterial replication assays were carried out.

Data: We show that wild-type L. pneumophila and flagellin-deficient Legionella, but not L. pneumophila lacking a functional type IV secretion system Dot/Icm, or heat-inactivated Legionella induced IFNβ production in human lung epithelial cells. We found that IFN-regulated factor (IRF)-3 and NF-kB-p65 translocated into the nucleus and bound to the IFNβ gene enhancer after L. pneumophila infection of lung epithelial cells. RNA interference demonstrated that in addition to IRF3, the caspase recruitment domain (CARD)-containing adapter molecule interferon-beta promoter stimulator 1 (IPS-1) is crucial for L. pneumophila-induced IFNβ expression, whereas other CARD-possessing molecules such as retinoic-acid-inducible protein I (RIG-I), melanoma-differentiation-associated gene 5 (MDA5), nucleotide-binding oligomerization domain protein 27 (Nod27) and apoptosis-associated speck-like protein containing a CARD (ASC) seemed not to be involved. Finally, bacterial multiplication assays in siRNA-treated cells indicated that IPS-1, IRF3 and IFNβ were essential for the control of intracellular replication of L. pneumophila in lung epithelial cells.

Conclusion: We demonstrated a critical role of IPS-1, IRF3 and IFNb in Legionella infection of lung epithelium.

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THE BLOOD SOLUBLE E-SELECTIN INCREASE IN ACUTE LUNG INJURY (ALI) AND TREATMENT OF THE SIVELESTAT SODIUM,A SELECTIVE NEUTROPHIL ELASTASE INHIBITOR DECREASE THE BLOOD SOLUBLE E-SELECTIN

Takashi Kobayashi, Takao Nakagawa, Yukihiro Soga, Hiroyasu Suga, Naohiro Terada, Kenji Okajima

Introduction: Neutrophils activated by tumor necrosis factor (TNF) injure the endothelial cells of pulmonary vascular vessels and cause acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). ARDS is considered to change to high incidences of multi-organ failure or disseminated intravascular coagulation syndrome. The blood soluble E-selectin concentration is considered to reflect the degree of intravascular endothelial cell disorder caused by TNF. If the degree of intravascular endothelial cell disorder can be grasped early by measurement of this concentration, it is very useful for clinical practice. Therefore, this institution studied soluble E-selectin in the blood of patients with SIRS by comparing the group showing high blood soluble E-selectin concentration at initial examination with the group showing normal concentrations. As a result, the incidences of respiratory failure, coagulation abnormality and renal failure and mortality were significantly high in the former group. Based on these results, we examined the effectiveness of blood soluble E-selectin measurement as an index of transfer from ALI to ARDS and the therapeutic results of administration of sivelestat sodium, a selective neutrophil elastase inhibitor.

Subjects: Among the ALI patients who were taken to the Critical Care Center of this hospital and showed the PaO2/Fio2 of 200 to 300 in arterial blood gas analysis, the patients showing high values of blood soluble E-selectin (above 29.74) were studied.

Method: The subjects were divided into two groups: a sivelestat sodium treated group and an untreated group. For each group, the blood soluble E-selectin concentration and the arterial blood gas were determined.

Results: Comparing before trea and after a week of treatment, the patients treated with sivelestat sodium showed a significant decrease in soluble E-selectin concentration but the patients that were not treated with the sivelestat sodium did not show a significant change. The P/F ratio showed a significant improvement in the patients treated, but the untreated patients did not show a significant change.

Conclusion: Blood Soluble E-selectin concentration is an effective therapeutic parameter in ALI patients. Early administration of sivelestat sodium effectively improved P/F ratio, and significantly decreased the soluble E-selectin concentration in the ALI patients.

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SMALL SPUTUM MACROPHAGES IN INFLAMMATORY DISEASES

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We have characterized macrophages in sputum samples from patients with inflammatory airway disease in order to determine their possible contribution to the inflammatory process. In flow cytometry we can distinguish macrophages from granulocytes based on light scatter properties and on marker molecules like CD14, CD68 and CD66b. With this approach we can define small and large macrophages in sputum. The small macrophages are rare in healthy individuals (<20%), they are increased in smokers and their percentage increases to > 50% of all macrophages in patients with COPD. By contrast patients with asthma have no or only a slight increase of the small macrophages. The expanded small macrophages show high level expression of CD14 and DR while CD68 is low. With respect to cytokine expression, production of TNF protein is increased and purified samples from patients with high percentages of small macrophages show high constitutive TNF mRNA. These data suggest that small macrophages in the inflamed lung are highly active and may contribute to the inflammatory process.

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HUMAN ATHEROMATIC FEMORAL ARTERY PLAQUES TRANSPLANTED TO SCID MICE ATTRACT MACROPHAGES

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Introduction. The etiology of atherosclerosis remains obscure. There are convincing scientific data that atherosclerosis is an inflammatory process. According to the present state of knowledge, the etiological factors are, beside of oxidized LDL, bacterial and viral antigens of Chlamydiae, Helicobacter, CMV, Herpes and others. We showed that arterial walls including adventitia of femoral and popliteal arteries contain, in contrast to carotid arteries, bacterial DNA of other than the above listed microbes. The most common bacteria turned to be Staphylococcus epidermidis. The question arises whether the detected microorganisms present in arterial plaques evoke immune reaction. One of the functional tests would be migration of macrophages to the infected plaques. Aim. The aim of study was to investigate recruitment of macrophages to atheromatous plaque harvested from femoral and carotid arteries and transplanted to scid mice.

Methods. Ten femoral and another ten carotid atheromatous plaques were transplanted subcutaneously onto the dorsum of scid mice. DNA was extracted from fragments of each plaque. PCR amplification was performed with primers for gene fragment coding bacterial 16s RNA, and for major outer membrane protein (ompA) of CP with positive and negative controls. Products were separated by PAGE electrophoresis and silver stained. Routine bacteriological cultures of specimens were also carried out. Seven days after transplantation plaques were removed with the surrounding tissue. Controls were implants of human saphenous vein. Specimens were stained for evaluation of recruited populations with mAbs against human HLA I, HLA DR and CD68, and mouse MHC I, PCNA, macrophages and neutrophils.

Results. Microbial DNA (16sRNA) was detected in 35% of femoral and 29%% of carotid arteries and CP in 69% and 29%, respectively ($p<0.001$). In 73% of femoral plaques bacterial isolates were found. Implanted fragments of femoral and popliteal arteries were surrounded by dense accumulations of mouse macrophages and neutrophiles. Some of them were penetrating plaques. These cells were MHC I and PCNA+ (dividing). Very few of them were HLA DR+ (human). Infiltrates around carotid plaques were less intensive. Venous implants evoked only minor reaction.

Conclusions. Human atheromatous plaques evoke major reaction of mouse macrophages.. Bacteria may, beside of oxLDL, be one of attractants of macrophages to atheromatous arteries.

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INTERACTION OF VASCULAR SMOOTH MUSCLE AND MONONUCLEAR CELLS IN COCULTURES IN-CREASES INTERLEUKIN-6 PRODUCTION

Harald Loppnow, Li Chen, Susanna Pippig, Bei Bei Li, Andreas Ludwig, Michael Buerke

Human smooth muscle cells (SMC) and mononuclear cells (MNC) are involved in the regulation of inflammatory responses during development of atherosclerosis by expression of cytokines. Interleukin-6 (IL-6) may play an important role in these processes by regulation of SMC and monocyte functions. SMC and MNC can produce IL-6 upon stimulation with lipopolysaccharide (LPS) or cytokines such as interleukin-1 (IL-1). In the vessel wall leukocytes and vascular smooth muscle cells may interact and this interaction may modify the cytokin production. Thus, the expression of IL-6 in cocultures of SMC and MNC was investigated. Compared to separately cultured SMC and MNC the cocultures contained up to 10.7 fold more IL-6, i.e. IL-6 was produced synergistically in the coculture system. The mechanism of this synergism was investigated. IL-1 receptor antagonist (IL-1ra) inhibited 49-100% of the IL-6 production in unstimulated and 18-100% in LPS-stimulated cocultures. Monospecific antibodies indicated that both IL-1α and IL-1β contributed to the system. Experiments using cell culture inserts which interrupt direct cell contact and allow only soluble factors to mediate interaction as well as experiments with fixed MNC, permit-ting cell contact, showed that soluble factors are responsible for the observed synergism. Inhibition experiments with IL-6 and TNF-α inhibitors showed only little reduction of the synergism. In conclusion, IL-1β may be a major activator of the synergistic IL-6 production in this system, whereas other mediators, such as IL-6 or TNF may contribute only to a low degree. These data indicate that interaction of leukocytes and vascular cells may contribute to regulation of local inflammatory processes during atherogenesis.

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THE EFFECT(S) OF FREE RADICAL SCAVENGERS ON PERITONIAL SEPSIS-INDUCED LUNG INJURY IN RAT

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Background: Excessive production of reactive oxygen species (ROS) has been associated with the development of the respiratory distress syndrome (ARDS) and organ failure through direct tissue injury and activation of genes integral to the inflammatory response. Plasma concentration of 3-nitrotyrosine (NT) which is produced by NO-mediated reaction elevate in patients of SIRS, ARDS and sepsis. 8-oxo-2′-deoxyguanosine (8-oxo-dG) has been implicated in a major type of oxidative damage in nucleic acid. MTH-1, an oxidized purine nucleoside triphosphatase, efficiently hydrolyzes oxidized dGTP, dATP and ATP such as 8-oxo-dG in nucleotide pools, avoiding their incorporation into DNA or RNA. Purpose: To assess the efficacy of free radical scavengers and elucidate their mechanisms of repair on nucleic acid in cytoplasm during sepsis-induced lung injury in rat by cecal ligation and puncture (CLP).

Method: Sepsis was induced by CLP in adult male Spraque-Dawley rat (n=25) which were anesthetized by ketamine. Rat subjected to CLP were administrated intraperitoneally with or without free radical scavengers (A: Sham, no laparotomy, n=5, B: CLP only, n=10, C: CLP/PEG-CAT;H_2O_2 scavenger, n=5, D: CLP /DMSO; OH radical scavenger, n=5) after 5 hours. Lung tissues examined were obtained from subjects which were sacrified after 24 hours . The content of each NT, 8-oxo-dG which was estimated by HPLC, and myeloperoxidase(MPO) were measured as indices of lung injury. mRNA of MTH-1contents on the gene encoding the 8-oxo-dG were quantified by RT-PCR.

Results: All indices(MPO ; 8-oxo-dG ; NT) of lung injury increased in CLP 24 only group compared with control group level (6.08±0,70 ; 1.71±0.20 ; 2.26±0.50 vs 2.76±0.49 ; 1.06±0.83 ; 0.33±0.14)($p<0.05$). However their all indices significantly decreased, furthermore 8-oxo-dG normalized in CLP/PEG-CAT group.(3.94±0.25 ; 1.07±0.62 ; 1.00±1.08 vs 6.08±0,70 ; 1.71±0.20 ; 2.26±0.50)($p<0.05$). DMSO had no influence on all indices of lung injury (5.23±1.54 ; 2.62±3.67 ; 1.98±0.95 vs 6.08±0,70 ; 1.71±0.20 ; 2.26±0.50). The mRNA of MTH-1 level of CLP/PEG-CAT group subjects were significantly higher than control group subjects ($p<0.05$). In histological examination, pulmonary edema and granular precipitate within alveolar spaces have improved in CLP/PEG-CAT group subjects compared with CLP group subjects.

Conclusion: H_2O_2 radical may be responsible for sepsis-induce lung injury by CLP. PEG-CAT can possibly attenuate the lung injury by not inhibiting the efficient sanitization of MTH-1.

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EX-VIVO DETERMINATION OF MITOCHONDRIAL FUNCTION IN LIVERS COLLECTED SIMULTANEOUSLY FROM A NUMBER OF ANIMALS

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Mitochondria are sensitive to storage and, therefore, their function should be measured within a short time after

organ withdrawal. This reduces the number of samples, which can be withdrawn simultaneously. One critical point in this area is the extrapolation of data obtained in mitochondrial suspension to an in vivo situation. The aim of this study was to develop an assay to determine mitochondrial function in livers collected simultaneously from a number of animals, as it usually takes place in many experimental protocols. Respiratory parameters (respiratory control and ADP/O ratio) were determined in liver homogenates and in isolated liver mitochondria using high-resolution respirometry. Mitochondria and homogenates were prepared from livers kept at 0°C for 72 hours in Custodiol. This experiment has shown that mitochondrial function measured in isolated mitochondria was impaired significantly earlier than those measured in tissue homogenate. Respiratory parameters in homogenate did not change after liver was kept for 12 hours at 0°C. For this analysis we have tested two kinds of respirometers, Oroboros and WPI. The advantage of WPI is the possibility to use small sample volumes. The advantages of Oroboros are two chambers operating simultaneously, high sensitivity and stability of measurements. Our data show that one can collect up to 10 livers simultaneously and estimate precisely their mitochondrial function one after the other in liver homogenates freshly prepared from livers kept on ice.

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CEREBROSPINAL FLUID INTERLEUKIN-1 RECEPTOR ANTAGONIST FOLLOWING SEVERE TRAUMATIC BRAIN INJURY

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Objective: Severe traumatic brain injury (TBI) induces inflammatory reactions with consequent secondary brain damage. As recently shown, a major issue of this inflammation is astrocytic and microglial activation and production of the cytokine interleukin-1 (IL-1). The physiologic inhibitor of IL-1 and its proinflammatory leukocyte attracting impact is the IL-1 receptor antagonist (IL-1ra), mainly synthesized by CD14+monocytes (MØ). Recently a significant correlation between IL1-ra levels and clinical outcome of patients suffering from subarachnoid haemorrhage (SAH) was reported. However the role of IL1-ra in patients suffering from TBI still remains uncharacterized. Therefore, the aim of our study was to analyze the intrathecal dynamics of IL-1ra in respect to CD14+ MØ in the early phase after TBI.

Patients and Methods: We enrolled 15 patients, suffering from severe TBI (initial GCS<8pts) and an intracranial lesion. After placement of an external ventricular drainage (45±30min after trauma) as well as 12, 24, 48 and 72hrs post trauma CSF samples were drawn. ICP was monitored at every sampling point. CSF of 8 healthy patients conceiving spinal anaesthesia, served as control group. For analysis of CD14+ MØ, CSF was stained using anti-CD14-TC (Caltag, Hamburg) and quantified using flow cytometry. Data are given as percentage value relating to the total CSF cell population. For detection of IL-1ra we used an ELISA technique (R&D Systems, Minneapolis, USA). Statistics were performed using the ANOVA followed by SNK-test vs. Baseline, and Mann-Whitney-U vs. control.

Results: IL-1ra in the CSF was already significantly increased initially after ICP-catheter placement with 540±120pg/ml in respect to the control group 44.5±6.9pg/ml (Mean±SEM). No significant increase of IL-1ra over time was observed within the first 72hrs post trauma. CD14+ MØ were continuously elevated over 48hrs with a significant increase of 5.6±0.9% (Mean±SEM) 72hrs post trauma in comparison to values at admission with 1.2±0.5% (Mean±SEM) as well as to the control group values 1±0.8 (Mean±SEM).

Conclusion: In this study we were able to demonstrate significantly increased levels of Interleukin-1 receptor antagonist (IL1-ra) in CSF of patients suffering from TBI directly after admission. In contrast, an increase of IL1-ra in patients with SAH is described starting the fourth day after haemorrhage. However, we also showed a significant rise of CD14+ MØ, the main source of IL1-ra 72hrs post trauma. Further studies need to be done to elucidate possible intrathecal sources of IL1-ra.

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BETA-2-DEFENSIN MRNA INDUCIBILITY IS DECREASED IN PATIENTS WITH SEVERE SEPSIS

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Objective: Defensins are part of the innate immune system. They show antimicrobial activities against bacteria, funghi and coated virus. Beta defensin gene expression was detected in epithelial and peripheral white blood cells [1]. In addition to antimicrobial properties immunomodulating activities have been detected [2]. The meaning of the inducible beta-2 defensin (hBD2) in patients with severe sepsis is unknown. This investigation quantified the ex vivo mRNA inducibility and protein plasma levels in patients with severe sepsis, critically ill non-septic patients and healthy controls.

Material and Methods: HBD2 protein levels were quantified in 16 patients with severe sepsis, 9 critically ill non-septic patients and 9 healthy controls by using ELISA technique. Inducible mRNA levels were quantified ex vivo by real time PCR.

Data: HBD2 plasma levels in septic patients were significantly higher compared to both other groups

($p<0.05$, Kruskal-Wallis Test mit Dunn's multiple comparison Test). MRNA inducibility was decreased in septic patients compared to both other groups ($p<0.05$, Kruskal-Wallis Test mit Dunn's multiple comparison Test).

Conclusion: HBD2 is involved in the systemic immune reaction and peripheral white blood cells are one source of circulating hBD2 protein molecules.

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THE DYNAMIC ANALYSIS OF CREATINE KINASE AND C-REACTIVE PROTEIN IN THE SERUM OF HYPERLIPOIDEMIA MODEL RABBIT

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Objective: To analyze the variability of creatine kinase and c-reactive protein in the serum of hyperlipoidemia model rabbit.

Methods: To establish the model of hyperlipoidemia rabbit.The biochemistry quotas such as Tch, TG, CK, CRP were assayed in the serum of hyperlipoidemia model rabbit of each stage (7,14,17,21,25,28 day feeding with high cholesterol).

Results: The hyperlipoidemia rabbit model was established successfully. The concentration of TG and Tch increased markedly, and there was significantly statistical difference compared with contrast group ($P<0.05$).The activity of CK and the concentration of CRP increased markedly with the development of hyperlipoidemia ($P<0.05$).

Conclusion: The increase of activity of CK and the concentration of CRP in the serum of hyperlipoidemia model rabbit may be well correlated with the development.

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PREDICTION OF SURGERY OUTCOME IN ELDERLY PATIENTS: USE OF INFLAMMATION MARKERS

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Objective: To determine if preoperative Interleukin 6 (IL-6), C Reactive Protein (CRP) and Alpha I-acid glycoprotein (AGP), are a prognostic indicators of outcome (morbidity and mortality) in ederly patients undergoing general surgery.

Material and Methods: We have reviewed 142 patients older than 80 years, included in a prospective observational study who underwent general surgery operations. The median age was 83 years old, and 72 patients were men.

Elective surgery was performed in 115 (80%) patients, and emergency surgery in 27 (20%).

Blood samples for IL-6, CRP and AGP were taken before surgery.

Complications and Mortality after surgery were collected.

Statistical analysis: Descriptive Statistis were performed and Non Parametricts Tests were used to compare the groups (complicated and non complicated patients).

Data: 9 (6.4%) patients died and 38 (27%) suffered a postoperative complication.

Conclusions: 1. Preoperative Interleukin - 6 and C Reactive Protein levels my be an excellent surgery outcome markers in elderly patients. 2. Alpha I- Acid Glycoprotein is not useful to discriminate morbimortality after surgery in patients older than 80 years.

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Table

	Mean	Mean	
Variables	Non Complicated Group	Complicated Group	
IL-6	136 pg/ml	658 pg/ml	$p<0.01$
CRP	47 mg/l	114 mg/l	$p<0.01$
AGP	112 mg/dl	141mg/dl	$p=0.26$

A 495

ELEVATED CRP LEVELS ARE ASSOCIATED WITH SEVERE SKIN AND SKIN STRUCTURE INFECTIONS: EXPLORATORY ANALYSIS IN A DOUBLE-BLIND, RANDOMIZED TRIAL COMPARING CEFTOBIPROLE WITH VANCOMYCIN FOR TREATMENT OF SKIN INFECTIONS

Richard Strauss, Gary Noel

Objective: Although elevated levels of C-reactive protein (CRP) have been associated with sepsis syndrome in subjects with infections, the association of CRP levels and severity of skin infection has not been established. In a recently completed large (n=784), double blind, comparative trial assessing the safety and efficacy of ceftobiprole (BPR) versus vancomycin as treatment for complicated skin and skin structure infections (cSSSI), CRP levels were analyzed in relationship to other markers of severe infection.

Materials and Methods: Subjects with cSSSI were randomized to each treatment arm and stratified based on type of cSSSI (cellulitis, wound, abscess) upon enrollment. CRP levels were analyzed and associated with 1) depth of infection, 2) WBC, 3) body temperature, 4) positive blood culture (present, absent), 5) pathogen (methicillin-resistant Staphylococcus aureus [MRSA] vs methicillin-susceptible S. aureus [MSSA]) and 5) clinical cure (cure vs failure, assessed at the test of cure visit, 7 to 14 days following end of therapy).

Data: Significantly elevated levels of CRP (CRP>50 mg/dl) were commonly associated with signs of the most severe infections. Patients with CRP>50 mg/dl were more than twice as likely to have a positive blood culture compared to subjects with CRP<50 mg/dl [OR: 2.4 (1.1 - 5.1)]. Staphylococcus aureus was identified as the primary pathogen in approximately 75% of microbiologically evaluable subjects and MRSA accounted for approximately one-third of all S. aureus isolates. CRP levels were not related to the presence of MRSA ($p = 0.8$).

Conclusion: CRP levels greater than 50 mg/dl were associated with deeper infections, higher WBC counts, higher temperatures, and increased likelihood of obtaining a positive blood culture. Clinical cure of subjects with cSSSI and elevated CRP was greater than 92% in subjects treated with ceftobiprole.

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A 496

KINETICS OF COMPLEMENT ACTIVATION IN PATIENTS WITH TRAUMA: ASSOCIATION WITH CLINICAL CORRELATES

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It is well known that severe injury is one of the most common stimulus generating a generalized inflammatory response in patients. Intensive research of the recent decades identified the participants of these process. The involvement of different cellular systems, protein cascades, cytokines and small molecules has been proved. Besides, we have only a limited set of data about the role of the complement system in trauma. The aim of the present study was to determine the characteristics of the

Table 1:

Severity criteria	CRP > 50 mg/dl	CRP < 50 mg/dl	
Depth of infection			
- muscle	58.6% (34/58)	41.4% (24/58)	
- subcutaneous tissue	41.1% (206/501)	58.9% (295/501)	$p < 0.02$
WBC			
> 12,000	71.4% (95/133)	28.6% (38/133)	
< 12,000	34.0% (181/553)	66.0% (352/553)	$p < 0.001$
Temperature			
> 38.0	53.0% (132/249)	47.0% (117/249)	
< 38.0	39.5% (196/496)	60.5% (300/496)	$p < 0.001$

Table 2: Clinical cure rates were similar in ceftobiprole- and vancomycin-treated subjects with and without significant elevations in CRP:

	CRP > 50 mg/dl		CRP < 50 mg/dl	
	Ceftobiprole	Vancomycin	Ceftobiprole	Vancomycin
Clinical Cure Rate				
- clinically evaluable (CE)	92.3% (108/117)	92.0% (115/125)	94.1% (143/152)	95.2% (139/146)
- intention-to-treat (ITT)	76.8% (126/164)	78.1% (128/164)	80.9% (169/209)	79.8% (166/208)

complement system activation in patients with severe injury during the first 24 hour and recognize possible correlations between the typicals of the complement activation and the clinical state and outcome.

Adult patients, who were admitted to the trauma center with severe injuries (ISS>12) were included. Beside regular blood samples and monitoring, serum samples were collected at admission and 1,3,6,12,24 hour later and frozen immediately. Serum levels of the complement components SC5b-9 (terminal complex) and Bb (alternative pathway) were measured by standardized ELISA assay. Type and severity of injury, hemodynamic parameters, base excess, serum lactate, hemoglobine, coagulation parameters and therapeutic interventions (fliuds, blood components, operation) were registered. Complications and outcome were followed until 30 day.

Our data present that severe trauma is associated with complement system activation and show the role of the alternative pathway in this process. We found that the mechanism and the energy of the injury are strong determinants of the complement activation. We observed, that the kinetics of the activation were different in patients and 3 main type of kinetics were identified: decelerating, accelerating, transient increase-plateau phase. Our preliminary results show a discrete association of these different activation kinetics with different clinical states and outcomes. Our results can help us to understand the role of the complement system in the trauma induced inflammatory response. These findings can support us to develop a reliable immunomonitoring procedure to help our therapeutic decisions and a tool with high prognostic value.

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TREM-1 LEVELS ARE BETTER INDICATOR OF INFECTION THAN CLINICAL AND LABORATORY VALUES IN TRAUMA PATIENTS

Fernando Rivera, Jureta Horton, Joseph Minei

The diagnosis of infection in the ICU or emergency room remains difficult. Many trauma and surgical patients have SIRS and do not necessarily have infection. Thus, we want to investigate the value diagnostic of sTREM in detecting infection in trauma and surgical patients. We measured plasma, BAL, and peritoneal fluid levels of sTREM in 27 trauma patients and 12 surgical patients. Multivariate multiple logistic regression analysis, showed that the sTREM-1 levels were the strongest independent predictor of infection (OR = 34.6, 95% CI = 12.8-64.6), when compared with clinical criteria and cmicrobiological cultures. Conclusion: TREM-1 measurements may be used as an adyuvant tool to detect infection in trauma ans surgical patients.

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A 498

S-100 PROTEIN LEVEL AND RADIATION INDUCED BRAIN INJURY

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Background:S100 is a calcium binding protein and potential marker for brain injury. Its biological function is not well known.

Patients and Methods: In this pilot study 47 patients (25 males, 22 females, median age 58.5 [28-77]) underwent total and partial cerebral radiation therapy. S-100 plasma concentrations were measured with an electrochemoluminescenz Immunoassay on admission and weekly during radiation therapy.

Results: S-100 plasma concentrations increased from median baseline values of 0,030 µg/l to levels of 0,053 µg/l. During radiation therapy most patients showed an increase of their values, but they were still within the normal range.

Conclusions: Partial- or total brain irradiation leads to a mild increase of S-100 protein plasma levels. Correlations between elevated plasma levels and late side effects of the radiation therapy will be investigated in the near future.

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A 499

INTERLEUCIN-8 A PREDICTION MARKER FOR SEPSIS IN POSTOPERATIVE INTENSIVE CARE UNIT PATIENTS

Edwin Boelke, Gerald Steinbach, Klaus Orth

Objective: Interleukin-8 (IL-8, also known as neutrophil-activating peptide 1, NAP1 and CXCL8, CXC chemokine ligand 8) is recognized as a potent effector of neutrophil functions. IL-8 is a major response factor following NfkB activation by cytokines or lipopolysaccharide and several different cell types T lymphocytes, monocytes, epithelial and endothelial cells secrete this polypeptide. IL-8 is not to be determined at significant concentrations in plasma due to its receptor binding but may play a major role in tissues. The prediction of sepsis is a major and current field of research in the treatment of surgical patients. The aim of this study was to compare the determination of IL-8 in whole blood cell lysates (IL-8 in hemolysate) and in plasma for the prediction of sepsis in postoperative intensive care.

Design: IL-8 in hemolysate, IL-8 in plasma, and CRP were measured in the daily routine monitoring of 84 patients in a surgical intensive care unit. Sepsis was defined by the criteria of the Society of Critical Care Medicine (SCCM). For comparison the APACHE II score (APACHE = Acute Physiology and Chronic Health

Evaluation) was calculated. The diagnostic value of the three tests was compared by receiver operating characteristic (ROC) curves.

Results: IL-8 in hemolysate showed higher areas under the curve (AUC) than IL-8 in plasma and CRP. The ROC curves for the APACHE II scores gave similar results.

Conclusions: Sepsis is a complex disease and is induced by systemic infection of patients suffering from systemic inflammatory response syndromes (SIRS). Therefore, the identification of infection or the host response to infection is of crucial importance. The prediction of an individual marker or interleukin or its binding to surface proteins is not necessarily indicative for sepsis. In cases with unequivocally identified bacterial infections, the current results suggest that whole blood IL-8 may have a similar diagnostic accuracy as plasma levels. Of note, this technique need less blood and is not being affected by hemolysis.

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A 500

IN VITRO EFFECTS OF FRANCISELLA TULARENSIS LIPOPOLYSACCHARIDES ON THE SURFACE EXPRESSION AND RAFT ASSOCIATION OF TOLL-LIKE RECEPTORS 2/4 AND CD14

Zsuzsanna Wolf, Evelyn Orsó, Thomas Langmann, Gerd Schmitz

The primary host defense against Gram-negative bacterial infection is initiated through the recognition of pathogen-associated molecular patterns by Toll-like receptors (TLRs) and by the lipopolysaccharide (LPS)-receptor CD14. The recognition of bacterial LPS induces the recruitment of CD14 and pattern recognition receptors (PRPs) within lipid raft microdomains.

To understand the role of LPS in the pathogenesis of Francisella tularensis infection we compared the in vitro potential of purified LPS from F. tularensis to Salmonella minnesota and Escherichia coli LPS. Expression levels and activation-dependent clustering of PRPs (CD14, TLR2, TLR4 and CD81) were characterized on peripheral blood monocytes after stimulation with different LPS species by flow cytometry and fluorescence resonance energy transfer (FRET).

Short time LPS stimulation (15-min) increased the surface expression of CD14, TLR2 and TLR4, independent of the LPS species. Interestingly, 1-hour LPS treatment with F. tularensis resulted in the downregulation of CD14, TLR2 and TLR4 expression. By contrast, expressions of these receptors were highly elevated by S. minnesota and E. coli LPS stimulation. Furthermore, we found a decreased expression of the CD81 receptor even after 15-min stimulation, irrespectively of LPS species. Finally, through FRET analysis we could demonstrate LPS-induced clustering of TLR-4 and CD81 in human monocytes into lipid rafts.

The present study demonstrates that all three LPS species induces equally the clustering of the given pattern recognition receptors within lipid microdomains. However, it is suggested that F. tularensis LPS triggers a distinct signalling pathway that results in a downregulated activation-dependent expression of CD14, TLR-2 and TLR-4, likely contributing to the survival of this intracellular pathogen.

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A 501

ROLE OF TOLL-LIKE RECEPTOR 4 SIGNALLING AND HYPOXIA INDUCIBLE FACTOR IN CARDIAC ISCHEMIA-REPERFUSION INJURY

Heidi Stapel, Stilla Frede, Stefan Ehrentraut, Pascal Knuefermann, Rainer Meyer, Georg Baumgarten

Lipopolysaccharides (LPS) bind to Toll-like receptor 4 (TLR4), thus inducing an inflammatory cascade in immune cells as well as in cardiomyocytes. LPS pretreatment is known to reduce the size of an ischemia-reperfusion injury (I/R). However, the signalling cascade by which LPS reduces I/R is not known. Therefore coronary artery occlusion was performed in a closed-chest model on mice with (C3H/HeN, C57BL/6) and without TLR4 signalling (C3H/HeJ, TLR4-/-). One week after instrumentation, animals were stimulated with LPS (1 mg/kg BW) or PBS 16 h ahead of ligation of the left anterior descending artery. As LPS is known to increase expression of Hypoxia inducible factor 1α (HIF1α), we investigated HIF1α expression and HIF1 target genes in the myocardium 6 and 16 h after LPS challenge. LPS pretreatment decreased infarct area by about 50% in animals with TLR4 signalling. Reduced infarct areas were also detected in all animals without TLR4 signalling. 6 h after LPS challenge HIF1α protein as well as its target genes iNOS and adrenomedullin were up-regulated only in hearts with TLR4 signalling. At the onset of ischemia iNOS and adrenomedullin were still high in those. In hearts without TLR4 HIF1α protein as well as its target genes were low independent of LPS stimulation. Thus we conclude that up-regulation of HIF1α is not necessary for a reduction of infarct size. However, iNOS and adrenomedullin may improve cardiac perfusion during ischemia after LPS pre-treatment, and thus reduce myocardial damage.

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ROLE OF TOLL-LIKE RECEPTORS IN PNEUMOLYSIN-INDUCED PULMONARY INFLAMMATION AND INJURY

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Background: Pneumolysin is an intracellular peptide of Streptococcus pneumoniae present in virtually all clinical isolates. At sublytic doses, pneumolysin exerts inflammatory properties, thereby affecting the function of neutrophils, macrophages and epithelial cells. At lytic doses, pneumolysin induces apoptosis and may form pores in the cell wall eventually leading to cell death. Toll-like receptors (TLRs) are pattern recognition receptors that recognize pathogens associated molecular patterns; both pneumolysin-induced cytokine production and pneumolysin-induced apoptosis are mediated through TLR4.

Objective: To determine the contribution of TLR2 and TLR4 in pneumolysin-induced pulmonary inflammation and injury in vivo.

Methods: Wild type (WT), TLR2 gene knockout (KO) and TLR4 KO mice were intranasally inoculated with a non-lytic and lytic dose of pneumolysin. Bronchoalveolar lavage fluid (BALF) was harvested 6 hours later for determination of leukocyte counts and differentials, and cytokine/chemokine concentrations.

Results: At non-lytic dose (25 ng/mouse) total cell counts tended to be lower in BALF of TLR4 KO mice compared to WT and TLR2 KO mice; no differences were observed between TLR4 KO mice and WT mice with respect to BALF concentrations of TNF-α or MIP-2 or neutrophil influx. At lytic dose (500 ng/mouse), TLR4 KO mice had significantly reduced total cell counts, neutrophil influx and IL-1β levels in BALF compared to WT and TLR2 KO mice. Surprisingly, both TLR4 KO and TLR2 KO mice displayed significantly reduced levels IL-6 and KC s well as hyaluronan in BALF and attenuated pulmonary leak as compared to WT mice.

Conclusion: Although pneumolysin is a known TLR4 ligand in vitro, both TLR4 and TLR2 contribute to the pulmonary response to lytic doses of pneumolysin in vivo.

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DUAL EFFECTS OF REACTIVE OXYGEN SPECIES ON TLR4 IN HUMAN CORONARY ARTERY ENDOTHELIAL CELLLS

Lihua Ao, Ning Zou, Yong Song, David Fullerton, Xianzhong Meng

Toll-like receptor 4 (TLR4) signaling is involved in cellular inflammatory response to non-bacterial pathogens and has been implicated in cardiac inflammatory response to ischemia and reperfusion (I/R) injury. While reactive oxygen species (ROS) contributes to I/R injury, the role of TLR4 in ROS-induced cardiac inflammatory response and the influence of ROS on cardiac TLR4 expression are unclear. The objectives of this study were to examine whether ROS-induced inflammatory response in human coronary endothelial cells (HCAECs) requires TLR4 signaling and whether ROS up-regulates TLR4 expression in HCAECs. Methods and results: HCAECs in culture were stimulated with hydrogen peroxide (0.25 mM/L) for 0.5-4 h. NF-kB activation, and the release of TNF-α and MIP-2 were analyzed to assess cellular inflammatory response. The role of TLR4 signaling in this response was determined by the application of TLR4-blocking antibody. TLR4 mRNA and protein levels were determined to assess the influence of ROS on TLR4 expression. Hydrogen peroxide stimulation induced NF-kB activation and the release of TNF-α and MIP-2. However, TLR4-blocking antibody pretreatment reduced the release of these pro-inflammatory cytokines. The reduction in cytokine release correlates with attenuated NF-kB activation. Interestingly, hydrogen peroxide stimulation also induced the expression of TLR4 mRNA at 1 and 2 h that was followed by an increase in TLR4 protein. Conclusion: These results highlight dual effects of ROS on TLR4 in HCAECs, activation of the receptor signaling and induction of its gene expression, and suggest that TLR4 signaling contributes to the mechanism by which ROS modulates cellular inflammatory response.

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A 504

LIPOPOLYSACCHARIDES FROM ATHEROSCLEROSIS-ASSOCIATED BACTERIA ANTAGONIZE TLR4, INDUCE FORMATION OF TLR2/1/CD36 COMPLEXES IN LIPID RAFTS AND TRIGGER TLR2-INDUCED INFLAMMATORY RESPONSES IN HUMAN VASCULAR ENDOTHELIAL CELLS

Kathy Triantafilou, Martha Triantafilou, Frederick Gamper, Philipp Lepper, Christian Schumann, Harokopakis Evlambia

Infection with bacteria such as Chlamydia pneumonia, Helicobacter pylori, or Porphyromonas gingivalis may be triggering the secretion of inflammatory cytokines that leads to atherogenesis. The mechanisms by which the innate immune recognition of these pathogens could lead to atherosclerosis remain unclear. In this study, using human vascular endothelial cells or HEK293 cells engineered to express pattern-recognition receptors (PRRs), we set out to determine Toll-like receptors (TLRs) and functionally associated PRRs involved in the innate recognition of and response to LPS from H. pylori or P. gingivalis. Using siRNA interference or recombinant expression of cooperating PRRs, we show that H. pylori and P. gingivalis LPS-induced cell activation is mediated through TLR2. Human vascular endothelial cell activation was found to be lipid-raft dependent and to require

the formation of heterotypic receptor complexes comprising of TLR2, TLR1, CD36 and CD11b/CD18. In addition, we report that LPS from these bacterial strains are able to antagonise TLR4. This antagonistic activity of H. pylori or P. gingivalis LPS, as well as their TLR2 activation capability may be associated with their ability to contribute to atherosclerosis.

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YERSINIA PESTIS V-ANTIGEN INHIBITS LIPOPOLYSACCHARIDE-INDUCED RESPONSES

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Yersinia pestis, the etiological agent of plague, and the enteropathogenic species of Yersinia pseudotuberculosis and Yersinia enterocolitica all possess a 70 kb-conserved virulence plasmid named pYV. Yersinia enteropathogenic species largely rely on the virulence antigen (V-antigen, LcrV) for pathogenicity. The virulence antigen LcrV has been shown to "silence" the innate immune responses against stimulation with TLR2 agonists. In this study we investigated whether LcrV is only able to "interfere" TLR2-dependent inflammatory responses, or whether it is able to inhibit other pattern recognition receptors. Our experiments demonstrate that LcrV is able to "silence" innate immune responses not only against TLR2, but also against TLR4 agonists in vitro. When tested an in vivo sepsis model caused by Gram-negative bacterial products such as lipopolysaccharide (LPS), it was shown that pre-treatment with LcrV was able to inhibit LPS-induced inflammatory responses and improve the survival of the LPS-treated mice. This study shows the ability of LcrV to inhibit TLR2 and TLR4 agonists and further demonstrates its potential use as a therapeutic intervention for sepsis and septic shock.

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SERUM LEVELS OF PROGRAMMED CELL DEATH IN ORGAN FAILURE

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Apoptosis and related forms of cell death have been suggested to contribute to a dysregulated immune response in shock and sepsis while their role in the development of end organ failure is discussed controversially. For a systematic evaluation of an activation of distinct cascades of apoptotic signaling, we analyzed serum levels of members of the TNF/TNFR family and the caspase cleavage product cytokeratin-18 neoepitope (CK-18) in patients with SIRS (n=49) or sepsis (n=38), which were subsequently correlated to disease severity. Serum concentrations of markers of the TNF/TNFR family were higher in infectious as opposed to non-infectious systemic inflammation, albeit only sFas was increased above while sFasL and TRAIL levels were below levels observed in healthy volunteers. Increased serum levels of the caspase-3 cleavage product CK-18 were restricted to overt multi organ failure. A detailed exemplary analysis of apoptotic signaling correlating with liver dysfunction revealed a similar restriction of CK-18 release to most severe forms of liver failure along with the appearance of cytochrome c, but lack of sFasL in serum indicative of hepatocellular apoptotic injury via the intrinsic / mitochondrial pathway. Our data are consistent with effective protection of solid organs against apoptotic injury in mild and moderate organ dysfunction while programmed cell death seems to contribute to overt organ failure, e.g. of the liver.

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TETRAHYDRO-4-AMINOBIOPTERIN INHIBITS KEY INTRACELLULAR SIGNALING PATHWAYS: POSSIBLE IMPLICATIONS FOR INOS-INDEPENDENT IMMUNOSUPPRESSION

Manuel Maglione, Thomas Ratschiller, Gabriele Werner-Felmayer, Ernst R. Werner, Sucher Robert, Gerald Brandacher

Objective: In different experimental settings pteridine derivates have been shown in the past to act as potent immunosuppressive agents. In the case of the selective iNOS inhibitor Tetrahydro - 4 - Aminobiopterin (ABH_4) an iNOS-independent attenuation of MHC class II expression on antigen-presenting cells (APCs) and T cell apoptosis have been observed. Since both processes are critically regulated by intracellular signaling, we analyzed the effects of pteridine derivates on two key regulators of cellular processes, the cytoplasmic or RAS-RAF-MEK-ERK pathway, and the PI3K/protein kinase B (PKB) entity.

Material and Methods: The human melanoma cell lines A375, WM-266-4, Colo829 and mouse fibroblast cell line NIH3T3-B-RAF^{V600E} carry an oncogenic mutant of B-RAF. Parental NIH3T3 cells, and normal human fibroblasts served as control. Cos7 cells were used in transient transfection experiments. Activation of signaling proteins was detected by the use of phosphorylation-specific antibodies for ERK, MEK and AKT. RAF kinase activity was measured following immunoprecipitation in a coupled assay using recombinant MEK and ERK as substrates. ABH_4/BH_4 were applied at concentrations of 500 and 1000μM. Apoptotic cells death was measured following propidium iodide (PI) labeling of cells. L-N^6-(1-Iminoethyl)lysine, L-NIL, a selective iNOS inhibitor, was used at a concentration of 100 and 250μM.

Data: To gain insight into possible effects on intracellular signaling, RAF-transformed and normal cells were compared for the effects of ABH_4/BH_4 and L-NIL on their survival. Following a 20 hour treatment with ABH_4, NIH3T3-B-RAF^{V600E} cells as well as several human melanoma cell lines carrying the same type of mutation, showed substantial apoptosis. Only a moderate effect was observed with BH_4, none with L-NIL. The survival of normal cells was not affected under these conditions. Following serum stimulation of NIH3T3 cells ABH_4 consistently blocked MEK/ERK activation. Pretreatment with ABH_4 also blocked the activity of mutant RAF. Inhibitory effects of ABH_4 were not limited to the cytoplasmic cascade but also the activation of PKB was affected. Moreover upon prolonged incubation with ABH_4 we observed the degradation of the signaling proteins analyzed.

Conclusion: Taken together these experiments demonstrate strong negative modulation of intracellular signaling pathways by ABH_4 in cells, where these pathways are hyperactivated and thus may explain previously observed iNOS-independent immunosuppressive effects of ABH_4.

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ATNF- B- AND P38 MAP KINASE-DEPENDENT GENE EXPRESSION PROGRAMS WITHκINDUCES NF- SPECIFIC PATTERNS IN HUMAN MICROVASCULAR AND MACROVASCULAR ENDOTHELIA

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Objectives: Inflammatory stimulation of endothelial cells (EC) by tumor necrosis factor a (TNF-a) is a key step in the pathogenesis of a systemic inflammatory response syndrome. Activation of endothelia results in the adhesion and recruitment of leucocytes to the place of inflammation and demands close regulatory control mechanisms to induce coordinated gene expression programs. Additionally, different types of endothelial cells lining the inner vessel surface fulfil distinct requirements. Understanding the diversity of ECs therefore is a prerequisite for the interpretation of data obtained from in vitro models with regard to their relevance for inflammatory responses in man. A systematic analysis of gene expression programs elicited by TNF-a in different endothelia and their assignment to distinct signalling pathways is not available.

Material and Methods: We compared global gene expression profiles of TNF-a-stimulated human microvascular (HMEC-1) and macrovascular endothelial cells (HUVEC) using oligonucleotide microarrays covering more than 13,000 genes. Blocking of signalling pathways by transfecting ECs with a dominant negative mutant of IKK2 or by the specific p38 MAP kinase inhibitor SB202190 elucidated the role of NF-kB and the MAP kinase cascade for the TNF-a-signal.

Data: A sophisticated analysis of the microarray data provided the first systematic and statistically validated gene profiles mediated by TNF-a-in different human ECs. Surprisingly, half of the TNF-a-regulated genes were only induced in HUVEC respective only in HMEC-1. Most of these cell type-dependently regulated genes encode for chemokines, cytokines and cell surface molecules. Virtually all TNF-a-inducible genes in HUVEC were dependent on IKK2/NF-kB activation while a minor number was additionally modulated by p38. Furthermore, novel genes induced or suppressed in a NF-kB- and/or p38-dependent mode were identified. All results were confirmed by quantitative RT-PCR and flow cytometry demonstrating reliability of data.

Conclusions: Thus, these studies sensitize to a cautious interpretation of in vitro data giving consideration of EC type-specific effects. Our results define a list of primary gene candidates with reference to the EC subtype and to involved signalling pathways. The data indicate important signalling junctions for targeted modulation of endothelial functions during systemic inflammatory responses and represent the basis for an EC type-specific development of therapeutic agents.

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HISTOPATHOLOGICAL CHANGES AND THERAPEUTIC EFFECTS OF GLUCOSAMINE, CHONDROITINE AND METHYL-SULFONYL-METHANE IN MONOIODOACETATE-INDUCED OSTEOARTHRITIS IN KNEE JOINT OF RAT

Mahsa Hadipour-Jahromy, Reza Mozafary-Kermani, Soha Noorafshan

Objectives: Glucosamine sulfate (GS), chondroitine sulfate (CS) and methyl-sulfonyl-methane (MSM) are among the most widely used food supplements for treatment of osteoarthritis. In the present study, histopathological alterations and their therapeutic effects, in high doses, were evaluated in monoiodoacetat (MIA)-induced osteoarthritis (OA) in Femorotibial joint of rat.

Materials & methods: To induce OA, single dose of MIA (1mg/knee) and saline were injected intra-articularly to the left and right knee joint of rat, respectively. Then, the beneficial effects of food supplements were studied in different groups, orally; Group 1: administration of GS (800mg/kg), Group 2: administration of GS+CS (800+ 500 mg/kg), Group 3: administration of GS+ MSM (800 + 200 mg/kg), Group 4: administration of GS+ CS+ MSM (800+500+ 200 mg/kg). Histopathological changes in knee joints were studied after two weeks.

Data: Histological findings such as disorganization of chondrocytes, erosion and fibrillation of cartilage surface, subchondral bone exposure, and loss of proteoglycan in cartilage were observed after fifteen days in iodoacetate injected knee, staining with hematoxilin/eosine and toluidine blue. Administration of GS alone, significantly prevented damage induced by MIA to chondrocytes and proteoglicane (PG) in OA knee. Chondrocytes organization was partly preserved, and their number was focally increased with less damage to proteoglycan in the epiphyseal plate. No inflammatory cells or cell proliferation in synovial were observed, either. However, Additional supplements such as CS and/ or MSM did not produce more improvement on cartilage degeneration and proteoglycan loss, over the period of two weeks.

Conclusion: Single intra-articular injection of MIA; make it possible to induce fast and progressive damage to articular cartilage, which mimic exactly human OA. In this study, the effectiveness of GS in improvement of histopathological alterations and its protective role against cartilage and subchondral bone damage is emphasized, however, neither additional nor synergistic effects observed when other supplements co-administered. More research is needed perhaps, using different doses or in longer period to guide us the correct usage of these food supplements.

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A 510

OXIDIZED PHOSPHOLIPIDS INHIBIT THE PHAGOCYTIC CAPACITY OF PROFESSIONAL PHAGOCYTES THEREBY IMPAIRING OUTCOME IN GRAM-NEGATIVE SEPSIS IN VIVO

Ulrich Matt, Sylvia Knapp, Leitinger Norbert, Tom van der Poll

Objective: Oxidized phospholipids that are generated during inflammation exert anti-inflammatory properties and prevent death during murine endotoxemia. Oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (OxPAPC) inhibits the interaction of lipopolysaccharide (LPS) with LPS-binding protein (LBP) and CD14. We here determined the functional properties of OxPAPC and potential interference with CD14 during abdominal sepsis caused by Escherichia (E.) coli.

Material and Methods: In vivo: CD14-/- and/or wild-type C57/BL6 mice were inoculated intraperitoneally (i.p.) with E. coli. Immediately before infection, mice received either vehicle (NaCl), control lipids (DMPC) or OxPAPC i.p. Survival was monitored over 5 days. In endpoint studies bacterial outgrowth in PLF (peritoneale lavage fluid), liver and blood was assessed. Cytokines and chemokines were evaluated in blood and PLF using ELISAs. Neutrophilic influx (cytospins stained with Giemas) was counted in PLF. In vitro: Primary macrophages or RAW 264.7 cells were incubated with FITC-labeled bacteria +/- OxPAPC; uptake was analyzed by FACS.

Data: Administration of OxPAPC rendered mice highly susceptible to E.coli peritonitis, as indicated by an accelerated mortality and enhanced bacterial outgrowth and dissemination. $CD14^{-/-}$ mice also displayed increased mortality and bacterial outgrowth and OxPAPC did not further impair host defense in these animals. The mechanisms by which OxPAPC and CD14 deficiency impaired the immune response differed: whereas $CD14^{-/-}$ mice demonstrated a strongly reduced recruitment of phagocytes to the site of the infection, OxPAPC did not influence the influx of inflammatory cells but strongly diminished the phagocytosing capacity of neutrophils and macrophages by a CD14 independent mechanism. Beside of inhibiting the uptake of Gram-negative bacteria, OxPAPC potently diminished the phagocytosis of Gram-positive bacteria such as Streptococcus pneumoniae. Furthermore, OxPAPC induced a dose dependent reduction of fluid-phase pinocytosis or internalization of fluorospheres.

Conclusion: These data suggest that oxidized phospholipids such as produced during inflammatory reactions may contribute to mortality during Gram-negative sepsis in vivo via impairment of the phagocytic capacities of professional phagocytes.

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ROLE OF LIVER AND SPLANCHNIC CIRCULATION IN ANAPHYLACTIC HYPOTENSION IN BALB/C MICE

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We determined the roles of liver and splanchnic vascular bed in anaphylactic hypotension using in vivo and isolated perfused BALB/c mouse liver preparations. An intravenous injection of ovalbumin antigen into the Intact-sensitized mice caused a decrease in systemic arterial pressure (Psa) from 92 ± 2 (SE) to 39 ± 3 mmHg but caused only a slight increase in portal venous pressure (Ppv) from 6.4 ± 0.1 cmH_2O to the peak of 9.9 ± 0.5 cmH_2O at 3.5 min after antigen. The elimination of the splanchnic vascular beds by ligation of the celiac and mesenteric arteries, combined with total hepatectomy attenuated anaphylactic hypotension. The ligation of these arteries alone, but not partial hepatectomy (70%), also attenuated anaphylactic hypotension similarly. In contrast, sensitized mouse liver perfused portally at constant flow did not show anaphylactic venoconstriction, but showed substantial constriction in response to the anaphylaxis-associated substance of platelet-activating factor, indicating that venoconstriction observed in in vivo mice may be induced by mediators released from extrahepatic tissues. These results suggest that splanchnic vascular beds are involved in BALB/c mouse anaphy-

lactic hypotension: They presumably act as sources of chemical mediators to cause the anaphylaxis-induced portal hypertension, which induced splanchnic congestion resulting in a decrease in circulating blood volume and thus systemic arterial hypotension. Mouse hepatic anaphylactic venoconstriction may be induced by factors outside the liver, but not by anaphylactic reaction within the liver.

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HEPATIC MICROCIRCULATION AND TISSUE OXYGENATION IN SEPSIS AND AFTER HEMIHEPATECTOMY IN A SMALL ANIMAL MODEL

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Objective: We tested the correlation between echo Doppler flow of arterial and venous influx of the liver in an animal model and hepatic microcirculation and oxygenation measured by reflection spectrophotometry in sepsis and after hemihepatectomy compared to a normal control group with the aim to identify specific flow and oxygenation differences.

Material and Methods: Thirty-six male Wistar rats, (BW 434 ±37 gr), were randomly allocated to control, endotoxaemia (LPS) or hemihepatectomy (HH). We inserted flow probes around the hepatic artery and portal vein (type 0,5V and 2,0S) coupled to a T-206 monitor (Transonic Systems Europe, Maastricht, NL). In the LPS group 5 mg/kg lipopolysaccharide (Esch.coli O127:B8, Sigma-Aldrich, St. Louis, USA) was administered. After stabilisation, hemodynamics, hepatic blood flow, and tissue flow and oxygenation (O2C«, Lea Medizin Technik, Giessen, Germany) measurements were performed during a 2h observation period.

Data: Mean arterial pressure did not change in the control group, but decreased significantly in the LPS group (89±11 mmHg to 73±10 mmHg, $p<0.01$) and in the HH group (89±10 to 76±13, $p<0.05$). Cardiac output did not change significantly in any group and were comparable. In the control group, hepatic arterial flow (HAF) decreased during the observation period (6,9±2,2 ml/min to 5,3±1,7 ml/min, $p<0,01$). In the LPS and the HH groups HAF did not decrease. Portal venous flow decreased in the control group (29,0±7,0 ml/min to 24,8±4,8 ml/min, $p<0.05$), whereas not in the LPS and HH groups. Hepatic tissue saturation in the control group was significantly different with LPS and HH groups after 1h (50±4%, 54±9% and 45±6% respectively). At 120 minutes hepatic tissue saturation in the control group was comparable with the LPS group and was significantly lower in the HH group (50±7%, 52±6% and 42±8% respectively). At 60 minutes, the microcirculatory flow was comparable between the control group and LPS group and was significantly higher in the HH group (141,1±54,3 AU, 151,9±42,3 AU and 181,5±41,6 AU respectively). In the control group tissue flow decreased over time. Flow velocity at 60 minutes was comparable between controls and LPS group but was significantly higher in the HH group (26,6±3,4 AU, 26,4±6,6 AU and 36,1±12,7 AU respectively).

Conclusions: (1) The lowered hepatic artery and portal flow in the control group might be explained by an anaesthetics effect as we observed a later steady state in the control group where 2% isoflurane was administered, than in the other groups were 2,5% was necessary. (2) Reduced MAP with constant CO and hepatic influx in the LPS group with an increased saturation was probably due to increased shunting and decreased oxygen extraction. (3) In the HH group more blood was flowing faster through the remaining part of the liver at a lower saturation. Perhaps congestion with increased oxygen extraction in the gut might be present but we did not measure gut circulation.

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NEUROGENIC SHOCK AND PULMONARY EDEMA INDUCED BY INTRACEREBROVENTRICULAR INJECTION OF BRAZILIAN SCORPION TOXIN IN RATS

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Scorpion poisoning is a major problem faced by health care workers at emergency rooms and intensive care units in tropical and subtropical zones. Scorpion toxins increase the serum levels of inflamatory cytokines in patients with severe shock and pulmonary edema. This study evaluated the hemodynamic and pulmonary effects of a purified scorpion toxin (ST) injected intracerebroventricular (i.c.v.) in anesthetized rats under mechanical ventilation. Forty-six rats were anesthetized with thiopental sodium and injected with atropine and submitted to sterotaxic surgery for guide-canulae implantation in the right lateral ventricle. A hypodermic needle fastened to the skull bone with zinc cement was used as a guide-canulae. After the surgical procedures, the animals went through a recovery period of at lest 3 days. Prophylactic antibiotic was done to prevent post surgical infection. The animals were allocated in six groups: 1) Control (Ct) (n=8): 5 μL of sterile saline was injected i.c.v.; 2) Scorpion toxin (Tx) (n=10): 5 μL of ST was injected i.c.v. without previous treatment; 3) Insulin (n=6): pre-treatment with insulin (1.5 iu/kg) previously to ST i.c.v. injection; 4) Enoxaparin (n=12): pre-treatment with Enoxaparin (2mg/kg); 5) Morphine (n=5): pre-treatment with morphine (2mg/kg); and 6) Vagotomy (n=5): cervical vagotomy was performed previously to ST i.c.v. injection. After i.p. anesthesia with xylasine and ketamin, tracheotomy, right jugular vein and carotid catheterization were done in order to connect to a ventilator and measure continuously the mean arterial pressure (MAP), central venous pres-

Table 1:

Parameter (96 hours)	CCI + Fe-Fx + Shock	WD + Fe-Fx + Shock	p
Mortality	21,4%	47,1%	< 0,01
Max. weight loss	6,2% of initial weight	8,5% of initial weight	n. s.
Max. temperature loss	1,9% of initial temperature	5,6% of initial temperature	< 0,05
CD4+ (%)	24,6	21,8	n. s.
CD8+ (%)	16	34,2	< 0,05
TNFa (pg/ml)	17,5	138,8	< 0,01
IL-6 (pg/ml)	23,8	280,9	< 0,01
Increase of ear lobe thickness	47,1%	54,6%	< 0,05

CCI: controlled cortical impact; WD: weight drop; Fe-Fx: femoral fracture

sure (CVP), and cardiac output (CO). Electrocardiography recording and heart rate (HR) were also continuously monitored. Systemic vascular resistance (SRV) was derived by dividing MAP-RAP/CO x 79.9 and is reported as dynes.s.cm-5. The mean stroke volume (SV) was calculated as CO/HR. After 60 min of the i.c.v. injection the animals were euthanized and the lung weighted to measure the lung/body index. In the Tx group, i.c.v. injection of ST induced a pronounced increase in MAP (80±3 to 147±7mmHg at 10min) followed by hypotension (39±3mmHg at 60min). This increase in the MAP was secondary to an increase in CO (97±2 to 128±10ml/min at 10in) and SVR (64899±4105 to 107827±11318 dynes.s.cm-5 at 10min). Following the inotropic phase, MAP progressively decreased below baseline and was accompanied by a reduction in CO (63±3ml/min at 60min), CVP (2±1 to -4±3 at 60min) but not in the SVR (50777±2213 dynes.s.cm-5). This decrease in CO was associated with a reduction in SV (0.37±0.01 to 0.19±0.02 ml at 60min) and an increase in HR (265±4 to 409±21 bpm at 60min). The previous administration of morphine prevented the hypertensive peak as well as the hypotension and the increase in SVR, but not the reduction in CO. Previous administration of heparin and vagotomy, but not insulin, prevented also the hypotension. A reduction in SV was observed in all groups when compared to control. Autopsy confirmed pulmonary edema after i.c.v. injection of ST with a lung-to-body weight ration of 0.80±0.05 (Ct group=0.51±0.03, $p<0.01$). Previous administration of insulin, but not morphine or enoxaparin, partially reduced the pulmonary edema (0.67±0.04, $p>0.05$). Vagotomy increased the lung-to-body ratio (1.437±0.1733, $p<0.001$) when compared to TX and control groups. We conclude that i.c.v. injection of ST can be a model to study the mechanisms of neurogenic induced-shock and pulmonary edema.

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CLINICAL AND INFLAMMATORY RESULTS 96 HOURS FOLLOWING MULTIPLE INJURIES IN A MOUSE MODEL OF HEAD INJURY, FEMORAL FRACTURE AND HEMORRHAGIC SHOCK

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Background: In multiply injured patients, injuries to the head and extremities and hemorrhagic shock are the most common clinical problems. In the past decade, a vital role during the clinical course and especially during critical care has been assigned to different immunological and inflammatory processes. Our goal was to develop a standardized model equivalent to the clinically predominant injury pattern for further investigation of pathophysiologic or potentially therapeutic concepts.

Methods: 20 male C57BL mice (22.5 ± 3.1 g) were anesthetized using Xylazine and Ketamine. Open head injury was done by controlled cortical impact (CCI) with the rounded tip of an air-pressure controlled rod to the left parieto-temporal cortex. Closed head injury was done by standardized weight drop (WD) to the left parieto-temporal cortex. Following each of the two methods without delay, a femoral fracture (Fe-Fx) was induced by a weight drop fracture apparatus as well as hemorrhagic shock by withdrawing 50% of the blood volume for one hour before resuscitation using Ringers' solution. The fracture was treated by splinting immediately after resuscitation. The following clinical status was monitored by activity, body weight and temperature and mortality. Finally, the animals were sacrificed at 96 hours post trauma and flow-cytometry and cytokine-analysis was performed.

Results: Multiply injured animals with CCI + femoral fracture and shock showed a significantly lower mortality and a lower inflammatory response to the trauma than WD + femoral fracture + shock did (see table 1). The WD-group clinically showed a greater decrease of body temperature post trauma than the CCI-group.

Conclusions: These findings seem to suggest that a closed head injury not only results in higher mortality but also in an increased inflammatory response in our model of multiple injuries in mice. Despite the primary craniotomy in the CCI-group, the decreased inflammatory response

might be connected to the decreased mortality, but could not be proven so. We feel that our models are readily reproducible and well standardized. Still, in comparison to the CCI-group, the closed head injury seems to be closer to clinical reality and might be a good basis for future research.

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CLINICAL SIGNIFICANCE OF THE DETECTION OF THE HOMOLOGOUS DELETION OF P16 GENE IN MALIGNANT PLEURAL EFFUSION

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Background: The homozygous deletion of p16 gene is frequent status in many types cancer including lung cancer. The pleural effusion is one of common status in patients with lung cancer. The cytology examination of the pleural effusion is one of the most reliable methods in the discrimination of benign or malignant pleural effusion, but the positive rate is only about 60%. The aim of this study was to explore the clinical significance in the diagnosis of malignant pleural effusion by the analysis of homozygous deletion of pleural effusion p16 gene.

Methods: The homozygous deletion of p16 gene$^{exon1\ and\ exon2}$ were determined in 34 specimens of pleural effusions associated with non small cell lung cancer and 21 cases tuberculous pleural fluids by polymerase chain reaction (PCR), the former compared with the determination of exfoliated cytology in the same specimens, from which the clinical value was analyzed.

Results: Our results showed that none of 21 tuberculous pleural fluids were found in homozygous deletion of $p16^{exon\ 1\ and\ 2}$ and $p16^{exon\ 1}$ did not deleted either in all of 34 malignant pleural effusions, but the homozygous deletion of $p16^{exon2}$ was present in 15 of 34 malignant pleural effusions(44.11%), including 8 cases were negative in cytology. Pleural fluid cytology was positive in 19 of 34 malignant cases (55.88%). If combined these methods, the diagnostic sensitivity was enhanced, from 55.88%(19/34) to79.41%(27/34), whose positive rate was higher than only the determination of $p16^{exon2}$ homozygous deletion or exfoliated cytology in malignant pleural effusions.

Conclusion: These data have suggested that combining the examination of exfoliated cytology with homozygous deletion of p16 gene^{exon2} in pleural effusion can recruit and enhance the diagnostic value of conventional pleural cytology. The detection of the homozygous deletion of p16 gene in pleural effusion may be a useful adjunct to cytological and histological examination of pleural effusions. In case of undiagnosed exudative pleural effusion with a high clinical suspicion for malignancy, it is reasonable to examinate the homozygous deletion of pleural fluid p16 gene.

Key Words: malignant pleural effusion, p16, homozygous deletion

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EVALUATION OF RESPIRATORY PARAMETERS IN THE COURSE OF MURINE SEPSIS

Uwe Senftleben, Jorg Thomas, Florian Wagner, Beatrix Hoffarth, Michael Georgieff

Objective: The mouse model of sepsis offers extensive possibilities to study basic pathophysiological principles. Moreover, the use of genetically engineered animals enables us to accomplish the bridging from molecules to physiology. Transgenic mice harbouring well described particular defects of signaling pathways provide a tool to investigate immunological and physiological functions of these pathways during critical diseases like sepsis. In this respect, murine sepsis models, such as cecal ligation and puncture (CLP), need to be carefully and well characterized regarding pathophysiological changes over time. For this purpose, techniques are needed which are as little invasive as possible. We therefore examined respiratory parameters of unrestrained septic animals in order to correlate respiratory changes with the severity of sepsis.

Material and Methods: Respiration of unrestrained adult female C57BL/6 mice was measured using a whole body plethysmographic device. Upon isoflorane anesthesia induction CLP surgery (double puncture with 18Gauge-, 20G-, 22G-, 26G-needles) and laparotomy only (Sham) was performed in 4-8 mice for each group. Subsequently, mice received 1ml 0.9% NaCl s.c. once and buprenorphine 25ng/g s.c. twice a day. Minute volume (MV), tidal volume (TV) and respiratory frequency (f) were detected for 30 min 2hrs after the operation followed by recurrent measurements every 12 hours for 5 days (except 18G: 24hrs).

Data: Normal values: MV 1,9 ± 0,2 ml/g/min, TV 6,1 ± 0,3 Ál/g, f 337 ± 75 /min. The 5-day-mortality rates of the 5 groups was similar to studies performed earlier in our lab. (Sham: 0%, 26G: 16%, 22G: 25%, 20G: 37%, 18G: 100%). Directly post-op all groups showed similar respiratory parameters. At 12 hrs after CLP MV in the 18G- and 20G group was decreased by 70 % and 35%, respectively. In contrast, MV was not decreased in the other groups. Interestingly, only those animals in the 20G-group that survived seemed to showed an increase of MV at 24h and 48h after CLP. The other groups demonstrated inconstant results. Thereafter, 20G-, 22G-, and 26G-treated mice showed similar reduced MV compared to sham mice, which exhibited normal values from 72hrs post-surgery on. From 84hrs on all surviving CLP mice started to increase their MV and fully recovered at 120hrs. Interestingly, the pattern of respiratory frequency closely resembled the course of MV in all groups throughout the observation period. TV did not show clear differences between the groups. In this context it has to be noted that telemetric observation of 22G- and 26G-mice revealed

that their overall activity is suppressed for 72hrs upon sepsis induction.

Conclusion: This technique provides a suitable tool to study the spontaneous course of respiration during murine sepsis. MV is mainly determined by changes of f and not TV. Especially during the early period severe grades of sepsis are indicated by reduced MV which possibly indicates fatal outcome. Presumably, increased activity after 72hrs leads to enhanced oxygen consumption and, hence, to increased MV. At this time, surviving septic mice appear to recover from this disease.

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EVALUATION OF ECG, TEMPERATURE AND ACTIVITY IN THE COURSE OF MURINE SEPSIS

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Objective: The mouse model of sepsis offers extensive possibilities to investigate basic pathophysiological principles. Moreover, the use of genetically engineered animals enables us to accomplish the bridging from molecules to physiology. Transgenic mice harbouring well described particular defects of signaling pathways provide a tool to study immunological and physiological functions of these pathways during critical diseases like sepsis. In this respect, murine sepsis models, such as cecal ligation and puncture (CLP), need to be carefully and well characterized regarding pathophysiological changes over time. For this purpose, techniques are needed which are as little invasive as possible. We therefore examined ECG, temperature and activity via telemetry in unrestrained septic animals in order to correlate these parameters with the severity of sepsis.

Material and Methods: ECG, temperature and activity of adult female C57BL/6 mice was measured using a sensor and telemetry device (DSI Systems). In particular, upon isoflorane anesthesia ECG wires of a sensor were surgically placed subcutaneously near the heart and guided to the connected sensor (ã 6mm), which was placed subcutaneously on the back. The mouse was kept unrestrained in a cage, which was placed on a receiver. This procedure was done 5 days before CLP and sham surgery. Upon isoflorane anesthesia induction CLP surgery (double puncture with 18Gauge-, 22G-, 26G-needles) and laparotomy only (Sham) was performed in 4 mice for each group. Subsequently, mice received 1ml 0.9% NaCl s.c. once and buprenorphine 25ng/g s.c. twice a day. ECG, temperature and activity were detected automatically every 6 hours for 30 minutes up to 10 days via a specific software.

Data: Heart rate of Sham mice demonstrated a characteristic diurnal rhythm with about 640 ± 70 beats/min during the day and 750 ±.50 beats/min at night. CLP surgery led to significantly decreased heart rates and a loss of diurnal rhythm. The diurnal rhythm turned back in 26G mice after 4 days but was not clearly detectable in 22G mice throughout the observation period. 18G mice did not survive longer than 55 hrs and showed massive suppression of heart rates. Interestingly, the diurnal rhythm of activity was clearly absent in all CLP groups except in the sham mice and only returned in 26G-treated mice after day 3. Body temperature was significantly decreased in CLP mice and returned to normal values after day 3 with the exception of 18G-treated mice, which were hypothermic throughout their observation period. Hyperthermia could not be detected.

Conclusion: This technique provides a applicable tool to study the spontaneous course of ECG, temperature and activity during murine sepsis. C57BL/6 mice do not present a hyperdynamic septic phase. Recovery of activity and body temperature negatively correlates with the severity of the insult. Overall activity is a suitable parameter to describe the severity and the disease course of a septic mouse.

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"IN VITRO / IN VIVO" MODELS FOR JOINT TRAUMATIC ARTHRITIS IN HORSE NEED THE MONITORING OF JOINT STATUS VIA MATRIX METALLOPROTEINASE (MMP) PATTERNS OF SYNOVIAL FLUIDS

Evgueni Karakine, Dirk Barnewitz, Michaela Neumann, Ingo Wilke

Molecular imaging of an acute joint dysfunction during the development of arthritic disease (AD) uses mainly two groups of cartilage matrix metabolytes namely cartilage derivates (fragments of collagens etc., Clark and Parker, 2003) and the enzymes of matrix degradation (the members of MMP- ADAMTS gene family, TIMP's etc., Kevorkian et al., 2004). A comparison of the joint status during "natural traumatic" AD with those after the implantation of artificial "engineered" cartilage is very inportant because of great clinical importance of the letter.The aim of our investigation was to estimate the usefullness of synovial MMP's as the markers of the disease during the course of horse traumatic arthritis (TA) in situ, in cell culture TA model in vitro and also in 2 models in vivo, namely after cartilage fragment extirpation / autoreparation and after extirpation / implantation. As a criterion for joint status we estimate the number and the activities of markers of the second group - i.e. synovial or cultural MMP's using the reverse zymography test for the analysis (Hibbs et al., 1985).

First, we studied MMP patterns of normal (N) and pathological (P) cartilage of TA and revealed the specific MMP patterns for P cartilage extracts and also for P synovial fluids. To support this conclusion, we created equine chondrocyte cell culture model for TA and found that MMP test also discriminate between N or P status, and could show that the in vitro data correspond well to the in situ ones. For MMP monitoring of autoreparation,

after extirpation of cartilage fragments we could find (after 2 days) an acute joint reaction (sharp activation of MMP's). The estimation of synovial MMP patterns afterwards showed a normalization of the joint status without a medicial therapy within 6-7 weeks. After cartilage implantation, it was possible to observe MMP patterns of traumatic arthritis in 100% of the cases during the first month after the implantation. Afterwards, a normalization of joint status (i.e. MMP patterns) was observed during different periods but not for all of the horses.

Our data clearly demonstrate the effectivity of synovial MMP test and our models for the estimation of the joint status after trauma or implantation of engineered cartilage.

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THERAPEUTIC INDUCTION OF HEME-OXYGENASE-1 IN THE LIVER VIA POLYMERIZED HEMOGLOBIN: PROTECTION FROM REPERFUSION INJURY

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Objective: In the setting of hepatic resection, transplantation or trauma, the liver is particularly susceptible to the injuries of ischemia and reperfusion (I/R). Cumulatively, these injuries may lead to hepatic dysfunction. Consequently, developing strategies to prevent this I/R injury will have diverse potential clinical benefits.

Endogenous and induced (hemin, hypoxia) overexpression of heme-oxygenase-1 (HO-1) provides protection against I/R injury experimentally, which is abolished with selective inhibition of this enzyme. Unfortunately, no clinically viable therapy exists to safely induce HO-1. We have previously found that a hemoglobin-based oxygen carrier (HBOC) upregulated HO-1 in isolated cell lines. We hypothesized that the in vivo administration of HBOC would upregulate the expression of HO-1 in liver and thereby confer protection against hepatic I/R injury. The purpose of this study was to examine the effect of HBOC administration in vivo on: (1) hepatic HO-1 expression and (2) hepatic I/R injury.

Materials & Methods: HBOC (PolyHeme®, human polymerized hemoglobin, pyridoxylated; Northfield Laboratories, Inc.) at 0% (instrumentation alone control), 5% or 10% of calculated blood volume (CBV), or normal saline (volume control), was administered intravenously (femoral vein) to anesthetized rats (N=5 per group). In phase I experiments, animals were sacrificed at 12 hours for dose response studies. For time course studies, animals were injected with HBOC and sacrificed at 0, 12 or 24 hours after injection. Livers were isolated, washed and flash frozen for tissue homogenization. Immunoblot of tissue samples were probed for HO-1 and heat shock protein 72 (HSP72, stress-inducible protein). Densitometry was performed using Kodak 1D software. Statistical analysis was ANOVA (*$p<0.05$). For liver I/R experiments, animals underwent the infusion protocol outlined above. Based on the results of the phase I studies, at 12 hours after infusion, rats were anesthetized and underwent laparotomy with partial liver ischemia induced by clamping all the contents of the portal triad to the left and median lobes of the liver for 45 minutes. Recovering animals were sacrificed at 6 hours after reperfusion. Blood samples were obtained for alanine aminotransferase (ALT) levels. Samples of liver were frozen in embedding medium for histological analysis.

Data: HBOC induced upregulation of HO-1 in a dose-dependent fashion at 12 hours. There was a 5.2 and 4.2 fold increase over baseline at 12 and 24 hours, respectively. Normal saline loading control did not change from baseline levels. In contrast, no group significantly altered levels of HSP72, suggesting the induction is specific to HO-1. Pretreatment with HBOC was protective against hepatic I/R injury. HBOC pretreatment conferred a 35% reduction in serum ALT levels 6 hours after reperfusion. Furthermore, histological analysis demonstrated a significant reduction in tissue necrosis in the HBOC-treated animals.

Conclusion: HBOC selectively induces expression of the protective enzyme HO-1 in the liver, without alteration of other stress-inducible heat-shock proteins. Furthermore, this up-regulation provides protection against hepatic I/R injury. We conclude that HBOC administration offers a novel clinical strategy to prevent liver dysfunction from diverse ischemic insults including liver trauma, elective resection, and transplantation.

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INITIAL POSTTRAUMATIC TRANSLOCATION OF STAT1 FOLLOWED BY SOCS1 MRNA EXPRESSION IS REDUCED IN MAJOR TRAUMA PATIENTS WITH ADVERSE OUTCOME

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Background: Destabilisation of the human immune system following severe trauma induces Systemic Inflammatory Response Syndrome, SIRS and Multiple Organ Failure, MOF. In this context, Suppressors of Cytokine Signaling, (SOCS) seem to play a substantial role for modulation of cytokine expression after trauma, which are controlled by Signal Transducers and Activators of Transcription, STAT1. Although it was demonstrated in animal models that i.e. SOCS1 mediates part of the posttraumatic immune modulation its role within human PMN obtained from multiply injured patients remains unclear, so far.

Patients and methods: Peripheral blood neutrophils (PMN) were isolated from whole blood samples of 26 multiple injured patients (Injury Severity Score, NISS≥16) within 90 minutes after trauma, as well as after 6, 12, 24, 48 and 72h standardized to the traumatic event. STAT1 translocation was analysed by Electrophoretic Mobility Shift Assay, EMSA in the nuclear protein fraction. EMSA results were quantified by densitometry. EMSA data [arbitrary units] is given as mean±SEM for nuclear translocation. Expression of the reporter gene SOCS1 was analysed by RT-PCR (Light Cycler, Roche). Results of RT-PCR are given as copies/50ng RNA as mean±SEM. Statistical analysis was performed by ANOVA on ranks and SNK-test. In addition, STAT1 activity as well as SOCS1 expression was analysed in PMN of 9 healthy volunteers were either native (negative control) or after LPS stimulation as positive control, respectively.

Results: The mean NISS of the enrolled patients was 41±8. 18 survived, 8 died within the posttraumatic period. Transcriptional activity of STAT1 in trauma patients was significantly increased over the whole observation period as compared to healthy controls ($p<0.01$). STAT1 activity in patients who died within the posttraumatic period was significantly reduced at 6h and 12h after trauma due to MOF as compared to surviving patients. In accordance to the transcriptional signalling the expression of the reporter gene SOCS1 was significantly reduced after 6h as well as after 48h and 72h in patients who deceased in the posttraumatic period as compared to the survivors.

Conclusion: Within this pilot study we analysed for the first time STAT1 nuclear translocation in respect of the reporter gene SOCS1 mRNA expression in PMN obtained from major trauma patients in the early posttraumatic period. Thereby, STAT1 activity is significantly increased in major trauma patients. Patients of adverse outcome show minor activity of STAT1 than those of good outcome. This reduced activation is accompanied by reduced mRNA expression of SOCS1 reporter gene. As preliminary data, further investigation will be performed in order to increase the number of enrolled patients as well as to correlate the demonstrated results with peripheral cytokine concentrations.

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INFLAMMATORY AND BIOCHEMICAL MODULATION FOLLOWING TRUNKAL SKELETAL SURGERY: PROSPECTIVE, COHORT STUDY TO QUANTIFY THE BURDEN OF SURGERY

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Purpose: The physiological and biochemical impacts of the primary trauma (first hit) and subsequent insult including surgical intervention (second hit) have been extensively described and widely debated for various types of skeletal trauma. Pro-inflammatory cytokines can be used as markers to monitor the development of variety of adverse outcomes in polytrauma patients. We previously demonstrated the strong correlation of these parameters with the risk of development of multiple organ failure in multiple injured patients. Moreover, we reported the significant increase of these pro-inflammatory parameters during the process of instrumentation in reamed femoral nailing (control group). Our aim, therefore, is to investigate the sub-clinical effect of various inflammatory variables in blunt trauma patients who underwent pelvic and acetabular surgical procedures.

Patients and Methods: Serially sampled central venous blood for peri-operative concentrations of IL-6 and IL- 8 (WAK Medical, Bad Homburg, Germany) have been evaluated by independent reviewer in 7 consecutive patients with different types of pelvic and acetabular fractures who underwent open reduction and internal fixation. The types of the pelvic fractures were as follows: 1 isolated symphysis pubis disruption, 1 sacral fracture with bilateral disruption of sacroiliac joints, and 1 isolated iliac wing fracture. The distributions of acetabular fractures according to AO classification were: 1 Type-A, 1 Type-B, and 2 Type-C.

The mean time of surgery for pelvic and acetabular fractures was 210 mins (92-370). In all patients the clinical and physiological variables were stable peri-operatively.

Results: Open reduction and internal fixation of pelvic and acetabular fractures is associated with a significant peri-operative increase in the concentration of serum IL-6 and IL-8 levels. These findings were comparable to the results in the control group. The highest concentrations of pro-inflammatory cytokines were evident significantly in

Table 1: Perioperative serum concentrations of IL-6 and IL-8

Group		B	End	7 H	24 H	48 H
Femoral Nailing ©	IL-6	52	78	110	250	200
	IL-8	40	-	100	90	75
Pelvic and Acetabulum	IL-6	13	23	99	119	44
	IL-8	19	28	38	80	14

B: Preoperative, End: End of operation, 7H: 7 Hrs postoperatively, 24H: 24 Hrs postoperatively, 48H: 48Hrs postoperatively, ©: Control group

Results: (mean±S.D., N=4)

GROUP	TNF		IL-6		Apoptosis
	Ap	Ba	Ap	Ba	
Caco-2 Control	0.5±0.1	1.0±0.4	2.1±0.2	2.4±0.4	2.6±0.2
Caco-2 + Et	6.9±0.1	1.2±0.2	2.0±0.5	0.5±0.2	3.2±1.0
Caco-2 + Et + EC	21.4±0.9*	11.0±0.6*	8.3±0.8*	1.0±0.5	4.3±0.4
Caco-2 + H/R	26.9±1.0*	4.8±0.4*	10.8±0.3*	8.6±0.6*	6.6±0.9*
Caco-2 + EC + H/R	19.8±1.0*	10.0±1.4*	72.3±1.3*#	12.9±0.5*	9.0±1.2*#

*p<0.001 vs Caco2 control, #p<0.001 vs. group IV

sacral fractures. The peak concentrations of IL-6 and IL-8 occurred at 7 and 24 hours postoperatively with IL-6 revealed to be the strongest associate (Table 1). These results are comparable to those in the control group.

Conclusion: Major trauma imposes changes to the inflammatory markers in patients with stable cardiopulmonary function. Reconstructive surgery inflict additional burden to the already traumatized patient. Although the clinical condition may appear stable, we feel it is prudent to respect the sub-clinical evidence of activation of inflammatory cascade system that may further harm the patient. Therefore we recommend, based on our primary results, that such surgical procedures should be carefully assessed and well planned to determine whether such measures can be performed safely and to appreciate the impact of these procedures, especially in complex polytrauma cases where second hit phenomenon is inherently hazardous, in order to improve the overall results and clinical outcome in the future. However, further research in this field is still required.

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SYNERGISTIC EFFECT OF ETHANOL AND SHOCK INSULTS ON GUT CYTOKINE PRODUCTION AND BARRIER DYSFUNCTION

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Introduction: Gut epithelial cells are important in orchestrating immunoinflammatory responses in the gut and may impact systemic immunocompetent cells following shock and trauma. Ethanol (Et) intoxication is an important etiologic factor in trauma and may increase the likelihood of post-traumatic septic complications. Both Et and gut ischemia-reperfusion impair intestinal barrier function. However, their combined effects on intestinal epithelial cell function and barrier integrity is unknown.

Methods: Confluent Caco2 cell monolayers were grown in a two chamber culture system and exposed to 0.1% Et and/or Escherichia coli C-25 (EC) under normoxic (21% O2) or hypoxia (5% O2) followed by reoxygenation (H/R). Apical and basal compartment supernatents were collected and TNF and IL6 quantitated by ELISA (pg/ml). Caco2 cell integrity was indexed by apoptosis and monolayer permeability.

Permeability changes were greatest in group V

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IS A SECONDARY OPERATION AFTER POLYTRAUMA REFLECTED BY ALTERED SYSTEMIC CYTOKINE RESPONSE?

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Objective: In multiple injured patients secondary operations are frequently necessary. These operative trauma represent further injury mechanisms which may immunomechanistically summarized as "second hit". The initial trauma and subsequent surgery lead to a complex activation of local and circulating leukocytes. The imbalanced release of inflammatory mediators during the immunological response to severe trauma leads to the systemic inflammatory response syndrome (SIRS) and may finally result in multiple organic failure (MOF).

Material and Methods: In a retrospective study, 71 (13 women and 58 men) severely injured patients (16,6% non-survivor, n=12) with a mean age of 41 ± 14 years (range 15-75) and an injury severity score (ISS) of 33 ± 12 points (range 16-66) were analyzed. All patients were primary admitted to the ICU and stayed for 28 ± 15 days (range 8-80). Plasma samples were daily collected and analyzed for cytokine content (IL-4, IL-10, IL-11, IL-18, IL-12p40, IL-12p70) by ELISA. The patients were divided into different groups: Non-survivor, survivor and into ISS 16-25, ISS 26-37 and ISS ≥ 38. Cytokine concentrations before surgery were correlated versus cytokine values one and two days after secondary surgery, in addition cytokine concentrations were correlated versus CRP and leukocyte count.

Results: In this study an influence of secondary operations on the systemic concentration of the analyzed cytokines was not observed. Our data revealed no significant differences in cytokine concentrations before and after secondary operation and additionally, there was no correlation between plasma IL-4, IL-10, IL-11, IL-

12p40, IL-12p70 concentrations and clinical parameters (CRP, leukocyte count).

Conclusion: These results may challenge immuno-mechanistic theories which have to be reevaluated in further clinical studies.

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EPIDEMIOLOGY AND CIRCADIAN ANALYSIS OF TRAUMA AND TRAFFIC ACCIDENTS IN TOKYO, JAPAN

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Objective: Previous studies have suggested circadian patterns for trauma including traffic accident, injuries and other exogenous diseases. To investigate epidemiology and circadian variation in ambulance use based on the occurrences of trauma, we aimed to describe the epidemiological characteristics of all ambulance use for trauma in Tokyo, Japan over one year study period.

Material and Methods: We reviewed all patients referred to all emergency departments of Tokyo, Japan, from January 2005 to December 2005 based on the ambulance records of the Tokyo metropolitan Fire department. We also analyzed the demographics, time, date, week, month, injury reason, initial diagnosis and severity based on the first impression of emergency physicians in patients with trauma including traffic accidents. Circadian patterns of trauma and traffic accidents were compared to those of patients other than trauma and traffic accidents.

Data: We identified total of 643849 patients who were transported to hospitals by ambulances during one year. There were 226339 patients of trauma including 94916 patients (42%) from traffic accidents. Trauma was one of the most frequent reasons for an ambulance use. The mean age was: 55 years old for non-trauma general patients; 46 for all patients with trauma; 37 for patients with traffic accidents. Patients with trauma and traffic accidents were significantly younger than non-trauma general patients ($p<0.001$). The proportions of male gender were about 51% for non-trauma general patients, 59% for trauma patients and 59% for patients with traffic accidents. The significantly higher proportions of male gender were noted among patients with trauma and traffic accidents than among non-trauma general patients ($p<0.001$). For the severity of trauma patients, 77.6% was mild, 20.0% was moderate, 1.4% was severe, 0.8% was the most severe and 0.3% was dead on arrival and for severity of patients. In traffic accidents, 88.4% was mild, 9.5% was moderate, 1.3% was severe, 0.6% was the most severe and 0.1% was dead on arrival. Patients with traffic accidents showed many more patients with milder severity ($p<0.001$). For the situations of injury, 9.6% was pedestrian walker, 28.7% was bicycle, 24.3% was motorcycle, 28.4% was car, and uncertain injury reason was 9.0%.There was a significant difference for severity distributions by injury situations ($p<0.001$). Traffic accidents involved with pedestrians included the more severe cases, while traffic accidents involved with cars included the less severe cases. There were significant circadian rhythms of trauma and traffic accidents for ambulance use. The highest ambulance uses of traffic accidents were identified in 8 AM and 5 PM. Ambulance use of trauma patients was high and stable from 9 AM to 7 PM. The least ambulance uses of both trauma and traffic accidents were noted in 4 AM.

Conclusion: In patients with ambulance use in Tokyo, trauma patients including traffic accidents are younger and male-predominant than non-trauma general patients. Patients with traffic accidents indicate lower severity than trauma patients other than traffic accidents, although pedestrian involvement shows the more severe cases. Significant circadian variations are identified in trauma and traffic accidents.

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REGULATION OF THE LIVER X RECEPTORS DURING EXPERIMENTAL SEPSIS

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Background: The "liver X receptors" (LXR) are nuclear transcription factors that act as cholesterol sensors by binding oxidized derivatives of cholesterol. LXR resides within the nucleus and binds to DNA as a heterodimer with retinoid X receptor (RXR). There are two isoforms, LXRα and LXRβ. LXRα is highly expressed in the liver, while LXRβ is expressed ubiquitously. Recent data have demonstrated that LXR activity suppresses systemic inflammation by inhibition of inflammatory gene expression, improves glucose tolerance through coordinate regulation of glucose metabolism in liver and adipose tissue, and promotes macrophage survival during infection, thereby enhancing immune defence.

Aim: On this background, we recently introduced the concept that LXR may be a node in the pathophysiology of sepsis. The aim of the present study was to study the gene regulation of LXR during experimental sepsis.

Method: A rat model of sepsis caused by cecal ligation and puncture (CLP) was used. By ligating and puncturing the cecum, rats develops severe peritonitis within 24 hours. In separate sets of experiments, rats were sacrificed at 6, 10, 18, and 24 hours following CLP or sham operation. Liver samples were snap frozen in liquid nitrogen, and RNA isolated. Gene expression of RXRα, LXRα and LXRβ was examined by real-time RT-PCR.

Results: We demonstrate that LXRα is down-regulated in the liver at 10 and 18 h following CLP, whereas LXRβ or RXRα mRNA were unchanged. No regulation of LXRα

or LXRβ mRNA was observed in the lung or in the intestine.

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CHANGES IN ENDOGENOUS HORMONE PRODUCTION FOLLOWING A MAJOR BURN

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Introduction: A severe burn injury is associated with increased levels of cortisol and catecholamines, while endogenous anabolic hormones are decreased. The purpose of this study was to determine in a large clinical prospective trial the acute and long-term pattern of urine cortisol and catecholamine expression in severely burned children, the association of changes to endogenous anabolic hormone levels and to assess gender differences.

Methods: Two hundred and twelve patients, admitted to our unit from June 1998 to May 2004 with burns greater than 40% were studied in a large prospective clinical trial. Each patient had 24hr urine collections during their acute admission as well as the reconstructive period. Urine was analyzed for Cortisol and blood drawn at the same time as urine collection analyzed for IGF-1, IGFBP-3, HGH, I-PTH, Cortisol, Osteocalcin, Total T4, T3 Uptake and FTI.Statistical analysis: ANOVA, Multiple Linear Regression analysis and Students t-test with Bonferroni's correction were used where appropriate.

Results: 70 female and 138 males were studied. To analyze the effect of cortisol levels on endogenous anabolic hormone levels patients were divided into those with normal or high urine cortisol levels. High urine cortisol levels were associated with decreased IGF, IGFBP-3, Osteocalcin, total T4 and FTI ($p<0.05$). High urinary cortisol levels were associated with increased serum cortisol and T3 uptake ($p<0.05$). Urine cortisol over time results were divided into groups of 0-10, 10.1-20, 20.1-40, 40.1-100, 100.1-200, 200.1-500 and >500 days after burn. Urinary cortisol levels showed a statistically significant increase in the initial stages followed by a steady decline (0-10 days: mean 283μg/24hrs, 10.1-20: mean 228, 20.1-40: mean 170, 40.1-100: mean 146) P=<0.001. Cortisol levels returned to near normal (2-90μg/24hrs) after 3 months. TBSA burn and depth of burn showed no significant correlation with urinary cortisol level at any time point. Male patients had significantly higher urinary cortisol compared to female patients in the same time period. (0-10: m=327μg/24hrs; f=205μg/24hrs, p=0.02. 10.1-20: m=253; f=193, p=0.04. 20.1-40: m=195; f=132, p=0.02. 40.1-100: m=158; f=132, p=0.7. 100.1-200: m=87; f=44 p=0.0007. 200.1-500: m=81; f=64, p=0.08, >500: m=86; f=64, p=0.05)

Summary: Urinary cortisol levels following a major burn are greater in male patients than in females however they both decline over time to near normal. Elevated urinary cortisol levels are associated with decreased endogenous anabolic hormone levels, indicating increased catabolic activity. This suggests that control of increased cortisol may lead to increased endogenous anabolic hormone levels leading to decreased recovery time and increased wound healing

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CHRONOLOGICAL EXPRESSION OF PAR ISOFORMS IN ACUTE LIVER INJURY AND ITS AMELIORATION BY PAR2 BLOCKADE IN A RAT MODEL OF SEPSIS

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Objectives: The liver can be injured and its functions altered by activation of the coagulation and inflammatory processes in sepsis. The objective of the present study was to: 1) investigate the pattern of protease-activated receptors (PARs) over time in a model of acute liver injury induced by lipopolysaccharide (LPS); and whether PARs play a role in this process and exert their effects through inflammation and coagulation.

Material and Methods: The effects of LPS (15 mg/kg) on inflammation, coagulation and fibrinolysis, PARs, and liver injury in rats, were investigated with and without the PAR2 blocking peptide (PAR2 BP).

Data: Levels of tumor necrosis factor-alpha (TNF-alpha) were significantly expressed 1 h after LPS administration followed by: 1) an increase in levels of tissue factor, factor VIIa, thrombin and plasminogen activator inhibitor-1; 2) unchanged or steady levels of tissue factor pathway inhibitor; and 3) subsequent deposition of fibrin in the liver tissue, that led to the elevation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), which are associated with liver injury. The expression of all PAR isoforms (1-4) was elevated, and each isoform had a distinct cellular localization (hepatocytes, Kupffer cells, the portal triad area, and central veins) and a time-dependent pattern of expression. The immuno-reactivity of PAR2 and 4 in Kupffer cells was intense. Interestingly, PAR2 BP improved the healing of liver injuries, an effect that was associated with suppression of TNF-alpha elevation, and normalization of coagulation and fibrinolysis. This ultimately led to decreased fibrin formation in the injured liver.

Conclusions: The present study reveals a distinct chronological expression and cellular localization of PARs in LPS-mediated liver injury and shows that blockade of PAR2 may play a crucial role in treating liver injury, via normalization of inflammation, coagulation and fibrinolytic pathways.

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DIAGNOSIS AND THERAPY OF CAUSTIC INJURIES OF THE UPPER GI TRACT IN A TERTIARY REFERRAL CENTER – A 5-YEAR RETROSPECTIVE ANALYSIS

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Background: Management of caustic injuries are still a matter of debate. Endoscopical and medical treatments are controversially discussed in the literature.

Aim: Our purpose was to analyze the management and outcome of the patients with caustic esophageal injuries admitted to our emergency and gastroenterological department.

Methods: A 5-year retrospective database analysis was performed to identify patients with caustic esophageal injuries. Clinical presentation on admission, results of esophago-gastro-duodenoscopy, therapy and outcome was documented.

Results: The diagnostic and therapeutic approach to our patients varied. An individual strategy was chosen based upon the severity of clinical presentation. In total, 43 adult patients with caustic injuries within the last 5 years were included in the study. Due to severe clinical symptoms (e.g. retrosternal or epigastric pain, hypersalivation) 17 patients underwent immediate upper gastrointestinal endoscopy. In spite of protective medical treatment 4 patients required surgical interventions with esophagectomy and colonic interposition. 6 patients developed relevant esophageal strictures with dysphagia and the necessity for endoscopic dilatation in the follow-up period. None of the patients developed esophageal cancer.

Conclusion: Based on our own experience of patients with caustic injuries the majority of patients could be effectively treated by conservative endoscopical and medical therapies without further need for surgical interventions.

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LETAL PNEUMONIA BY STREPTOCOCCUS PYOGENES 4 WEEKS AFTER SIMULTANEOUS PANCREAS- KIDNEY-TRANSPLANTATION – A CASE REPORT

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Objective: Pneumonia is the main reason for infection-associated death. Transplant patients are at an increased risk due to immunosuppression and due to an extended bacterial and viral spectrum.

Methods: Case report

Data: A 30 years old female patient underwent a simultaneous pancreas-kidney transplantation because of complicated diabetic disease July 2005. Following an uncomplicated postoperative course the patient was discharged 4 weeks postoperatively in good condition and with stable transplant organ function under standard immunosuppression including steroids, tacrolimus and mycophenolatmofetil. Three days later the patient was submitted with high fever and severe airway infection. Immediate antibiotic therapy was initiated with ceftriaxon and ciprofloxacin following diagnosis of a pneumonia on same day plain x-ray and CT-scan. The patient developed severe hemorrhagic pneumonia with bronchial hemorrhage. Despite early aggressive therapy she died within 24 hours exhibiting multiple organ failure. Microbiology discovered streptococcus pyogenes at a concentration of > 1.000.000/ml at broncho-alveolar lavage. Pathology demonstrated a most severe hemorrhagic pneumonia.

Conclusion: Simulare case reports have been presented so far in immunosuppressed patients with lung infection with streptococcus group A, staphylococcus and haemophilus influenzae. In case of such an infection it is suggested to treat immediately with penicillin G und clindamycin (for toxin suppression) in addition to the established antibiotic regime of the individual hospital/unit.

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CARDIAC FUNCTION DERANGEMENTS IN PEDIATRIC BURN PATIENTS

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Background: Severe thermal injury is followed by a period of hypermetabolism and catabolism that has been shown to last up to 24 months. However, little is known of the long term effects of this response on cardiac function. This study investigates cardiac derangements in massively burned children for a period of 2 years following the initial injury

Methods: Eighty-three pediatric patients with greater than 40% total body surface area (TBSA) burns were enrolled into the study. Cardiac Output, Stroke Volume, Cardiac index and Heart Rate were collected weekly on each patient during acute admission and subsequent follow up visits at 6, 9, 12, 18 and 24 months under controlled conditions. Patient results were divided into standard timepoints 0-10, 11-20, 21-40, 41-100, 100-200, 201-300, 301-400, 401-600 and 601-800 days post burn. Differences between timepoints and age groups were measured. Age groups were defined as 0-3, 4-10 and >10yrs old. The measured results were calculated as a percentage of predicted value, taken from normograms for age matched non-burned individuals. Statistical anal-

ysis was performed using ANOVA with Bonferoni's correction

Results: Cardiac Output, Cardiac Index and Heart rate all decrease with time ($p<0.05$), while Stroke volume increases over time. Cardiac Output and Heart rate remained above 120% of predicted at 24 months following injury in the two youngest groups. When divided into age groups Cardiac Output, Cardiac Index and Stroke Volume were all inversely proportional to age ($p<0.05$). Significant differences were shown between age groups at each timepoint.

Conclusions: Cardiac function is markedly deranged following a severe thermal injury in pediatric patients. The derangement in function persists up to twenty four months. This study suggests the need for the use of pharmacotherapeutic options in the control of the hypermetabolic response for at least two years following injury, which is longer than previously thought.

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MANAGEMENT OF ABDOMINAL EMERGENCIES BY A SURGICAL HIGH-DEPENDENCY UNIT

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Background: A minority of hospitals in Spain have a high-dependency unit (HDU), managed by surgical specialists. This study sought to compare the outcomes of patients undergoing major emergency abdominal surgery with regard to HDU utilization.

Methods: Data were collected prospectively from two groups of patients over 2 years. Patients in the no-HDU group underwent major abdominal surgery in a hospital with an ICU managed by intensive's and returned to a general surgical ward. The other group was managed initially in an surgical HDU. Data collected included Physiological and Operative Severity Score for enUmeration of Mortality and morbidity (POSSUM) scores, complications, deaths and length of stay, for every case-mix grouped by Diagnosis Related Groups (DRG)

Results: Physiological and operative scores calculated by POSSUM scoring were similar in both groups. The HDU group comprised 213 patients: expected (O : E) morbidity ratio was 1.09, whereas (O : E) mortality was 0.83. 58 per cent of patients stayed in hospital shorter than was predicted. The no-HDU group comprised 171 patients. expected morbidity was significantly higher (O : E ratio 1.74). Mortality ratio was also significantly higher (O : E ratio 1.13). The O : E ratios for morbidity were significantly different ($P < 0.0005$). Some 63 per cent stayed longer than predicted. ($p< 0{,}001$). Patients in HDU Unit presented higher reoperation rates than ICU patients, when morbidity scores where over 50%, and stay in HDU was significantly lower for every given case-mix.

Conclusions: Postoperative management on an HDU was associated with fewer complications, morbidty and mortality rates with shorter hospital stay. Implication of surgeons in critical care of surgical patients provides a more aggressive approach for patients with high expectatives of complicated surgical recovery.

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A MULTIPLEX REAL-TIME PCR ASSAY FOR RAPID DETECTION AND DIFFERENTIATION OF 25 BACTERIAL AND FUNGAL PATHOGENS FROM WHOLE BLOOD SAMPLES

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Objective: Early detection and adequate treatment of bacterial or fungal bloodstream infections (BSI) are crucial for patient outcome. Recently, real time PCR based assays have been introduced for rapid detection of several microorganisms involved in BSI. However, these assays currently suffer from important limitations: Commonly, fungi are not part of the pathogen detection panel and DNA is mainly prepared from plasma and not from whole blood thereby missing bacterial cells or bacterial DNA already engulfed by phagocytes such as granulocytes and macrophages. In addition, most of these assays lack adequate assay and process controls, and usually do not use high quality DNA-free reagents or, do apply alternatively, proper reagent decontamination procedures, giving rise to questions of workflow contamination and consequently lower sensitivity and specificity. Here, we present a multi-target, multiplex real-time PCR based assay developed for rapid detection and identification of bacterial and fungal microorganisms directly from whole blood.

Material and Methods: A real time PCR assay using DNA free reagents and possessing full process controls was developed. The assay uses internal transcribed spacer region (ITS) as the target region for specific amplification of bacterial and fungal DNA. It is located between the 16S and the 23S ribosomal DNA sequences of all gram-negative and gram-positive bacteria and between the 18S and 5.8S ribosomal sequences of all fungi. Each target of the specified species was amplified with either generic or specific primers. The amplicons were detected using specific pairs of hybridization probes. Assay precision, analytical sensitivity and specificity (inclusivity/exclusivity) was evaluated.

Data: Assay precision: The maximal intra-assay variance observed was 0.22°C (S.D.: 0.03–0.22°C). The overall inter-assay precision varied between 0.19°C and 0.54°C (S.D.: 0.12–0.27°C). Analytical sensitivity and specificity: The detection limit determined with EDTA-blood samples spiked with bacterial and fungal reference strains was 30 CFU/ml for all microorganisms except for C. glabrata (100 CFU/ml). To further study the inclusivity of

the assay the uniformity and homogeneity of the ITS target region 1548 isolates in total were analyzed. The overall sensitivity (number of detected isolates / number of isolates tested) is 98.8%, with an overall accuracy of 98.8% (number of correctly detected isolates / number of isolates detected).

Conclusion: By use of this assay DNA from 25 clinically important microorganisms involved in BSI can be identified from whole blood samples to the species level within a turn around time of less than six hours.

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EFFECTS OF SURGICAL STRESS AND ENDOTOXIN ON GASTROINTESTINAL MYOELECTRIC ACTIVITY AND TRANSIT

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Objective: The combined effects of surgical stress and endotoxemia on gastrointestinal myoelectric activity and transit were investigated.

Materials and Methods: Experimental models of surgical trauma and endotoxemia were established in rats. Animals were divided into three groups: a control group (n = 10), LAP group (n = 10) and LAP + LPS group (n = 10). Rats in LAP and LAP + LPS groups received laparotomy. Rats were implanted with three bipolar electrodes positioned at antrum, 3 and 30 cm distal to the pylorus. An infusion cannula was inserted into the stomach. Rats in LAP + LPS group received injection 0.2 mg/kg i.v lipopolysaccharide from E. coli, serotype O55:B5. Recordings of antroduodenojejunal electromyograms and intestinal transit studies were performed on postoperative day (POD) 1 and 2. The phases of the migrating myoelectric complex (MMC) and the occurrence of the migrating action potential complex (MAPC) were measured. The intestinal transit was calculated as the percentage of the distance traveled by the marker Evans blue relative to the total length of the small intestine.

Results: The MMC phase II duration on POD 1 in the LAP and LAP + LPS groups was increased at the duodenum and phase III duration was decreased at the antrum, duodenum, and proximal segment of the jejunum ($P < 0.05$). The transit index on POD 1 in the LAP group (24.8 ± 4.8 %) and LAP + LPS group (16.2 ± 4.4 %) was shorter than that in the control group (35.8 ± 11.1 %, $P < 0.05$). Although rats in the LAP group recovered the MMC phase III duration at the jejunum on POD 2, no rats in the LAP group recovered MMC at the antrum and duodenum. The transit index on POD 2 in the LAP group was recovered (37.8 ± 10.3 %). The occurrence of the MAPC at the duodenum and jejunum on POD 2 was observed only in the LAP + LPS group. Moreover, the transit index was significantly increased in the LAP + LPS group (50.9 ± 9.3 %) when compared with the control group (35.8 ± 11.1 %, $P< 0.05$).

Conclusions: Full recovery of liquid intestinal transit precedes the return of MMC activity after abdominal surgery in the rats. The combined effects of surgical stress and endotoxemia may cause abnormal increased intestinal transit and may cause the delayed recovery of MMC activity.

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THE RELATIONSHIP BETWEEN THE DEPTH OF THE SEDATION AND THE MORTALITY RATE OF THE PATIENTS UNDERGOING MECHANICAL VENTILATION

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Introduction: In the intensive care unit (ICU), sedation is used to improve the comfort and safety of patients undergoing mechanical ventilation. We usually use continuous administration of sedatives for the patients who are under mechanical ventilatory support. However, sedation has many adverse effects on the patients. We have analyzed the relationship of the depth of the sedation and the mortality rate of the ICU patient undergoing mechanical ventilation.

Hypothesis: The severity of the respiratory failure may affect the depth of the continuous sedation and furthermore the depth of the sedation may affect the mortality rate of the critically ill patients.

Methods: Thirty six patients who had mechanical ventilatory support over 3 days were analyzed retrospectively. The patients who had the problem of the central nervous system were excluded. All the patients were provided continuous sedation during their mechanical ventilatory support period according to the local guideline of the continuous sedation. Sedation agitation scale (SAS) was used to evaluate the depth of the sedation and recorded by nurse at least 3 times a day during their mechanical ventilation periods. The recorded SAS during the first 3 days, APACHE II score, P/F ratio at the start of the mechanical ventilation and the mortality rate was examined.

Results: The average SAS of the first 3 days of the patients who suffered lung injury (P/F ratio < 300) and the patients of P/F ratio < 300 were 2.32 ± 0.20, 3.42 ± 0.15, respectively ($p < 0.01$). The average SAS of the first 3 days of the patients who survived was significant higher than those of the patients who died.

Conclusions: In severely lung injured patients, they tend to be oversedated during mechanical ventilation. The depth of the sedation may affect to the mortality rate of the severely ill patients.

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MANNHEIM PERITONITIS INDEX: PROGNOSTIC SCORE IN INTRA-ABDOMINAL ORGAN PERFORATION

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Background/Aim: Prognostic evaluation of patients with intra-abdominal organ perforation is useful to provide classification of the severity of disease and in predicting mortality. Mannheim peritonitis index (MPI) is a simple scoring system and it appears to be practical than the other scoring system. In this study, we used MPI to select high-risk patients, to predict prognosis and to search it's usage without the need of intensive care units.

Method: To determine the prognostic effect of MPI, 179 patients treated with surgical operation were studied in our clinic. Patients categorized according to age, male/female ratio, etiology, MPI, morbidity and mortality. Patients were grouped into three categories based on disease severity, those with MPI less than 21 (Group I), between 21-29 (Group II) and greater than 29 (Group III).This study based on the prospective analysis of the patients. Patients were followed up until death or discharge.

Results: The patients with a mean age of 45.5 years (35-72) admitted to our Surgical Unit. Male and female ratio is 1.1/1. The perforated gastric ulcer was found the leading cause of peritonitis in 59 (32%) patients followed by appendicitis (31%), perforated duodenal ulcer (17%), small bowel perforation (5%) and perforated gall bladder, perforation of colon diverticulum, anastomosis discharge. We examined 117(65%) patients in Group I. Group II and III contained 29 (16%) and 33 (18%) patients. All patients underwent laparotomy. Postoperative complications appeared in 24 (13%) patients. These included respiratory failure (13 patients), renal failure (5), dysfunctional nervous system (4) and pulmonary embolus (2). Postoperative mortality was seen in nine (1.1%) patients. The male /female death ratio was 8/1 and mean age was 44. No mortality was seen in Group I. Mortality rate was 44 per cent (perforated ulcer in three, small bowel perforation in one patient) in Group II and 56 per cent (colon , small bowel, peptic ulcer perforation in 2,2 and one patient) in Group III. From nine patients, seven of them died with multiorgan failure and another two with embolus. Morbidity and mortality was seen significantly increased in Group II and III.

Conclusion: The MPI is shown as a prognostic index for intra-abdominal organ perforation with high accuracy in high-risk patients and it could be an easily clinical practice.

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CLINICAL DYNAMIC OBSERVATION OF LIDOCAINE'S FUNCTION IN PATIENTS WITH ACUTE CEREBRAL CONTUSIONS

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Objectives: It is reported that lidocaine can alleviate edma, reduce encephalic pressure, this is partially due to lidocaine's function of slowing down cerebral metabolism, stabalizing cell membranes, and improving blood circulation. This study is to test the effect of lidocaine on traumatic hydrocephalus and also to seek an effectual way to treat traumatic brain damage.

Method: The patients receiving emergency treatment that are diagnosed as brain contusion sufferers after a CT scan should be GCS graded and those who with the same level are divided into two groups, A and B. A is the subject group, and patients receive standard treatment as well as the application of lidocaine hydrochloride of 3mg/kg in saline solution at a slow speed for three to five days. Group B patients receive only standard treatment. The cerebral blood flow (CBF) of the two test groups of patients are measured simultaneously in the middle cerebral arteries peak mean flow velocity (VmMCA), anterior cerebral arteries peak mean flow velocity (VmACA) on either side of the brain at 2 h (hour), 12 h, 24h and 72 h intervals after the occured damage, in order to monitor the effect of lidocaine on brain blood flow.

Result: 1) CBF observation of groups A: VmACA at 2 hours was similar to that of the groups B. However, VmMCAs of the injured sides increased significantly within the first 12 hours after the contusions, compared with the controls ($P<0.05$). Since the 24h of the cerebral contusions, VmACAs were significantly faster than those of the groups B ($P<0.01$). At 72, both VmMCA and VmACA decreased a little bit but still kept higher than the groups B (both $P<0.01$).2)CBF observation of the contralateral sides: VmACAs of the contralateral sides, were significantly faster than those of the groups B. This indicates that lidocaine hydrochloride can improve the blood quantity of patients of acute local brain contusion. This function probably has to do with lidocaine's ability to reduce the passing of ions through membranes, to stop the passing of Na+ into the cell and the exchange of Na+ and K+ through cell membranes, thus relatively stablizing the function of cell membranes as well as reducing energy use of cell membranes. Lidocaine can improve the blood flow of contusion sufferers and help maintain normal blood supply, reduce harms done to the brain and can be used for clinical treatment of local brain contusion.

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INFLUENCE OF DIRECT HEMOPERFUSION USING POLYMIXIN B IMMOBILIZED FIBER (PMX- DHP) ON CARDIAC VARIABILITY IN THE PATIENTS WITH SEPTIC SHOCK

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Background: We have reported that direct hemoperfusion using polymixin B immobilized fiber (PMX-DHP) could absorb endotoxin and decrease inflammatory cytokines. On the other hand, cardiac variability has been reported to be impaired in the patients with sepsis.

Objectives: To evaluate the influence of PMX-DHP on cardiac variability in the patients with septic shock.

Patients: Three patients (76-year-old male, 58-year-old male, and 64-year-old female) with septic shock were enrolled into this study. Underlying diseases of septic shock were pyelonephritis, necrosis of small intestine, and group B streptococcal bacteremia. All patients were treated with PMX-DHP on admission day and day 1. All patients could be survived.

Measurements and Main Results: Entropy (En), low frequency power (LF), high frequency power (HF) were measured for 5 minutes before and after PMX-DHP and day 2. Serum interleukin (IL)-6 and IL-8 were measured at the same measurement points. En increased from 12 ± 5 to 26 ± 2 after PMX-DHP ($p<0.05$). LF and HF had a trend to increase after PMX-DHP though they didn't have statistical significance. In addition, En and LF had inverse correlation with IL-6 and IL-8 (En/IL-6: $r = -0.56$, $p<0.05$, En/IL-8: $r = -0.59$, $p<0.05$, LF/IL-6: $r= -0.63$, $p<0.05$, LF/IL-8: $r= -0.67$, $p<0.01$; Spearman's rank correlation coefficient).

Conclusion: PMX-DHP might increase cardiac variability in the patients with septic shock. Cardiac variability might be suppressed under the circumstance of hypercytokinemia.

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PROSPECTIVE ASSESSMENT OF HEPATIC FUNCTION AND POTENTIAL MECHANISMS OF DYSFUNCTION IN SEVERE SEPSIS

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Objective: To assess impairment of hepatic function in severe sepsis with respect to patient outcome and to assess underlying mechanisms of sepsis-induced liver dysfunction.

Material and methods: Incidence and course of impaired hepatic partial functions in severe human sepsis was assessed prospectively, and potential mechanisms of dysfunction were studied in cultured precision-cut human liver tissue. Markers of hepatocellular integrity, synthesis, and excretory function were measured daily in 48 consecutive patients fulfilling criteria for severe sepsis. In addition, plasma disappearance rate of the anionic dye indocyanine green (PDR_{ICG}) was determined.

In precision-cut human liver tissue two series of stimulation experiments were performed to address prototypic cellular events associated with septic shock, either with mixed inflammatory mediators or with phorone to achieve depletion of the cellular glutathione content. After incubation for 0, 6, or 24 hours slices were harvested, weighed and homogenized immediately in a commercial buffer to prepare total RNA. First strand cDNA synthesis for real time PCR was performed. Real-time PCR was run with up- and down-stream primers for various transporter proteins.

Demographic data and APACHE II-, MOD- and SOFA-scores, values obtained from laboratory tests and COLD measurements at inclusion were evaluated with unpaired t-test or Mann-Whitney rank sum test for continuous variables or Fisher's exact test for categorical variables. For serial measurements analysis of variance (ANOVA) was used. For receiver operating characteristics areas under the curve (AUC) were calculated. All p-values are two-tailed. Statistical significance is defined as $p<0.05$.

Data: All-cause mortality was 37.5% with an incidence of liver dysfunction of 42 % and 74% as assessed by hyperbilirubinemia or PDR_{ICG}, respectively. While conventional markers for liver injury (e.g. ALT: AUC 0.48, $p=0.084$; bilirubin: AUC 0.43, $p=0.412$) failed to predict poor outcome, a $PDR_{ICG}<8\%$ (AUC 0.81, $p=0.006$) predicted death with a sensitivity of 81% and specificity of 70%. Weak but significant correlations were obtained between PDR_{ICG}, surrogates of altered blood flow, and inflammation. In the translational studies using cultured human liver tissue rapid alterations in gene expression of various basolateral (e.g. multidrug resistance-associated protein (MRP)3) and canalicular (e.g. MRP2 and bile salt export pump) transporter proteins could be demonstrated. Particularly sensitive proteins regarding stress were the basolateral transporter MRP3 as well as the canalicular transporters MRP2, bile salt export pump (BSEP) and the acid loader AE2, while OATP1, a transporter for ICG on the basolateral membrane, displayed increased expression in both stimulation tests. Overall, the canalicular transporter seemed to be more sensitive to either stress event.

Conclusion: Our data suggest early occurrence of, and a key role for excretory dysfunction, which is underestimated by conventional laboratory markers. PDR_{ICG} better reflects liver dysfunction in the light of diagnostic uncertainty regarding clinical signs of liver failure, such as coagulopathy and encephalopathy in the septic host. Excretory function is important for biotransformation of xenobiotics. This might be an underrated problem in pharmacotherapy in patients presenting with severe sepsis potentially leading to accumulation of cytotoxic xenobiotics in hepatocytes.

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KLEBSIELLA PNEUMONIAE AND CHRONIC SEPSIS: AN INVESTIGATION OF ANTI-INFLAMMATORY IL-10 CYTOKINE INDUCTION IN MACROPHAGES

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An acute, highly lethal peritoneal **Klebsiella** mouse model was converted to a chronic peritonitis model with bid gentamicin. With gentamicin (5 mg/kg, bid) and peritoneal injection of 103 colony forming units (CFU) of **Klebsiella pneumoniae**, 50% of mice survived for two weeks. Without gentamicin (saline control) all mice died in three days. Data showed that in vivo, peritoneal macrophages coexisted with **Klebsiella** without eliciting peritoneal neutrophils until bacteria levels exceeded 105 CFU. Neutralization of the inhibitory cytokine interleukin-10 (IL-10) further increased survival and bacterial clearance. This suggested the possibility that **K. pneumoniae** stimulates macrophage IL-10 production, thus allowing chronic peritoneal infection. RAW 264.7 is a mouse macrophage cell line that is able to respond to bacterial products **in vitro** and produce both TNF-α and IL-10. RAW cells were cultured with **E. coli** endotoxin, **K. pneumoniae** endotoxin or whole **Klebsiell**a bacteria and TNF-α and IL-10 production was determined. Little evidence of inhibition was seen as all **K. pneumoniae** products tested produced an early TNF-α response (~1.5 x104 pg/ml). Both endotoxins and whole **Klebsiella** stimulated a dose dependent TNF response. Although **E.coli** and **K. pneumoniae** endotoxins (1μg/ml) induced IL-10 production (2-4 x103 pg/ml), only high concentrations of whole **Klebsiella**, (107 CFU but not 105 CFU) evoked IL-10 production. The diminished TNF response seen from the lower **Klebsiella** CFU suggests that lack of activation may be an important mechanism of bacterial persistence. Further experiments with individual bacterial components are underway to determine the time course of TNF and IL-10 production and their role in stimulation and inhibition.

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INTERLEUKIN 17 IN ACUTE PANCREATITIS

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Objective. Respiratory failure and hematologic changes are typical for acute necrotizing pancreatitis. Unfortunately, the pathogenesis of these changes is fully unknown. IL-17 is a relatively recently described T-cell derived cytokine. Many of its effects are similar to, although in isolation less potent than, those of IL-1β and TNF-α.

Materials and Methods. Nineteen patients with severe acute pancreatitis with respiratory complications were studied. Quantity of neutrophils, levels of interleukins (IL) 1β, 6, and 17, MCP-1, TNF-α in the plasma and bronchoalveolar fluid were measured.

Results. The IL-17 levels clear correlated with the quantity of total leukocyte, polymorphonuclear leukocytes and hemoglobin level. The significant increase of neutrophils quantity and concentration of all cytokines in bronchoalveolar fluid were noted in all patients with pancreatitis-associated lung injury. The clear correlation between IL-17 levels and concentration of IL-1β, IL-6, TNF-α, MCP-1, and neutrophils quantity was noted. IL-17 has prominent proinflammatory properties due to the induction numerous genes that associated with inflammation, such as IL-1β, IL-6, TNF-α, and GM-CSF. Hematological effects of IL-17 may connect with its ability induced the GM-CSF expression which also enhanced in severe pancreatitis.

Conclusion. These data pointed on the role of IL-17 in the pathogenesis of pancreatitis-associated lung injury and hematological changes.

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ANALYSIS OF SYSTEMIC INTERLEUKIN-17 (IL-17) AFTER SEVERE INJURY

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Objective: IL-17 is the prototype member of a cytokine family that is generally thought to increase inflammation by recruiting other immune cells to peripheral tissue. IL-17 is a cytokine that induce multiple proinflammatory mediators, including chemokines, cytokines and metalloproteasen from epithelial and fibroblast cells. IL-17 may contribute to prediction of outcome after severe injury.

Materials and Methods: We studied the IL-17 and IL-6 plasma levels in 71 multiple injured patients admitted to the surgical ICU (55 male, 16 female) with a mean age of 43 ± 16 years (range 17-82) and an Injury Severity Score (ISS) of mean 33 ± 12 points (range 16-66). Plasma samples were daily collected and analyzed for cytokine content by ELISA. For IL-17 a threshold for normal values was set, because as described in the literature, healthy normal donors has systemic IL-17 concentrations up to 40pg/ml. Patients with at least three values over 40pg/ml were evaluated as patients with revealed IL-17 concentrations.

Results: After cytokine analysis we observed in 94% of the patients IL-17 concentrations under the detection limit (8pg/ml) or under the normal value of 40pg/ml. Only in 6 % (n=9) of the patients we could measure increased

systemic IL-17 concentrations (median: 74.8pg/ml). Our analysis demonstrated in this small subgroup a positive correlation of systemic IL-6 (median: 40.5pg/ml, range 2.6-708.8pg/ml) concentrations and systemic IL-17 (median: 75pg/ml, range 0.1-1822pg/ml) concentrations ($r=0.258$; $p \leq 0.01$). These patient group showed similar pattern of injuries with an ISS of 27 ± 12 points (range 16-41).

Conclusions: On the basis of these data the systemic IL-17 concentration after severe injuries is not suitable as a prognostic marker for outcome prediction after trauma. The systemic IL-17 values seem to cause by the major producer TH-17 cells. However, the role of a posttraumatic modulation of systemic IL-17 in certain patients has to be further analyzed.

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INTERLEUKIN 18 AND ADHESION MOLECULES IN CLINICAL COURSE OF SEVERE PANCREATITIS

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Objective: Migration leukocytes to the site of inflammation one of the initial steps in the development of acute pancreatitis. Frequent observations have confirmed that cytokines are markers of severity and major mediators involved in the pathogenesis of acute pancreatitis. One of the most important targets of cytokine action is the blood vessels, which undergo some structural and functional changes that result in activation of endothelium. Activated endothelium expresses several adhesion receptors, which control the leukocyte recruitment at the inflamed zone. The goal of this study was to examine the relationship between IL-18 and adhesion molecules (ICAM-1 and E-selectin) in acute pancreatitis.

Materials and Methods: Applying the ELISA technique, levels of IL-18, ICAM-1 and E-selectin were studied in 77 patients with acute pancreatitis. Mediators' levels were studied in arterial, venous and pancreatic ascites samples. According the Atlanta criterion the mild pancreatitis was established in 33 patients and severe - in 44 patients. All patients have the alcoholic pancreatitis and admitted in clinic not later 48 hours after disease onset.

Results: The highest levels of IL-18 were noted in ascites and lowest in arterial samples. The highest concentration of adhesion molecules was in venous samples and lowest in ascites. It was a clear correlation between levels of IL-18 and adhesion molecules and severity of pancreatitis. During first week the levels of IL-18 gradually increased in patients with severe pancreatitis, while in patients with the edematous pancreatitis its levels decreased starting from the third day. ICAM-1 levels gradually increased during first three day with the following decrease after this term. The highest levels of E-selectin were noted at the time of admission. The clear correlation between IL-18 and adhesion molecules was noted in both groups of patients. Besides that, the clear strong correlation was observed between IL-18 and quantity of circulating granulocytes and between E-selectin and hematocrite in patients with necrotizing pancreatitis.

Conclusion: Our study confirms the importance of activation of endothelium as a part of the systemic inflammatory response in patients with acute pancreatitis. The inflamed pancreas is the source of proinflammatory cytokines, while the activated venous endothelium - of adhesion molecules.

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SUBSTITUTION OF INTERLEUKIN-2 (IL-2) FAILS TO RESTORE NEONATAL T CELL ACTIVATION TO THE EXTENT OF ADULTS

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Background: IL-2 is a crucial cytokine to mount antiviral reactions of the specific immune system. Paralleled by impaired T cell activation, neonatal T cell IL-2-, TNF-α–, and IFN-γ–production were found to be lower in cord lymphocytes than in blood lymphocytes from adults. T cell reactions may be orchestrated by monocyte-derived macrophages (MΦ), equipped with costimulatory and cytotoxic receptors and cytokines.

Hypothesis: An IL-2 –substitution to neonatal cells fails to restore T cell activation to the extent of that in adults. Methods: Cord blood mononuclear cells and MΦ from neonates (CBMNC; CBMΦ) and adults (PBMNC; PBMΦ) were isolated and stimulated with IL-2, a polyclonal T cell mitogen (anti-CD3 mAb). Receptors influenced by IL-2 (CD25, CD28, CD152, CD80, CD86) were phenotyped by flow cytometry. Cytokine production was analyzed by ELISA; apoptosis was detected by Annexin V-stain; T cell proliferation by CSFE.

Results: CBMNC IL-2 production was lower ($p < 0.05$ vs. PBMNC). In contrast to CBMNC, initial anti-CD3-mediated T cell deletion could be inhibited in PBMNC by IL-2 ($p < 0.05$). Adding IL-2 to anti-CD3 in CBMNC resulted in an increased fraction of deleted T cells ($p < 0.05$ vs. anti-CD3 only). IL-2 increased T cell blast formation more strongly in PBMNC ($p < 0.05$ vs. CBMNC). T cell CD25 and CD28 up-regulation in PBMNC was stronger in the presence of IL-2 (all $p < 0.05$ vs. CBMNC). In contrast, CD152 on surviving neonatal T cells was up-regulated earlier than on T cells from adults. In contrast to CBMΦ, IL-2 dose-dependently up-regulated corresponding CD80 and CD86 receptors on PBMΦ ($p < 0.05$).

Conclusion: The substitution of IL-2 to CBMNC fails to restore T cell activation, suggesting an impaired neonatal cellular immune response.

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DEPLETION OF REGULATORY T-CELLS TO AMPLIFY IN VITRO RESPONSE OF NICKEL SPECIFIC T-CELLS

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NiTi-shape memory alloys (NiTi-SMA) are of biomedical interest due to their unusual properies (superelasticity, shape memory effect). However, the potential release of Ni-ions requires preclinical in vitro testings of implant samples to predict potential allergic reactions. Beside the cytotoxicity of nickel at high concentrations nickel is the most frequent allergic metal known. It is known that $CD4^+$ $CD25^+$ T-cells (T_{reg}s) downregulate the proliferation of specific T-cells after antigen stimulation.

Thus it was the purpose of this study to improve the readout of in vitro testing methods on T-cell reactions towards nickel by the depletion of T_{reg}s from peripheral blood mononuclear cells (PBMC).

PBMC from allergic and non-allergic subjects were cultured in presence and absence of different concentrations of $NiCl_2$ (50 to 500 ng/ml) for 24 h, 7 d and 14 d. Activation and proliferation was analyzed by flow cytometry via $CD3^+$ $CD69^+$ labeling and AlamarBlue assay. The release of cytokines was determined by ELISA.

T_{reg}-depleted PBMC ($PBMC_{Td}$) from allergic subjects showed an increased number of CD3+CD69+ cells. Additionally, there was an increase in proliferation of $PBMC_{Td}$ from allergic subjects in the presence of nickel ions. Also the secretion of IL-5 and IL13 was increased from $PBMC_{Td}$ of allergic subjects compared to non-allergic donors.

Our data suggest that depletion of T_{reg}s from PBMC may be a useful tool for in vitro biocompartibility testings of nickel containing implant materials.

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CHANGES IN REGULATORY T CELL AND MYELOID SUPPRESSOR CELL POPULATIONS AFTER BURN INJURY

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Objective: Burn injury disrupts the mechanical and biological barrier that the skin presents against infection. In addition, burn injury also results in immune suppression that places the host at increased risk of subsequent infection, although the mechanisms of this immune suppression are not known. In this study, we examined two populations of suppressor cells (myeloid suppressor cells (MSCs) and endogenous Tregs) that might play a role in the immune suppression and susceptibility to infection that is evident after severe burn injury.

Material and Methods: We generated a 12.5% TBSA scald burn in C57BL/6 female mice (10-12 weeks of age). The popliteal, inguinal, axillary, cervical and mesenteric lymph nodes were harvested as well as the spleen on post burn days three and seven. The percentages of endogenous Tregs ($CD4^+CD25^+FoxP3^+$) and MSCs ($GR1^+CD11b^+$) were determined by flow citometry. All experiments were conducted with n=4 for the control groups and n=5 for the burned groups on day 3 and day7.

Data: In the spleen, MSC numbers increased from 3.89 ±1.20% (mean ± SD) in the control group to 6.75 ± 2.94% on post-burn day 3 to 6.07±1.80% on post-burn day 7, although these differences did not achieve statistical significance. The percentages of MSCs in lymph nodes also increased modestly at 0.19 ± 0.04% in controls, 0.22 ± 0.04% on post-burn day 3, and 0.28 ± 0.09% on post-burn day 7. The percentage of $CD4^+$ cells that were endogenous Tregs in the spleen declined modestly from 15.40 ± 3.83% in controls to 14.09 ±1.57% on post-burn day 3, and 14.61 ± 2.17 % on post-burn day 7. In pooled lymph nodes, the percentages of endogenous Tregs increased modestly from 12.26 ±1.17% in controls to 12.82 ± 0.33% on post-burn day 3, and 13.88 ± 0.81% on post-burn day 7 ($p<0.05$ by ANOVA and Tukeys post-hoc test).

Conclusion: A 12.5% scald burn injury was associated with small and insignificant increases in the percentages of MSCs in the spleen and lymph nodes. Although Treg numbers significantly increased in the lymph nodes, but not the spleen, the increased numbers were also relatively modest. These results suggest that either a 12.5% burn injury is an insufficiently modest insult in itself to affect substantially the expansion of these suppressor cell populations; and/or that an infectious component most be present to trigger the expansion of such suppressor populations. Increasing the surface area of the burn and/or adding an infectious component such as Pseudomonas infection may be required for the full expansion of these suppressor cell populations.

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MYOCARDIAL ISCHEMIA-REPERFUSION INJURY DURING CARDIOPULMONARY BYPASS

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Cardiac ischemia-reperfusion injury represents a formidable clinical problem after cardiac bypass surgery.

Leukocytes and inflammatory mediators play a critical role in the myocardial tissue during ischemia-reperfusion. While an increase in neutrophil adhesion and transmigration can already be observed during cardiac ischemia, this process increases significantly during reperfusion. This results in myocardial cell death and subsequent loss of contractile function. Interestingly, reperfusion leads to a significantly higher rate of myocyte death than ischemia alone. Clinically, this may be observed as the "stunned myocardium" post-CABG. Since reperfusion injury during cardiac bypass cannot be completely avoided, strategies are needed to minimize its potentially deleterious effects. Aprotinin, a nonspecific serine protease inhibitor that has long been used in cardiovascular surgery because of its antifibrinolytic properties, has been shown to decrease infarct size and to increase contractile function in several animal models. The perioperative use of aprotinin in humans resulted in decreased troponin T levels in the postoperative phase. Aprotinin exerts its anti-inflammatory effects through inhibition of neutrophil extravasation and decreased production of cytokines and proteases.

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THE CRITICAL ROLE OF INFLAMMATION IN PULMONARY DYSFUNCTION AFTER CARDIAC BYPASS

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Lung injury is a common consequence of cardiopulmonary bypass. Much of the pulmonary damage associated with this condition is believed to be mediated through neutrophil infiltration. Elevated neutrophil counts have been demonstrated in lung tissue as well as bronchoalveolar lavage fluid (BALF) from patients undergoing cardiopulmonary bypass. Activated neutrophils subsequently release cytokines and proteases which degrade proteins in human tissue. Elastase is one of the most potent of these proteases. The proteolytic cell damage ultimately leads to loss of integrity of the alveolocapillary membrane. Several studies have demonstrated that arterial oxygenation in patients undergoing cardiopulmonary bypass is negatively correlated with BALF neutrophil counts as well as IL-8 and elastase concentrations. The perioperative administration of aprotinin, a nonspecific serine protease inhibitor that has long been used in cardiovascular surgery because of its antifibrinolytic properties, has been shown to decrease pulmonary neutrophil infiltration as well as IL-8 and serum elastase levels. In addition, aprotinin decreases alveolar-arterial oxygen gradient, $PaCO_2$ and mean airway pressures after cardiopulmonary bypass.

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APROTININ ATTENUATES THE INCIDENCE OF STROKE DURING THE PERIOPERATIVE PERIOD OF CARDIOPULMONARY BYPASS

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Stroke is a potentially devastating consequence of cardiopulmonary bypass surgery (CPB). Although rare, the risk of stroke following CPB is more frequent in high risk patients who are predisposed to comorbidities such as hypertension, diabetes, or history of previous stroke. As the prevalence of cardiac patients who manifest these conditions continues to rise, methods to prevent or reduce perioperative stroke associated with CPB are becoming increasingly paramount.

Aprotinin, a nonspecific serine protease inhibitor used to decrease bleeding during cardiac surgery, has been found to confer neurologic protection in the perioperative period of hypothermic circulatory arrest. Aprotinin is thought to exert its beneficial effects on neural tissue via the inhibition of neutrophil extravasation, as well as by subsequently decreasing the production of proinflammatory cytokines and proteases. Aprotinin has been shown to decrease the postoperative brain edema associated with deep circulatory arrest, as well as harmful metabolic byproducts such as lactate. In addition, aprotinin decreases neural tissue bradykinin, increases available energy stores such as ATP, and decreases neutrophil extravasation and platelet plugging in the neural microvasculature. The benefits that aprotinin exerts during hypothermic circulatory arrest have not only aided in operative hemostasis, but have also succeeded in attenuating the rate of perioperative stroke during cardiopulmonary bypass surgery.

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THE SPECTRUM OF INFLAMMATION-INDUCED COAGULATION IN SEPSIS CAUSED BY *BURKHOLDERIA PSEUDOMALLEI* AND ITS CORRELATION WITH MORTALITY

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Objective: Melioidosis, which is caused by an infection with the Gram-negative bacterium *Burkholderia pseudomallei*, is an important cause of sepsis in South-East Asia and Northern-Australia with a mortality up to 40% in endemic areas. Knowledge of the involvement of coagulation and fibrinolysis in the pathogenesis of melioidosis is highly limited.

Material and Methods: Therefore we analyzed important parameters of coagulation and fibrinolysis in 34 patients with culture proven septic melioidosis and 32 healthy local controls.

Data: Tissue factor (TF), which is considered the central activator of the clotting system, was upregulated on the cell surface of granulocytes of patients with melioidosis (FACS analysis) which corresponded with increased TF mRNA levels in blood leukocytes. Accordingly, high plasma levels of soluble TF (sTF) were measured in patient plasma. Furthermore, significant increases in plasma levels of thrombin-antithrombin complex (TAT) and prothrombin fragment F1+2 were seen in patients compared to healthy controls. Anticoagulant pathways were downregulated in patients: protein C, protein S and antithrombin levels were decreased in patients with melioidosis compared with healthy controls. Patients also demonstrated evidence of activation and inhibition of fibrinolysis, as reflected by elevated plasma concentrations of tissue-type plasminogen activator (tPA), and plasminogen activator inhibitor type I (PAI-1). The extent of coagulation activation (TAT and F1+2 levels) was correlated with mortality.

Conclusion: Strong activation of the coagulation system is an indicator of a poor outcome in patients with sepsis caused by *B. pseudomallei*.

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IMMUNE SEQUELAE IN THE SURGICAL PATIENT

Eugen Faist, Siegfried Zedler

In spite of the progress in diagnostics and therapy of critically ill surgical patients the incidence of severe late complications like multi organ failure and sepsis remains consistently high. Why with identical injury severity degree, some patients develop MOF and others not, is not clear so far. Indeed, the severity and duration of post-traumatic SIRS correlates with the incidence of MOF, though a causal connection between crucial alterations of the immune status and the development of post-traumatic organ failure or sepsis remains unclear. Up to now no early biochemical warning parameter exists, which should indicate reliably the progress of a systemic inflammatory reaction or immune paralysis, respectively, and further distinguish specifically bacterial from non-infectious inflammation. Basically, markers of inflammation and immunodysfunction should reflect the clinical course of seriously ill patients. Predominantly with IL-6 there is a damage associated marker of inflammation available, which, however, cannot discriminate between inflammation and infection. According to actual studies, also from our own group, the determination of IL-6 allows a good early prognostic statement concerning the clinical course of the patients. The increase of IL-6 precedes the development of MODS 2-3 days and thus clearly outruns CRP. Indeed, after severe injury and in sepsis the highest levels of the namely biomarkers are reached, but the severity of inflammation within one patient population with comparable injury pattern is still subjected to considerable variations due to genetic variability. IL-6 clearly counts concordantly as one of the most potent predictive mediators to assess the clinical course of critically ill patients. As a real diagnostic alternative the expression of HLA-DR on the surface of monocytes is favored nowadays as a surrogate marker indicating cellular immunocompetence.

We do further work on new biomarkers of the acute phase reaction and their diagnostic importance as a risk-/warning parameter for the development of sepsis and/or organ failure.

Beside the maintenance of proinflammatory mechanisms, IFN-γ stimulates the production of neopterin in human macrophages and the degradation of the essential amino-acid tryptophan onto kynurenin. Both immunobiochemical processes are narrowly associated with immune stimulation (Th1 type) after trauma leading to increased concentrations of neopterin and accelerated degradation rates of tryptophan. The multiligand surface molecule RAGE serves as a pattern recognition receptor for diverse pro-inflammatory mediators like HMGB-1 and S100/Calgranulins and its engagement results in sustained cellular dysfunction via long-term activation of NF-kappa B leading to increased expression of cytokines. Soluble forms of RAGE, namely the endogenous secretory receptor (esRAGE), as well as the proteolytically cleaved variant sRAGE, were shown to act as decoys binding inflammatory RAGE ligands. Our findings that both markers are circulating in blood under stressful conditions will be discussed with respect to the severity of injury and clinical outcome. Active secretion of intracellular synthesized ubiquitin as well as passive release with tissue damage have to be considered to represent a cellular source of endogenous ubiquitin, an cytokine-like mediator with anti-inflammatory properties. Own preliminary data of our collaborative partner precisely indicate a clear correlation between the plasma levels of extracellular ubiquitin and the survival rate in patients after severe tissue trauma suggesting ubiquitin as a candidate for a biomarker that could also be useful in the estimation of cellular damage after traumatic injury.

Even if a single parameter will never be probably able to display the pathophysiologic situation in the critically ill surgical patient with even sufficient selectivity, increasing efforts must be carried out in the future in order to shape up a suitable panel of sensitive and in particular specific markers. The long-term intention of individual diagnostics must be a timely and dynamic assessment of the extremely complicated illness events with differentiation between bacterial and sterile inflammatory reactions (sepsis/SIRS).

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MONITORING OF SELECTED IMMUNOLOGIC MARKERS AFTER SEVERE BURN INJURY

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In principle any molecules that are released in response to infection and tissue damage, and mediate innate immunity and tissue repair are potential candidates serving as risk/warning parameters for the development of organ failure in severely burned patients. Classical vital and laboratory parameters, however, like leukocyte number, differential blood count, CRP and temperature are less useful to recognize early adverse or beneficial alterations with intensive care patients. Tryptophan is an essential aminoacid and its depletion might contribute to sepsis, organ failure and death following trauma and burns. Therefore, we investigated early after injury on consecutive days the IFN-gamma stimulated indoleamine 2,3-dioxygenase (IDO)-mediated tryptophan degradation via the kynurenin pathway. Blood concentrations of kynurenin and tryptophan were analyzed in plasma samples of 30 patients after severe burns (mean TBSA 41%) by our collaboration partner. In addition the kynurenin to tryptophan ratio (kyn/trp) was calculated to estimate IDO activity. In addition IDO-activation was correlated with plasma concentrations of Neopterin, a marker of Th1-type immune activation. Very recently it has been demonstrated that RAGE-mediated sustained inflammation contributes to irreversible tissue injury. These findings suggest the possibility that soluble RAGE, as decoy for the cell surface receptor, binding inflammatory RAGE ligands such as AGEs, HMGB-1 and S100 proteins, may serve as a biomarker of inflammation and perhaps be useful in the prediction of organ failure. Thus, we will report on the relationship of plasma s/esRAGE levels in the early postburn course to the development of organ failure and clinical outcome of the patients.

It is the central purpose of our work to analyze the dynamics of potentially novel systemic biomarkers in the temporal course and to evaluate their prognostic value with regard to the development of early MOF after severe burn injury and if necessary to scrutinize as a decisive help in the clinical routine. The results of our studies should flow into the advancement and improvement of clinically relevant state of the art immunomonitoring procedures with special emphasis on the question how other known indices of acute stress, such as circulating levels of IL-6 and CRP do correlate with levels of these novel markers.

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DIFFERENTIATION OF IMMUNE RESPONSE PATTERNS IN SURGICAL PATIENTS WITH AND WITHOUT INFECTIOUS COMPLICATIONS

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The inflammatory response to extensive tissue-trauma alters cellular immune function, which renders the patient susceptible to infection. Monitoring this response may allow early recognition of patients being at risk to suffer septic complications. Thus, we analyzed the influence of natural infection on the capacity of PBMCs in whole blood to secrete inflammatory cytokines following major surgery. To our knowledge, little is known about the LPS-induced secretion capacity of IFN-γ, which plays a key role in the functional behaviour of monocytes during an inflammatory process. Next to the fact that recent experimental studies demonstrated that macrophages can be a source of IFN-γ there haven't been any studies in clinical settings looking at consequences of surgical trauma.

In this observational clinical study 41 patients undergoing major surgery received perioperative immune-monitoring. During the postoperative course 11 patients developed sepsis according to the Bone-Criteria. Blood was taken before and on day 1, 3, 5 and 7 after surgery. Mean expression of HLA-DR on the surface of monocytes was analyzed by flowcytometry and whole blood samples were stimulated with LPS for 24 hours. Using Luminex-100-technology we measured LPS-induced levels of TNF-α, IFN-γ and IL-10 in the supernatants. The statistical analysis was done using ANOVA (ANOVA-on-ranks or One-way-ANOVA with the suitable post hoc tests) and t-test or rank sum test as appropriate.

Septic patients showed both decreased HLA-DR expression and LPS-induced TNF-α levels after surgery with significant differences between day 5 and 1, respectively (HLA-DR d0: 2,1 ± 0,45 ,d5: 0,89 ± 0,19; TNF-α d0: 3491,62 ± 427,07 , d1: 2139 ± 295 pg/ml, d7: 1721 ± 266 pg/ml; $p<0.05$) . In contrast HLA-DR expression and LPS-induced TNF-α of non-septic patients returned to baseline after an initial decrease (HLA-DR d0: 2,43 ± 0,31,d1: 1,5 ± 0,2, d7: 2,0 ± 0,4; TNF-α d0: 3573 ± 211pg/ml, d7: 3501 ± 186 pg/ml). LPS-induced IL-10 levels were hardly changed in both groups, except for day 7 post surgery with slightly lower levels in septic patients (Non-septic vs. septic patients d0: 215 ± 25 pg/ml vs. 157 ± 32 pg/ml, d7: 222 ± 15 pg/ml vs. 140 ± 35 pg/ml). Furthermore, LPS-induced IFN-γ was significantly decreased during the postoperative course. In contrast to non-septic patients whose levels recovered from day 1, septic patients showed a continuous decrease with a significant difference on day 7 ($p<0.05$) with respect to controls (septic vs. non-septic patients d0: 1153,99 ± 449,65 pg/ml vs. 758 ± 127,27 pg/ml, d7: 28,56 ± 21,28 pg/ml vs. 359 ± 95,17 pg/ml).

Postoperative sepsis induced alterations of well-known markers of the inflammatory response in this whole blood assay, such as reduced antigen-presenting capacity as indicated by diminished HLA-DR expression on monocytes and decreased LPS-induced TNF-α levels. The LPS-

induced secretion of anti-inflammatory IL-10 showed perioperatively more or less static levels. Furthermore, we found septic patients to have markedly reduced LPS-induced IFN-γ levels. These findings support the concept of monocytes playing an important role in the inflammatory response following tissue trauma. Apart from T-cells and Natural Killer cells, monocytes may be a potential source of IFN-γ and thus significantly contribute to an impaired immune function. Further investigations, in particular on a single cell level, are required to confirm our assumption.

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LEVELS OF SOLUBLE AND ENDOGENOUS SECRETORY RAGE AFTER SEVERE ACCIDENTAL TRAUMA

Robert Kraft, Siegfried Zedler, Eugen Faist

Background: The cell surface receptor for advanced glycation end products (RAGE) is supposed to be involved in inflammatory processes like SIRS and sepsis and may contribute to the development of cellular dysfunction and organ failure. This multiligand receptor also acts as a pattern recognition and signal transduction receptor for diverse other molecules like proinflammatory HMGB-1 and S100/Calgranulins, which are associated with inflammatory conditions. Soluble forms of RAGE consist of a novel splice variant lacking the transmembrane domain of the receptor, named endogenous secretory receptor, esRAGE, as well as proteolytically cleaved forms mediated by extracellular metalloproteinases (sRAGE).

Objective: Evaluation of the prognostic value of systemic sRAGE and esRAGE levels in patients suffering from major burns and after severe multiple injury as early risk-/warning parameter for the development of organ failure or death.

Methods: In a prospective study 31 severely burned patients were included with a mean TBSA of 41.3% (partial and/or full thickness injury) as well as 25 polytraumatized patients with a mean ISS of 31 points. The control group consisted of 30 healthy volunteers. Blood samples were drawn immediately after injury (d 0) and on consecutive days 1, 3, 5 and 7 post trauma. Organ failure has been determined during the entire stay on the ICU according the guidelines of the German Association for the Surgery of Trauma (DGU).

Results: Using commercially available ELISA-kits, our data revealed that the concentrations of total sRAGE were significantly higher on admission (2426±337) and day 1 (1866±300) after severe multiple injury, returning to control level (611±39) on days d 3 (767±137), d 5 (542±68) and d 7 (437±39), obviously due to the non-surviving population [(3615±245 on d 0 and (2789±518) on day 1, respectively]. The determination of esRAGE yield similar results, but on a significant lower level compared to sRAGE (522±102 [d 0] - 189±28 [d 1]). Of note, the concentrations of both soluble receptors remain more or less static in the early posttraumatic period after severe burn injury and were not unrelated to the controls. Finally the subtraction of esRAGE from total sRAGE and the ratio will be analyzed and discussed.

Conclusion: Both forms of soluble RAGE have been found circulating in blood after severe tissue trauma. Thereby elevated concentrations in the early posttraumatic course, in particular after severe multiple injury, seemed to be associated with adverse clinical outcome. Appropriately powered clinical studies are warranted to verify the diagnostic importance of systemic RAGE levels for the development of sepsis and/or organ failure.

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PREOPERATIVE "CARBOPEPTIDE-LOADING"

Edwin Rant

Background: Carbo-loading is generally accepted as a standard procedure in preoperative care to reduce metabolic stress during the surgery-process.

Recently it has been suggested that addition of peptides to the carboloading-drinks has beneficial effects on the patient's mobilization.

Methods: We investigated the consequences of applying carbo-peptide - drinks during preoperative treatment, particularly focussing on the blood-sugar and the gastric residual volume. Fluid volumes of 400 ml versus 200 ml were applied to 14 versus 10 arbitrarily selected patients.

Results: We found that the drink must be given 90 to 120 minutes before induction of anaesthesia, so that a critical gastric residual volume of 200 ml is not exceeded. The carbopeptide-loading - drink had no negative influence on postoperative nausea or vomiting. Thus, the preoperative oral application of carbopeptide solutions is safe and can be recommended.

Key words: Carbopeptide-loading, blood-sugar, gastric residual volume

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SIRNA: NOVEL DESIGN AND PROKARYOTIC GENE SILENCING

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In eukaryotes, small interfering RNA (siRNA)-mediated RNA interference (RNAi) represents a powerful reverse genetic tool and a promising strategy for drug development. Progresses achieved in the field of functional siRNA design significantly contributed to the understanding of cellular RNA silencing pathways and vice versa. We investigated the role of RNA structure in RNAi and developed a structure-based program for selection of active siRNA[1,2]. Major challenges in the design of siRNA and small hairpin RNA include reliable prediction of immunostimulation and off-targeting as well as the avoidance of interference with processing and action of cellular regulatory RNA[3].

Prokaryotes lack the RNAi pathway or major components thereof. Using potent computationally designed siRNA and complementing the prokaryotic repertoire with eukaryotic components, specific functional silencing of chromosomal and episomal genes was achieved in Gram-positive and Gram-negative bacteria as well as in mycobacteria[4]. Prokaryotic gene silencing is distinct from, and excels, antisense effects. Observed phenotypes ranged from transient gene knockdown to persistent gene knockout. This powerful technology can substitute for expensive conventional knockout strategies and opens promising perspectives regarding validation of prokaryotic gene functions and the development of novel antiinfectives.

Acknowledgements: This work was supported by grants from the Fonds Chemie and the RNA Network, funded by the EFRE, the BMBF, and Chiron Corp. which is now part of the Novartis Group.

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A 556

NONINVASIVE MAGNETIC RESONANCE IMAGING OF THE VULNERABLE/HIGH-RISK CAROTID ATHEROSCLEROTIC PLAQUE

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"Vulnerable Plaques" are atherosclerotic plaques which have a high likelihood to cause thrombotic complications, such as myocardial infarction or stroke. Plaques which tend to progress rapidly are also considered to be "vulnerable". Besides luminal stenosis, plaque composition and morphology are key determinants of a plaque's vulnerability to cause cardiovascular events. Noninvasive magnetic resonance imaging (MRI) has great potential to characterize carotid atherosclerotic plaque composition and morphology and thus to assess a plaque's vulnerability. A classification for clinical as well as pathological evaluation of vulnerable plaques was recently put forward, which proposed 5 major and 5 minor criteria to define vulnerable plaques. The purpose of this talk is to summarize the status of MRI to identify the criteria which define vulnerable plaques in the carotid arteries by use of existing MRI techniques.

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THE PATIENT WITH CARDIOGENIC SHOCK (CS) COMPLICATING ACUTE MYOCARDIAL INFARCTION (AMI) – WHICH PATIENT FOR THE CARDIAC SURGEON ?

Hans-Reinhard Zerkowski, Robert Von Wattenwyl

Cardiogenic shock occurs in 7-10% of all AMI (without any dependable predictors). Its mortality reaches 60% and more despite all improvements in treatment during the last decades. There is no debate, that first line treatment is immediate PCI of the AMI related vessel (with/without simultaneous circulatory support). In case of impending or established CS every effort is justified to improve outcome. Based on best available evidence, today, there are two main problems representing targets for aggressive surgical intervention:

1. remaining (significant?) multi-vessel disease despite PCI of the infarct-related stenoses
2. mechanical/functional complications (i.e. acute mitral insufficiency, post-MI VSD, acute "near-complete " heart failure)

Timing of surgery is crucial under all circumstances; main cause of death in CS remains subsequent MOF. Therefore, stabilization of hemodynamics before initiation of multi-organ damage must be first goal. Circulatory support by use of IABP or more advanced systems

(percutaneous CPB, MCS) should be used as soon as available.

CABG is underused in CS, but is followed usually by acceptable results when completed within 12(-24) hours after coronary occlusion.

In case of acute mitral insufficiency due to papillary muscle rupture immediate valve replacement combined with CABG is the treatment of choice; repair can be done in selected cases.

The timing of post-MI VSD is object to controversial discussion due to the fact that trials (RCT) do not exist. Best available evidence from registries suggest under stable hemodynamic conditions (under IABP) some delay makes surgery safer avoiding re-VSD; when renal or liver failure is impending immediate repair under CPB is mandatory, type of repair depends on localization of VSD as well as infracted area, combination with complete revascularization must be strongly recommended.

The use of L- or BiVADs or CPB-based post intervention hypothermia should be considered in selected cases.

To improve data bases for guidelines and recommendations founding registries should be strongly considered.

Disclosure statement: Dr. Zerkowski is member of the supervisory board of Lifebridge AG, Germany.

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MALNUTRITION

John E. Morley

Weight loss has been shown to be the most powerful predictor of death and disability in community dwelling, hospitalized and institutionalized older individuals. There are three major causes of weight loss viz. anorexia, sarcopenia and cachexia. Older persons develop a physiological anorexia of aging due to declining taste and smell, alterations in adaptive relation of the stomach, increased cholecystokinin and the age related decline in testosterone with an increase in leptin. Pathological anorexia occurs with depression, numerous medications and a variety of other conditions. Management includes caloric supplements, treatment of the underlying cause and in selected cases orexigenic agents.

Sarcopenia is the loss of muscle without anorexia. Persons with obese sarcopenia have a particularly poor outcome. The major causes of sarcopenia appears to be mild cytokine excess especially IL-6 and TNFα, and low testosterone. The muscle isoform of IGF-1 also plays a role in the pathogenesis of sarcopenia. Leucine and creatine appear to be important dietary supplements to maintain muscle mass. Ostarine, a SARM, increases muscle mass and strength. Antimyostatin antibodies may enhance muscle strength. Ghrelin agonists are underdeveloped for the treatment of malnutrition.

Cachexia is severe muscle and fat wasting in the presence of underlying disease such as cancer, COPD or congestive heart failure. Cytokines result in proteolysis through the ubiquitin-proteasome system. This leads to amino acids going to the liver to produce acute phase proteins such as CRP. Lipolysis occurs with hypertriglyceridemia. Anorexia and sickness behavior also occur. Treatment is with anticytokine agents such as megestrol.

The management of weight loss is a complex key component of the care of frail older persons.

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METABOLIC AND ENDOCRINE FUNCTIONS IN THE ELDERLY

John E. Morley

Aging is associated with multiple changes in hormones and cytokines. These changes lead to an anorexia of aging and a loss of muscle mass (sarcopenia) with fat infiltration into the muscle (myosteatosis). They are also associated with an increase in diabetes mellitus. In addition, alterations in metabolic function can lead to a decline in cognition. The major hormones involved in the anorexia of aging are cholecystokinin and leptin. Caloric supplements should be given as liquids between meals because of the alterations in compliance of the fundus of the stomach that occurs with aging. Sarcopenia has multiple causes and leads to functional disability. Cytokine excess represents a major cause and, as such, glutamine and fish oils may play a role in retarding age related muscle loss. Oligo fructosaccharides decrease mRNA for TNF and interleukin suggesting a role for prebiotics. Older persons need a high protein intake to protect muscle. The metabolic syndrome leads to myosteatosis. Hypertriglyceridemia appears particularly important in this regard. The hypertriglyceridemia can be limited by giving mono or poly unsaturated fatty acids or fiber. The gut modulates cognition through cholecystokinin and ghrelin, the so called gut-brain axis. Oxidative damage in the brain causes memory disturbance, which can be reversed by alpha-lipoic acid, N-acetylcysteine or fish oils. Decreased testosterone leads to weight loss and possibly hyperglycemia. The understanding of hormone changes with aging can play an important role in attenuating the deleterious effects of inflammation.

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ESTROGEN RECEPTOR BETA (ERB) AGONISM REDUCES DAMAGE IN EXPERIMENTALLY INDUCED LUNG INJURY

James C. Keith, Steven M. Opal, Patricia A. Cristofaro, Emily C. Lutterloh, Jhung Jhung, Glenn Peer

Estrogen receptor beta (ERβ) is expressed in the lung at similar levels in both males and females. Upon binding to its ligand, ERβ mediates a number of cytosolic and transcriptional effects that may protect the host in pro-inflammatory conditions.

An ERβ-selective agonist (WAY-202196) was studied in the murine cecal ligation and puncture (CLP) model of polymicrobial sepsis and in an intravenous *E. coli* challenge model in baboons. WAY-202196 was given at a range of doses either orally (po) or intravenously (iv) at time 0, 24 and 48 hours following CLP in male and female BALB/c mice and intravenously at a dose of 10 mg/kg in baboons 5 minutes before and then at 2, 24, 48, 72 and 96 hours following *E. coli* challenge. Survival, inflammatory markers, lung histopathology, and microbiologic parameters were assessed in both models.

WAY-202196 (1-50 mg/kg PO and 1 & 3mg/kg IV) provided a significant survival advantage ($p<0.01$) following CLP. There was no difference in effectiveness between males and females (studied at 50mg/kg po or 1 & 3mg/kg iv). The ERβ agonist lowered levels of bacteremia and decreased injury in the lung following CLP. Analysis of pulmonary gene expression in satellite animals at 48 hours after CLP revealed attenuation of several gene products by WAY-202196 when compared to vehicle treated animals. The ERβ agonist attenuated clinical signs of pulmonary injury in the baboon model, and histopathologic examination revealed decreased pulmonary damage.

ERβ-selective agonism provided a significant reduction in lung injury during experimental polymicrobial intra-abdominal sepsis in mice and in baboons receiving a live, *E coli* intravenous challenge. This new treatment modality may be useful prophylactically in patients at risk for lung injury, severe sepsis and septic shock.

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DECIPHERING INTERACTIONS AMONG ACUTE INFLAMMATION, TISSUE DAMAGE, AND HEALING USING MATHEMATICAL MODELS

Yoram Vodovotz

Various stresses elicit an acute, complex inflammatory response, leading to healing but sometimes also to organ dysfunction and death. We constructed both equation-based models (EBM) and agent-based models (ABM) of various degrees of granularity–which encompass the dynamics of relevant cells, cytokines, and the resulting global tissue dysfunction–in order to begin to unravel these inflammatory interactions. The EBMs describe and predict various features of septic shock and trauma/hemorrhage (including the response to anthrax, preconditioning phenomena, and irreversible hemorrhage) and were used to simulate anti-inflammatory strategies in clinical trials. The ABMs that describe the interrelationship between inflammation and wound healing yielded insights into intestinal healing in necrotizing enterocolitis, vocal fold healing during phonotrauma, and skin healing in the setting of diabetic foot ulcers. Modeling may help in understanding the complex interactions among the components of inflammation and response to stress, and therefore aid in the development of novel therapies and diagnostics.

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INSULIN IMPROVES HEPATIC MITOCHONDRIAL FUNCTION AND STRUCTURE, DECREASES HEPATOCYTE APOPTOSIS AND ER STRESS AFTER BURN VIA PI3K/AKT SIGNALING

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Introduction: Mitochondrial structure and function is essential for organ integrity and recovery after severe stress. Burn induces hepatocyte apoptosis and causes a significant impairment in mitochondrial function and structure. We further showed that a burn induces ER stress and the unfolded protein response (UPR). We hypothesized that insulin improves mitochondrial structure and function, ER stress and UPR via the pro-survival pathway PI3K/Akt.

Methods: Thermally injured rats received either insulin (5 IU/kg body weight q.24 hours i.m.) or saline. Eight animals per group and per time point were sacrificed after 24 and 48 hours and state-3 respiration, respiratory control index (RCI), and mitochondrial pore transition (MPT) were determined. Hepatocyte apoptosis, proliferation hepatic caspases-3, and -9 and Bcl-2 were measured. Major ER stress and UPR markers were determined by Western blotting, intracellular calcium signaling by fluoresecence microscopy. Significance was accepted with $p<0.05$.

Results: Burn significantly decreased mitochondrial state-3 respiration and RCI at 24 and 48 hours postburn. Insulin significantly improved state-3 respiration and RCI compared to burn, $p<0.05$. Calcium dependent onset of MPT was markedly accelerated indicating severe mitochondrial structure damage at 24 and 48 hours postburn, which was significantly attenuated with insulin administration $p<0.05$. Postburn hepatocyte apoptosis was increased along with increased caspases-3 and -9 and decreased Bcl-2 concentration at 24 and 48 hours. Burn injury causes almost complete loss of the calcium storage protein calsequestrin and the calcium-dependent chaperone calnexin. SERCA1

expression is greatly increased, suggesting a compensatory response to ER calcium depletion. SERCA1 expression is normalized with insulin administration. In concordance with our hypothesis that burn injury induces ER stress, the UPR markers p-PERK and p-elf2α are elevated after burn injury. These changes are reversed with insulin administration, $p<0.05$. Insulin significantly decreased apoptosis and caspases-3 and -9, and increased Bcl-2 and hepatocyte proliferation at the two time points, $p<0.05$. This pro-survival effect was associated with increased phosphorylated Akt expression, $p<0.05$.

Conclusion: Burn injury is associated with ER calcium store depletion and subsequent ER stress, which may mediate hepatocyte apoptosis after burn injury. Insulin improves mitochondrial structure and function postburn via Bcl-2, caspases-3 and -9. Mitochondrial improvement is associated with decreased hepatocyte apoptosis and increased proliferation indicating improved hepatic function and recovery.

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ENDOGENOUS SECRETORY RAGE: A POTENTIAL BIOMARKER FOR METABOLIC SYNDROME AND ATHEROSCLEROSIS

Hidenori Koyama, Hiroshi Yamamoto, Yoshiki Nishizawa

Receptor for AGEs (RAGE) is involved in macro- and microvascular complications in diabetes. RAGE expression is upregulated in atherosclerotic plaques of diabetic animals, and augmentation of atherosclerosis in diabetic mice is inhibited by the competition of RAGE. Diabetic nephropathy is augmented in RAGE-overexpressing mice, but is ameliorated in RAGE-null mice. An endogenous secretory RAGE (esRAGE) has been identified as a novel splice variant carrying all of the extracellular domains but devoid of the transmembrane and intracytoplasmic domains. esRAGE is released outside from the cells, binds AGEs, and is capable of neutralizing AGE actions on endothelial cells in culture (Yonekura H: Biochem J 2003). We have also shown in mice that adenoviral overexpression of esRAGE restores diabetes impairment of vascular dysfunction (Shoji T: Diabetes 2006), suggesting that esRAGE may be an important inhibitor of RAGE signaling in vivo and potentially be useful for prevention of diabetic vascular complications. We have recently developed ELISA system specifically measuring esRAGE in human plasma, and examined its pathophysiological role in age- and gender-matched 203 type 2 diabetic and 134 non-diabetic subjects (Koyama H: Arterioscler Thromb Vasc Biol 2005). Plasma esRAGE was significantly lower in diabetic patients (0.176 ± 0.092 ng/ml) than non-diabetic controls (0.253 ± 0.111). Of note, in all, diabetic or non-diabetic group, plasma esRAGE was significantly and inversely correlated with components of the metabolic syndrome including body mass index, blood pressure, triglyceride, glycated hemoglobin A1c, or an insulin resistance index. Moreover, plasma esRAGE levels are inversely associated with carotid or femoral atherosclerosis particularly in non-diabetic subjects. In patients with chronic kidney diseases, plasma esRAGE levels are higher than those without it. In a cohort of 206 (171 non-diabetic) patients with end-stage renal diseases (ESRD) which were followed for a median of 111 months, cumulative incidence of cardiovascular death by Kaplan-Meier estimation is significantly higher in subjects in the lowest tertile of plasma esRAGE than those in the middle or the highest tertile (Koyama H: Arterioscler Thromb Vasc Biol 2007). As compared with the lowest tertile of plasma esRAGE, the hazards ratios (HR) for the highest and middle tertile are 0.40 (95% CI, 0.18-0.89) and 0.26 (0.10-0.66), respectively. The higher risk for lower esRAGE is still significant even after adjusted either with body mass index, hypertension, dyslipidemia and vascular complications, but is only confounded by age and diabetes. Thus, we postulate that plasma esRAGE is a potential protective factor and a novel biomarker against atherosclerosis and metabolic syndrome.

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HEAT SHOCK PROTEINS AS DANGER SIGNALS

John H.H. Williams

Hsp72 and Hsp60 can both be detected in serum, despite being intra-cellular proteins. Their presence in serum suggests that they are either released by damaged cells, or that they are being secreted by healthy cells. We have previously demonstrated that heat shock proteins (Hsps) are released from cells. An increasing number of cell types, including peripheral blood mononuclear cells (PBMCs), have been demonstrated to release Hsps. This talk will investigate the hypothesis that Hsps have a role as danger signals, and challenge the paradigm that only necrotic cells release Hsps.

PBMCs and Jurkat cells released Hsp70 (0.22 and 0.7 ng/10^6 cells respectively) into medium over 24 h at 37°C. The release occurred in the absence of any significant cell death. Release of Hsp72 was inhibited by methylamine, methyl β cyclodextrin and monensin, but not brefeldin A. The inhibitor data suggests that secretion is *via* a non-classical route, possibly involving lipid rafts. Release of Hsp70 from PBMCs is stimulated by both bacterial antigens (LPS and GroEL) and by pro-inflammatory cytokines (Il-6, TNF-α), again in the absence of significant cell death. Hsp60 could only be detected in extracellular media when Jurkat cells were exposed to GroEL (2.88 ng/10^6 cells) or LPS (1.40 ng/10^6 cells). The data are consistent with the hypothesis that Hsp70 and Hsp60 are part of a danger signalling cascade in response to bacterial infection.

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PULMONARY CYTOMEGALOVIRUS REACTIVATION PATHOLOGIC IN IMMUNCOMPETENT HOSTS

Charles H. Cook

Although CMV is a known pathogen in immunosuppressed transplant patients, it is unknown whether reactivated CMV is a pathogen in immunocompetent hosts. We have observed that CMV reactivates in lungs of critically ill surgical patients, and that this reactivation may be triggered by bacterial sepsis. It is intriguing that a ubiquitous herpes virus might reactivate during critical illness, causing pathology and possibly worsening outcome.

We have previously observed that inflammatory mediator expression in lungs of mice latently infected with MCMV is abnormally elevated following bacterial sepsis. To further test this hypothesis, cohorts of immunocompetent BALB/c mice with or without latent murine CMV (MCMV+/MCMV−) underwent cecal ligation and puncture (CLP). Lung tissue homogenates were evaluated 1, 2, 3, 7, 14, and 21 days after CLP for TNF-α mRNA and protein expression by real time quantitative RT-PCR and ELISA. Lungs of latently infected animals were found to have significantly elevated TNF-α expression at days 1-3, and after MCMV reactivation at day 21 when compared to MCMV− controls ($p<0.003$).

Pulmonary TNF-α expression is known to cause pulmonary fibrosis, and we therefore hypothesized that overexpression of TNF-α during CMV reactivation may cause pulmonary injury. Trichrome stained sections of lung tissues were analyzed using computer image analysis to quantitate pulmonary fibrosis. This showed that MCMV+ mice had significantly increased pulmonary fibrosis compared to MCMV− mice 3 weeks after CLP ($p<0.001$).

To confirm CMV as the causative agent, latently infected mice received ganciclovir (10mg/kg/day sq) following CLP. Ganciclovir treatment following CLP prevented MCMV reactivation. Further, ganciclovir treated animals did not demonstrate abnormal pulmonary expression of TNF-α mRNA, and subsequently did not develop the pulmonary fibrosis previously seen following MCMV reactivation.

Taken together, these data support the hypothesis that CMV reactivation causes pathology in immunocompetent hosts. Fortunately, it appears that antiviral therapy may prevent this pathology.

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INFLAMMATION AND THE RENIN-ANGIOTENSIN-SYSTEM

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The renin angiotensin system is an important peptide hormone system regulating multiple cardiovascular functions. Most of the physiological angiotensin II (AngII)) effects such as vasoconstriction and fluid homeostasis regulation have been attributed to angiotensin type 1 (AT1) receptor activation. With regard to inflammation the angiotensin type 2 (AT2) receptor is gaining functional importance.

It has been demonstrated that AngII directly stimulates proinflammatory processes in endothelial cells and monocytes/ macrophages via AT1 receptor activation. These processes involve upregulation of adhesion molecules, production of cytokines and activation of pro-inflammatory transcription factors. AngII-mediated pro-inflammatory actions are important mediators of the development of atherosclerosis in rodents and humans. Blood pressure independent pro-atherosclerotic actions of Ang II are corroborated by clinical studies where blockade of AngII responses either by angiotensin-converting enzyme inhibition or selective AT1 receptor blockade lowers the risk of cardiovascular end points in the absence of major changes in blood pressure when compared to control groups. In contrast, the AT2 receptor appears to induce anti-inflammatory effects involving also actions on endothelial and inflammatory cell.

In summary, the renin-angiotensin sytem has been characterized as a molecular network involved in the regulation of inflammation by actions on vascular and inflammatory cells. The diverse function of AT1- and AT2-receptors with regard to inflammation provides a new platform for future pathophysiological and pharmacological strategies.

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TRIPHOSPHATE-RNA IS THE LIGAND FOR THE CYTOSOLIC RECEPTOR RETINOIC-ACID INDUCIBLE GENE-1 (RIG-I)

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Receptor-mediated detection of pathogen-derived nucleic acids assists in protecting the host genome from invading foreign genetic material. Retinoic-acid-inducible protein I (RIG-I) recognizes a specific set of RNA viruses (Flaviviridae, Paramyxoviridae, Orthomyxoviridae and Rhabdoviridae) whereas a second member of this protein family, MDA-5, is responsible for the antiviral defense against a reciprocal set of RNA viruses (Picornaviridae). The four members of the TLR family (TLR3, TLR7,

TLR8 and TLR9) involved in viral nucleic acid recognition are located in the endosomal membrane. While TLRs are largely dispensable for effective antiviral defense, the two cytosolic helicases MDA-5 and RIG-I are essential for controlling viral infection. The structural basis for the distinction of viral RNA from abundant self-RNA in the cytoplasm of virally infected cells is largely unknown. Here we demonstrate that the 5′-triphosphate end of RNA generated by viral polymerases is responsible for RIG-I-mediated detection of RNA molecules. Detection of 5′-triphosphate RNA is abrogated by capping of the 5′-triphosphate end or by nucleoside modification of RNA, both occurring during posttranscriptional RNA processing in eukaryotes. Genomic RNA prepared from a negative strand RNA virus and RNA prepared from virus-infected cells, but not RNA from non-infected cells triggered a potent IFN-? response in a phosphatase sensitive manner. 5′-triphosphate RNA directly binds to RIG-I. In conclusion, uncapped 5′-triphosphate RNA present in viruses known to be recognized via RIG-I serves as the molecular signature for the detection of viral infection by RIG-I. However, Picornavirus-like supergroup of viruses use a RNA-dependent RNA polymerase that exclusively employs a protein as a primer for both positive and negative strand RNA production; as a consequence, during the lifecycle of Picornaviruses uncapped, triphosphorylated 5′ ends are absent. Consequently, RIG-I cannot detect Picornaviruses.

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INFLAMMATORY BOWEL DISEASES

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The inflammatory bowel diseases (IBD: Crohn's disease and ulcerative colitis) are relapsing inflammations of the gastrointestinal tract not due to specific pathogens. Although the precise etiology of the diseases is still unknown, recent data from animal model strongly suggest that predisposing genetic factors, (NOD2, OCTN, IL-23R), barrier defects and bacterial antigens lead to an unbalanced activation of the mucosal immune system that in turn causes chronic intestinal inflammation. There has been a growing interest in understanding the role of cytokines and cytokine signaling events in IBD models in recent years. T-bet expressing Th1 cells, GATA-3 expressing T cells producing IL-13 and THIL-17 cells are key effector cell populations with major relevance for the design of novel therapeutic approaches for IBD. Furthermore, IL-12 family cytokines such as IL-23 and IL-27 appear to play a prominent role in modulating the activity of effector T cells in experimental colitis. Finally, various proinflammatory cytokines and transcription factors in the gut have been shown to regulate the development and progression of colitis associated colon cancer in murine models. These data provide a rationale for selective targeting of cytokines and transcription factors in IBD. Such targeting has the potential advantage of targeting the activity of various cell types simultaneously rather than of a single cell type. In any case, the findings in animal models of chronic intestinal inflammation have provided new insights into the pathogenesis of IBD and are important for the development of novel immunotherapeutic approaches.

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V.A.C ABDOMINAL DRESSING – A RETROSPECTIVE STUDY IN THE TREATMENT OF THE OPEN ABDOMEN FOLLOWING SECONDARY PERITONITIS

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Introduction: Treatment of an open abdomen following secondary peritonitis is a challenge for surgery and intensive care units (ICU). The goal of this study was to compare three different treatment strategies for the open abdomen: V.A.C. Abdominal Dressing (AD), conventional V.A.C. therapy (CV) and conventional open therapy (OP).

Methods: All patients suffering from an open abdomen following surgery for secondary peritonitis between 2001 and 2006 at 5 different surgical departments in Austria were retrospectively analyzed. Parameters that were analyzed: survival, length of stay (LOS) in ICU, nursing requirements (change of dressing / day), BMI, age, Apache II Score and integrity of abdominal wall after discharge. Treatment strategies included: open packing (OP), classic vacuum assisted closure (VAC) -therapy with silicone net protection for the intestines (CV) and VAC- therapy with "abdominal dressing", a newly developed dressing containing an encapsulated foam with non-adherent layer (AD).

Results: In total 215 patients were analyzed: 64 patients were treated with OP, 100 patients with CV and 51 patients with AD. Mortality rates were 48/64 (75%) for OP vs. 38/100 (38%) for CV vs. 18/51 (35%) for AD ($p<0.01$ Kruskal-Wallis Test).

There was no difference in mean LOS in the ICU: 37.3 days for OP, 29.6 days for CV and 39.9 days for AD ($p<0.107$ Kruskal-Wallis Test). The ApacheII scores were higher in the AD group, AD: 21.1; OP: 18.9 and CV: 16.7 ($p<0.0001$ Kruskal-Wallis Test) and there were no significant differences in age between the groups.

Conclusion: We found a significant reduction in mortality for patients treated with VAC therapy, the mortality rate decreased by half. The VAC "abdominal dressing"-therapy appeared to be a more efficient treatment option in patients suffering from open abdomen following secondary peritonitis. The results from this retrospective study indicate the need for further prospective evaluation of VAC therapy using the VAC abdominal dressing to determine whether the intervention has set a new standard for managing the open abdomen.

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