Digestive Diseases and Sciences, Vol. 50, No. 10 (October 2005), pp. 1959–1998 (© 2005)
DOI: 10.1007/s10620-005-2996-8

ABSTRACTS

CYTOKINE AND CHEMOKINE IN ALCOHOLIC AND NON-ALCOHOLIC LIVER DISEASE

ETHANOL METABOLISM AND LIVER INJURY: INFLUENCE ON GENE EXPRESSION. S. Zakhari, PhD. National Institute on Alcohol Abuse and Alcoholism NIH, Bethesda, Maryland, USA

The phenotypes of numerous diseases (e.g., type 2 diabetes, Parkinson's, heart failure, and muscular dystrophy) are the result of disorders of metabolism, and not primarily by gene mutation. The concept that DNA and proteins can specify phenotypes is oversimplified, and does not take into account different metabolic pathways, in which key metabolites within a pathway bind to transcription factors and alter gene expression patterns that contribute to the phenotype. Simple metabolites dictate the actions of specific transcription factors that sense the minute to minute cellular environment to determine which parts of, and the extent to which, the genetic code will be transcribed. Thus, metabolic pathways define the part of the genome to be transcribed, and thereby shape the emergent phenotype. For example, following caloric restriction (CR), entire sets of genes may be silenced by NAD-dependent histone deacetylase, thus influencing obesity and longevity. The metabolic NAD/NADH ratio that controls gene transcription with CR is also changed by ethanol metabolism, and hence ethanol metabolism may influence gene transcription leading to disease phenotypes.

Altered states of chromatin structure and histone modification (acetylation, phosphorylation, methylation or ubiquitination) also influence exposure of DNA to transcription factors, and are considered important factors in the regulation of gene transcription, which is referred to as epigenetics. Ethanol causes selective acetylation of histone in the hepatocyte nucleus, and may regulate chromatin remodeling and gene expression. Ethanol also affects folate levels, the perturbation of which affects DNA methylation and DNA synthesis. Methylation of DNA is an important epigenetic factor in regulating gene expression. This presentation will review ethanol-induced tissue damage in view of these plausible changes in gene transcription.

NONALCOHOLIC STEATOHEPATITIS: ADAPTATION AND MAL-ADAPTATION. S.H. Caldwell, MD,* J.A. Redick, MD,† L.A. Krugner-Higby, MD,‡ A.M.S. Al-Osaimi, MD,* C. Chang, MD,* C.A. Davis, PhD‡. *The University of Virginia Gastroenterology & Hepatology Division, Charlottesville, Virginia; †University of Wisconsin, Research Animal Research Center, Madison, WI; ‡Center for Advanced Microscopy, University of Virginia School of Medicine, Charlottesville, VA

Human non-alcoholic fatty liver (NAFL) is variable in terms of severity and course. Because of familial clustering of the more severe form known as NASH (non-alcoholic steatohepatitis) and ethnic variation in the prevalence of hepatic steatosis, it is likely that genetic factors play a role in its pathogenesis. Similar variation, attributed in part to altered lipoprotein metabolism, has been described in different strains of migratory geese where it is thought to represent a ready source of energy during strenuous activity. In human fatty liver, the fat storing hepatocytes become ballooned and takes on an appearance similar to that of brown adipose tissue (BAT) with centrally located nuclei and multiple small droplets of fat. From studies using osmium fixed specimens, these osmiophilic lipid droplets, which appear membrane bound, coalesce to form single large white adipose tissue (WAT) like cells. Lipid peroxidation, the 'second hit' in NASH and the major source of cell injury, can be identified in fat loaded cells which express some markers of adipocytes including adiponectin. However, most such cells appear healthy by electron microscopy in spite of increased peroxides suggesting that they have become adapted to function as cytokine-producing fat storing cells. Taken with studies showing expression of uncoupling protein in NAFLD, the mitochondrial changes characteristic of NAFLD (especially the formation of parallel crystal inclusion which appear contiguous with cristae and resemble the stacked cristae of BAT), suggest that plasticity of adipocytes extends to the fatty hepatocyte and involves progression of microvesicular steatosis to macrovesicular and likely involves thermogenesis. The fact that these mitochondrial changes are not related to areas of injury as indicated by light microscopic findings suggesting that these structures represent a diffuse adaptive change. Taken with observations regarding sympathetic nervous system (SNS) activity in the development of obesity and SNS influence on fatty liver, these findings suggest that NAFL represents another evolutionary adaptation to the 'feast or famine' conditions endured by humans exposed to extreme cold and lack of resources as seen in the Ice Age when severe cold placed a remarkably potent evolutionary stress on human populations living in latitudes away from the equator. Conceptually, NAFL can be viewed as an adaptation to these stressful conditions. At some point in the past, the 'ability' to develop fatty liver offered a survival advantage. However, in the presence of over abundance and lack of environmental stress such as cold exposure, the development of fatty liver becomes potentially injurious. Thus, NAFL can be viewed as an extension of the energy and thermoregulatory function of the adipose organ (including the liver) and non-alcoholic fatty liver *disease*

(NAFLD) can be viewed as a form of fat inflammation (panniculitis or lipodystrophy). This paradigm provides a different perspective for understanding both the stable fatty liver and the diseased fatty liver as well as the relationship of steatosis to other parameters of the metabolic syndrome and the concept of the evolutionarily adapted thrifty genome.

INTRAHEPATIC GENE EXPRESSION PROFILING IN HUMAN AND THE BABOON MODEL OF ALCOHOLIC LIVER DISEASE. P. Haber, MD, D. Seth, MD, M. Leo, MD, M. Gorrell, MD, C.S. Lieber, MD, G. McCaughanm, MD. Royal Prince Alfred Hospital, Camperdown, Australia and Alcohol Research and Treatment Center, Bronx Veterans Affairs Medical Center, New York, USA

Background: Alcohol-induced liver disease (ALD) remains a major problem in Australia and worldwide. For the established disease there is no widely accepted treatment [1]. Our approach has been to profile hepatic gene expression to study the effects of alcohol on the molecules and molecular pathways that are important in this disease [2, 3, 4]. To exclude confounding by nutritional or other pathological processes we also performed similar studies in the well-characterized Lieber-DeCarli baboon model of ALD.

Aims: To define differentially expressed genes in the baboon model of ALD and in human alcoholic steatosis (AS), alcoholic hepatitis (AH) and end-stage (ES) ALD. To explore functional significance of differentially expressed genes.

Methods: RNA was extracted from liver biopsies of alcohol-fed & control baboon (BB: $n = 2$ each), AS ($n = 7$), AH ($n = 7$), ES ($n = 7$) ALD and non-diseased (ND, $n = 7$) were hybridized to human DNA microarrays. Expression status of selected molecules was confirmed by real time RT-PCR and immunoflourescence. To investigate the direct effect of alcohol on gene expression *in vitro*, hepatocyte (Huh7 and HepG2) and stellate (LX2) cell lines were incubated with 10 and 50 mM alcohol and for *in vivo* studies, a single dose of alcohol (6g/kg) was administered to C57BL6 mice. RNA was extracted for qPCR for annexin A1, A2 and p11 and osteopontin. Fibrinolysis assay was performed in a 96-well plate for 5×10^4 cells treated with alcohol.

Results: The global gene expression profile of the Lieber-DeCarli baboon model of ALD was similar to that of human ALD. Cluster analysis allowed differentiation of AH from AS. The number of differentially expressed genes increased as the severity of disease increased. Molecules involved in fibrogenesis/ECM were upregulated in all groups. Genes involved in energy/alcohol metabolism and specifically, CYP2E1, were upregulated in the baboon. A number of differentially expressed genes previously reported in other models of ALD were identified [2, 3]. Several genes involved in fibrinolysis were identified, in particular annexin A2, and were associated with increased fibrinolysis activity in ethanol treated LX2 cells. Osteopontin, a Th1 cytokine, was up-regulated in relation to the severity of liver disease. In mice, osteopontin transcripts were up-regulated as early as 4 h after administration of alcohol.

Conclusions: This is the first study to define global intrahepatic gene expression in human ALD. The study identified known and novel molecules involved in the ALD pathogenesis. The studies implicate early activation of fibrinolytic pathways and osteopontin in ALD.

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THE ROLE OF ADIPOCYTOKINES IN PROGRESSION OF ALCOHOLIC AND NON-ALCOHOLIC STEATOHEPATITIS. K. Ikejima, K. Okumura, K. Kon, S. Yamashina, N. Enomonto, Y. Takei, N. Sato. Juntendo University School of Medicine, Tokyo, Japan

Lines of evidence indicate that obesity and insulin resistance are the key factors for progression of hepatic fibrosis in various chronic liver diseases including alcoholic liver disease (ALD) non-alcoholic steatohepatitis (NASH). There is a paradox, however, that ob/ob mice and Zucker rats, which are the obese and diabetic strains, showed minimal profibrogenic responses in the liver, most likely because they lack leptin and its receptors. To establish more clinical relevant model to study the mechanism of fibrogenesis under steatohepatitis, we investigated fatty changes and profibrogenic responses in the liver caused by methionine-choline deficiency (MCD) in the KKAy mouse, which is an obese and diabetic strain. C57Bl/6 mice fed MCD diet for 4 weeks developed hepatic steatosis as expected. In KKAy mice fed MCD diet, however, the fatty changes in the liver were more prominent as compared to those in Bl/6 mice. Further, overt hepatic fibrosis was observed in KKAy mice fed an MCD-diet for 8 weeks, while Bl/6 mice developed less fibrosis in the liver. Indeed, a1(I)procollagen and TGF-b1 mRNA levels 4–8 weeks after feeding with MCD-diet were significantly higher in KKAy mice. Serum adiponectin levels were elevated nearly 2-fold when Bl/6 mice were given MCD-diet for 4 weeks, which were inversely correlated with the loss of body weight as expected. In sharp contrast, serum adiponectin levels in KKAy mice fed both the control- and MCD-diet were almost the same, reaching the values almost 1/2 of those in Bl/6 mice, even significant body weight loss was observed in MCD-diet group. These findings indicated that KKAy mice not only develop more severe hepatic steatosis but also demonstrate increased profibrogenic responses due to steatohepatitis caused by MCD diet. Importantly, KKAy mice lack phys-

iological up-regulation of adiponectin levels, suggesting that adiponectin plays a pivotal role not only in regulation of insulin sensitivity but also in modulation of inflammatory and profibrogenic responses in dietary steatohepatitis. This animal model may serve a new insight toward the comprehensive understanding of steatosis-related progression of hepatic fibrosis including the pathogenesis of ALD and NASH.

MARKERS OF FIBROSIS IN NON-ALCOHOLIC STEATOHEPATITIS. Luca Miele, MD, Alessandra Forgione, MD, Giovanni Gasbarrini, MD, Antonio Gasbarrini, MD, Antonio Grieco, MD. Department of Internal Medicine, Catholic University of the Sacred Heart. Rome, Italy

Non alcoholic fatty liver disease (NAFLD) associated with inflammation and fibrosis is referred to as non-alcoholic steatohepatitis (NASH). Fibrosis is the single most important determinant of the natural history and progression of NAFLD and NASH. It involves remodeling of the extracellular matrix (ECM) characterized by an imbalance between the breakdown and synthesis of collagen types I, III, and IV. Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) participate in ECM degradation. Transforming growth factor β 1 (TGFβ1) also plays a crucial role by stimulating the conversion of hepatic stellate cells into myofibroblast-like cells capable of secreting ECM proteins. A simple, reliable, non-invasive marker of fibrosis is thus essential for evaluating NAFLD patients in clinical practice. Ultrasound-guided liver biopsy is currently the gold standard for diagnosing NASH, but it is too invasive for monitoring therapeutic responses and disease evolution. Serum enzymes (ALT, AST and GGT) are markers used to screen for NASH. Recent reports detail that advanced fibrosis may well be present in patients with normal transaminase levels. Steatosis is a prerequisite for the development of steatohepatitis, but steatosis alone is sufficient. Fibrogenesis occurs, triggered by cytokine or noncytokine stimuli, such as such as products of lipid peroxidation, while the progression of the fibrotic process is a consequence of, tumor necrosis factor (TNF)-α and other cytokines whose activities it stimulates, such as IL-6, TGF-β, and platelet growth factor (PDGF). Generation of oxidative stress-related molecules (e.g., reactive oxygen intermediates, reactive aldehydes) is considered by some to be a critical factor in the progression from simple steatosis to NASH and fibrosis. An excess of reactive oxygen species triggers lipid peroxidation of cell membranes and stimulates the release of the proinflammatory cytokine TNF α, from hepatocytes, Kuppfer cells, and adipose tissue. Circulating levels of soluble TNF receptors (sTNF-R) have been proposed as a marker of TNF-system activation, since elevations in these levels persist longer that those of TNF itself. Blood levels of sTNF-R display significant correlation with the histological grade of fibrosis. TGF β promotes hepatic fibrosis by accelerating the transdifferentiation of hepatic stellate cells (HSC) into myofibroblast-like cells. This process leads to the synthesis of various ECM components (collagens I, II and IV, fibronectin, and laminin) and upregulated expression of protease inhibitors, such as TIMP-1 and plasminogen activator inhibitor, which protect the matrix from degradation. HSCs are also responsible for the secretion of cytokines and other soluble that modulate recruitment of inflammatory cells and of additional HSC, thus amplifying the inflammatory and fibrotic processes. Our findings indicate that the dynamic ECM remodeling NASH-related fibrogenesis is associated with increased serum levels of TGF-β as well as laminin, TIMP1, and leptin. Hyaluronic acid type IV collagen and procollagen III propeptides (PIIINP) and the metalloproteinases (MMPs) and their inhibitors (TIMPs) have all been evaluated as direct markers of fibrosis. The N-terminal propeptide of type III collagen, hyaluronic acid, and TIMP1 are considered surrogate markers of liver fibrosis while platelet counts or the prothrombin index, are considered as indirect markers of fibrosis. Diagnoses of liver fibrosis based on a combination of several blood markers have been shown to be more reliable than those based on a single parameter. The progression of liver fibrosis is associated with increases in the levels of total bilirubin, γGT, and α_2 macroglobulin, which inhibits the protease activity responsible for the catabolism of ECM proteins; in contrast, levels of hatoglobin and apolipoprotein $_{A1}$, which is trapped in the ECM, decrease. Patient age, serum hyaluronic acid, procollagen-III N-terminal propeptide, and TIMP-1 are the most useful. This algorithm used in this test can identify patients with little or no fibrosis as opposed to those with clinically significant hepatic fibrosis.

CYTOKINE–NUTRIENT INTERACTIONS IN ALCOHOLIC LIVER DISEASE. C.J. McClain, MD, S. Barve, MD, L.S. Marsano, MD. Ohio State University, Department of Internal Medicine, Division of Digestive Health, and University of Louisville and Jewish Hospital Liver Research Center, Louisville VAMC, University of Louisville, Departments of Medicine and Pharmacology and Toxicology

Alcohol liver disease (ALD) remains an important complication and cause of morbidity and mortality from alcohol abuse. Major developments in our understanding of the mechanisms of ALD over the past decade are now being translated into new forms of therapy for this disease process, which currently has no FDA, approved treatment. Cytokines are low molecular weight mediators of cellular communication, and the proinflammatory cytokine tumor necrosis factor (TNF) has been shown to play a pivotal role in the development of experimental ALD. Similarly, TNF levels are elevated in the serum of alcoholic hepatitis patients. Abnormal methionine metabolism is well documented in patients with ALD, with patients having elevated serum methio-

nine levels, but low S-adenosylmethionine levels. On the other hand, S-adenosylhomocysteine and homocysteine levels are elevated in ALD. Recent studies have documented potential interactions between homocysteine and S-adenosylhomocysteine with TNF in the development of ALD. Altered zinc metabolism also is now well documented in ALD, and decreased zinc can play a role in the increased gut permeability and liver injury in ALD, including abnormal TNF metabolism. This talk will review nutrient cytokine interactions in ALD.

NON-ALCOHOLIC FATTY LIVER IN OCTOGENARIANS. S. Malnick, MD. Kaplan Medical Centre, Hebrew University, Rehovot, Israel

Background: Non-alcoholic fatty liver (NAFLD) is regarded as the hepatic manifestation of the metabolic syndrome. While body weight and serum cholesterol decline in the aged, there is an increase in the prevalence of both diabetes and hypertension. The aims of the current study were to determine in the aged, the prevalence and the clinical presentation of NAFLD as well as the relation to the underlying metabolic abnormalities.

Method: In this prospective study we evaluated 91 octogenarians with a mean age of 85.56 ± 3.76 years, who were admitted to the rehabilitation departments of a geriatric hospital. Clinical evaluation included: abdominal ultrasound (US), fasting glucose and lipid levels, serum liver enzymes, ferritin, iron and transferrin saturation. Elderly patients with NAFLD were compared with the 46 young patients with NAFLD.

Results: NAFLD diagnosed by US was a common finding in this aged population present in 42/91 patients (46.2%). No significant differences were observed between the patients with or without NAFLD in the following: age, gender, chronic illnesses, anthropometric parameters, lipid profile, fasting glucose levels, metabolic syndrome prevalence, serum levels of transaminases, ferritin and iron. Young patients with NAFLD had significantly higher serum levels of triglycerides and a significantly higher prevalence of glucose intolerance, obesity and the metabolic syndrome compared with the elderly patients with NAFLD.

Conclusions: NAFLD was a common finding in our group of elderly patients and the prevalence was higher than reported in the general population. In contrast to the association between the metabolic syndrome and NAFLD in the general population, we did not find this association to exist in this age-group. In addition, none of the patients had stigmata of advanced liver disease. These data suggests that NAFLD is a common and benign finding in the elderly population, but is not associated with the metabolic syndrome.

SERUM ADIPOKINES AND THE ASSOCIATED GENE EXPRESSION IN THE ADIPOSE TISSUE OF OBESE PATIENTS WITH AND WITHOUT INSULIN RESISTANCE. A. Baranova, S. Gowder, R. Collantes, H. Elariny, K. Schlauch, A. Afendy, J.P. Ong, Z. Goodman, MD, V. Chandhoke, MD, Z.M. Younossi, MD. Center for Liver Diseases, Inova Fairfax Hospital, Center for the Study of Genomics in Liver Disease, George Mason University and Armed Forces Institute of Pathology

Adipose tissue is an active endocrine organ that secretes a variety of metabolically important substances, including cytokines. Adipocyte-secreted factors affect insulin sensitivity and represent an important link between obesity, insulin resistance (IR), type 2 diabetes and probably non-alcoholic fatty liver disease (NAFLD). We performed Real-Time PCR quantification of mRNAs encoding for adiponectin, leptin and resistin in the snap frozen samples of intra-abdominal adipose tissue obtained from morbidly obese diabetic patients ($N = 11$; serum glucose 151.49 ± 40.56 mg/dL and serum insulin 8.28 ± 3.52 μU/mL) and non-diabetic patients without IR ($N = 10$; serum glucose 102.2 ± 8.43 mg/dL and serum insulin 3.431 ± 1.162 μU/mL) who were undergoing bariatric surgery. Adiponectin mRNA level in the intra-abdominal adipose tissue of the obese diabetic patients was significantly lower than those obese patients without IR (55.47 ± 19.16 vs. 99.56 ± 45.64, $p < 0.01$). On the other hand, mRNA levels of leptin and resistin were not different between the two groups. Measurements of resistin concentration in the serum revealed pronounced discordance between the gene expression in the adipose tissue and its serum concentration (diabetics vs. non-diabetic without IR 6.91 ± 3.28 ng/mL vs. 3.90 ± 1.24 ng/mL, $P < 0.01$). In conclusion, this preliminary data suggests decreased gene expression of adiponectin in the adipose tissue of patients with DM. This low adiponectin expression in the adipose tissue could contribute to the low level of serum adiponectin which has been reported in the progressive form of NAFLD. On the other hand, the discordance between the gene expression and serum concentration of resistin suggests that the intra-abdominal adipose tissue is not the only source of resistin secreted into the serum.

MODULATING EFFECT OF SESAMIN, A FUNCTIONAL LIGNAN IN SESAME SEEDS, ON THE TRANSCRIPTION LEVELS OF LIPID AND ALCOHOL-METABOLIZING ENZYMES IN RAT LIVER. Y. Kiso, PhD. Suntory Institute for Health Care Science, 1-1-1 Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618-8503, Japan

Sesamin is one of the components of sesame seed, which has been known as a traditional health food or a medicinal plant in the East in particular. The content of sesamin in sesame seed is approximately 0.5%. A bulk of sesamin is removed from the edible sesame oil during the refining process. Various pharmacological activities of sesamin have been reported so far including the stimulation of alcohol metabolism in mice and human and the prevention of ethanol-induced fatty liver in rats.

Sesamin may exert these effects by up- and down-regulation of the gene expression for various proteins. In this study, we systematically investigated the changes of gene expression in rats given 250 mg/kg of sesamin (sesamin rats) or vehicle (control rats) for 3 days by a DNA microarray analysis. At 4 h after the final ingestion, the profiles of gene expression in rat livers were compared.

The analysis showed that 36 transcripts were up-regulated with a significant change of more than 2-fold and 10 transcripts were down-regulated with a significant change to less than half in the livers of sesamin rats versus control rats. The gene expression levels of the early stage enzymes of β-oxidation including long-chain acyl-CoA synthetase, very long-chain acyl-CoA synthetase and carnitine palmitoyltransferase were not changed, however, those of the late stage enzymes of β-oxidation including trifunctional enzyme in mitochondria, and acyl-CoA oxidase, bifunctional enzyme and 3-ketoacyl- CoA thiolase in peroxisomes, were significantly increased by sesamin ingestion. Also, the expression of acyl-CoA thioestelase genes was markedly increased. On the other hand, the transcription of the genes encoding the enzymes for fatty acid synthesis was decreased by sesamin. Moreover, in sesamin rats, the gene expression of aldehyde dehydrogenase was increased about 3-fold, whereas alcohol dehydrogenase, liver catalase and CYP2E1 were not changed. Changes in the gene expression of alcohol- and aldehyde-metabolizing enzymes observed in a DNA microarray were also confirmed by a real-time PCR method. These results suggested that sesamin ingestion regulated the transcription levels of hepatic metabolizing enzymes for lipids and alcohol.

Sesamin is also known to be resistant to oxidation, though it has no antioxidative activity *in vitro*. Sesamin can be absorbed from the intestine and reaches the liver via the portal vein where it is metabolized to catechol derivatives prior to being secreted as glucuronic acid conjugates into bile. Recently, we have shown that catechol metabolites from sesamin exert strong antioxidative activity in the liver, suggesting a beneficial physiological role for ingested sesamin in the body.

VIROLOGY, IMMUNOLOGY AND PHARMACOLOGICAL DOSING FROM THEORY TO PRACTICE

FIBROSIS, CYTOKINES AND HEPATITIS. Scott L. Friedman. Chief, Division of Liver Diseases, Mount Sinai School of Medicine, New York, NY 10029, USA

Hepatic fibrosis is a wound-healing response to chronic liver injury, which if persistent can lead to cirrhosis and liver failure. Hepatic stellate cells (HSC) are the primary source of extracellular matrix in normal and fibrotic liver. Activation of HSCs is the central event of fibrogenesis. Exciting progress has been made in understanding the molecular basis of this process. Major advances include: (a) elucidation of effects of key cytokines on and their signaling pathways in HSC; (b) understanding of transcriptional regulation of HSC activation; (c) characterization of matrix proteases and their inhibitors; (d) demonstration of apoptosis as an important event in resolution of hepatic fibrosis and identification of its mediators; (e) elucidation of the complex and dynamic interaction between HSC and matrix; (f) understanding of the role of other cellular elements in hepatic fibrosis and their interaction with HSC. In addition, clinical studies have begun to identify host genetic polymorphisms that may soon predict risk of fibrosis progression. Such data will be valuable in stratifying patients for trials of antiviral and antifibrotic therapies.

Our recent studies of fibrosis have begun to focus on interactions between immune cells and hepatic stellate cells, and have identified key mediators and lymphocyte subsets that may modulate fibrosis. Adoptive transfer studies suggest that fibrogenic activity can be conferred in naïve liver following administration of lymphocyte subsets from animals with liver injury. Additionally, upregulation of receptors that mediate apoptosis during stellate cell activation point towards the use of apoptotic ligands as potential therapies for hepatic fibrosis to clear activated stellate cells, thereby reducing fibrogenesis.

Collectively the data indicate a previously overlooked role of interactions between immune cells and stellate cells in the pathogenesis of hepatits and fibrosis. They further suggest that perhaps host-determined variations in immune phenotype might account for variability in fibrosis progression that is well recognized in clinical practice. Ongoing research with gene analysis using cDNA or oligonucleotide microarrays or transcriptional profiling has further increased our knowledge in the regulation of stellate cell activation. Ultimately, advances in the understanding of the molecular biology of hepatic fibrosis are critical to the development of effective, targeted antifibrotic therapy that may benefit millions of patients with chronic liver disease worldwide

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MECHANISM BASED DESIGN OF SYNERGISTIC THERAPEUTICS FOR HEPATITIS C. John A. Thomson, PhD. Research, Vertex Pharmaceuticals Incorporated, Cambridge, MA, USA

Chronic HCV infection is a serious disease that causes debilitating inflammation of the liver. According to the Centers for Disease Control and Prevention and the World Health Organization, HCV affects as many as 2.7 million people in the U.S. and as many as 185 million people worldwide. Vertex currently has two novel drug candidates in development for the treatment of hepatitis C virus infection, one targeting the virus directly and another targeting a human viral response mechanism. Merimepodib (MMPD) is Vertex's most advanced oral drug candidate. Targeting the human enzyme IMPDH, MMPD has the potential to boost clinical activity for existing and experimental HCV treatments. VX-950 is an orally administered HCV protease inhibitor under clinical evaluation and is one of the most advanced drug candidates in this new class of direct antivirals. In a recent Phase 1b clinical trial, some patients receiving VX-950 (750 mg, TID) achieved a median reduction in HCV-RNA of greater than 4 log10, or a more than 10,000-fold decrease in viral levels, at the end of 14 days of treatment.

The current presentation will review the biology of Vertex's two anti-HCV approaches and our strategic approach to anti-HCV target selection. A brief update on our most exciting recent clinical findings will be provided.

NEW THERAPIES FOR VIRAL HEPATITIS. Zhi Hong, PhD. Valeant Pharmaceutical International, Costa Mesa, CA, USA

Chronic viral hepatitis is caused primarily by hepatitis B virus (HBV) and/or hepatitis C virus (HCV). It represents a major global health issue with a combined prevalence of 570 million infected individuals. Current therapies are able to suppress viral replication, control disease progression or even achieve sustained viral eradication in a proportion of patients. However, drug tolerability, resistance, prolonged treatment duration and suboptimal efficacy still pose significant challenges to the clinical management of viral hepatitis. Newer therapies are developed to address these limitations but the benefits or treatment outcomes continue to be incremental over the existing therapies. Long term therapies with poorly tolerable agents remain to be the option in the next 5 years.

Creative use of current therapies as well as those in the late stage development pipelines will be the theme in the years to come. Curative medicines, especially in the context of HBV therapy, require a changing paradigm in the drug discovery process.

PEGINTERFERONS FOR CHRONIC HEPATITIS C: NEW PARADIGMS. M.W. Fried, MD. Hepatology and General Clinical Research Center, University of North Carolina at Chapel Hill, NC, USA

Therapy for chronic hepatitis C has advanced rapidly over the last 15 years. Sustained virological response rates using peginterferon and ribavirin combination regimens are over 50% for all of those treated. For patients with favorable virological characteristics, such as genotype 2, sustained response may be as high as 90%. Despite this unprecedented success, it is recognized that many patients fail to achieve a sustained response or are considered poor candidates for therapy due to the rigors of the treatment regimen. This has fueled the need for refined treatment regimens using currently available medications and the search for newer agents to treat hepatitis C. Recent evidence suggests that genotypes 2 and 3 do not respond equally well to standard regimens as previously believed. Genotype 3 patients have a higher relapse rate than those with genotype 2. The greatest risk for relapse occurs in patients with genotype and high levels of viremia, steatosis, and increase fibrosis. Patients with these characteristics may benefit from a longer course of therapy. Similarly, the general recommendation to treat all patients with genotype 1 for a total of 48 weeks, in those achieving early virological response, is also being reevaluated. The greatest chance of SVR occurs in those who have a rapid virological response (undetectable HCV RNA at week 4 of therapy) while those who are slower to clear HCV RNA may benefit from prolonged treatment. Thus, in the short term, treatment decisions will rely upon a precise interpretation of viral kinetic response for an individual patient. A better understanding of the relative resistance to therapy for certain special populations will also lead to new treatment paradigms that will further enhance the rate of sustained virological response.

DEVELOPMENT OF NOVEL INTERFERON MOLECULES FOR THE TREATMENT OF CHRONIC HEPATITIS C INFECTION. Julian A. Symons. Viral Diseases Therapeutic Area, Roche Palo Alto LLC, Palo Alto, CA, USA

Interferon alfa-2a played a valuable role in the treatment of chronic hepatitis C but its efficacy was limited by the short in vivo half-life that contributed to low rates of viral eradication. To overcome these drawbacks, a modified form of the drug, peginterferon alfa-2a (PEGASYS®), was developed by attaching a 40-KD branched-chain polyethylene glycol moiety to the interferon alfa-2a molecule. Peginterferon alfa 2a (40KD) has a sustained absorption, slower rate of clearance and a longer half-life resulting in sustained levels and once-weekly administration. Results of large randomized, double-blind trials have confirmed that treatment with PEGASYS and ribavirin is more effective than conventional IFN alfa and ribavirin in patients with chronic hepatitis C and in patients co-infected with HCV and HIV. Analysis of the HCV viral dynamics during treatment with IFN alfa suggests that the antiviral and Th1 immunity inducing activities of IFN alfa play a critical role in early and late viral dynamics, respectively. In order to optimize these properties we have employed the process of in vitro directed molecular evolution by DNA family shuffling to the human IFN alfa gene family. DNA family shuffling is a method for permutation of

natural genetic diversity. It mimics and extends classical breeding methods by recombining more than two parental genes in a single reaction. The power of this technology is that large improvements in phenotype can be achieved by recursively screening only a small subset of all theoretically possible progeny. In a proof of concept study, DNA shuffling of a family of over 20 human IFN alfa genes was used to derive variants with increased antiviral activities on murine cells. Interestingly, the most active clones derived from this screen were more active than the native murine IFN alfa proteins. The potential use of these rapidly evolved IFN alfa proteins in the treatment of chronic hepatitis C infection will be discussed.

RECENT CONCEPTS IN ANTI-HIV THERAPY: CHEMOKINE RECEPTOR ANTAGONISTS. Jaime E. Hernandez, MD, FACP. GlaxoSmithKline, Research Triangle Park, NC

Antiretroviral therapy (ART) has transformed HIV infection and disease from being universally fatal to a manageable chronic state. Despite this progress, new ART agents are increasingly needed due to the development of resistance to existing classes of drugs. Binding, attachment, and fusion are important steps in the entry of HIV into a susceptible host cell that precede the intracellular replication of HIV. Attachment of the virus to the cell occurs via CD4 binding, followed by chemokine co-receptor interactions that facilitate fusion of the cellular and viral membranes. CCR5, CXCR4 and other chemokine receptors have been identified as co-receptors for HIV entry. CCR5 is a β-chemokine receptor and member of the 7TM superfamily. The natural ligands for CCR5 (RANTES, MIP-1α, MIP-1β) bind to CCR5 and transmit signals for cellular activation and chemotaxis. 873140 (aplaviroc) is a spirodiketopiperazine CCR5 allosteric antagonist that binds specifically to human CCR5 and demonstrates potent anti-HIV activity in vitro in the subnanomolar range. Short-term, placebo-controlled, dose-ranging studies of aplaviroc monotherapy in HIV-infected subjects demonstrated promising antiviral activity. In addition, aplaviroc binds to human CCR5 with a unique profile as evidenced by the selective inhibition of monoclonal antibody binding to CCR5. The effect of short-term administration of aplaviroc in both HIV-negative and HIV-positive subjects on chemokines and TH1/TH2 cytokines is being investigated. Aplaviroc has demonstrated antiretroviral activity and has been generally safe and well tolerated in short-term studies conducted in treatment-naïve and treatment-experienced HIV-1 infected patients. Phase 3 studies of aplaviroc are underway.

EFFECT OF INFLIXIMAB IN HEPATITIS-C GENOTYPE 1 NAÏVE PATIENTS WITH HIGH TNF-α ON THE EFFICACY OF PEGYLATED INTERFERON αt2b /RIBAVIRIN THERAPY. M.G. Neuman, A. Nabil, C. Cooper. University of Toronto, Toronto, ON; Schering Canada, PQ; University of Ottawa, The Ottawa Hospital, Ottawa, ON, Canada

Tumor necrosis factor alpha (TNF-α) may have an important role in the natural history of HCV and in response to pegylated-Interferon α2b /ribavirin therapy. Neuman *et al*. demonstrated a significantly higher serum TNF-α levels in those with chronic HCV infection. A positive correlation between the degree of inflammation as indicated by the histological activity index and TNF-α levels was reported ($r = 0.92$, $p = 0.001$) in non-cirrhotic patients with minimal to moderate histological activity index scores. Furthermore, a low level of serum TNF-α (i.e. $<$300 pg/mL) was identified as a predictor for SVR to interferon-based HCV antiviral therapy. Based on these observations, we hypothesize that patients with high baseline serum TNF-α levels ($>$300 pg/mL) may achieve improved EVR and SVR (over that which can be attained with pegylated -interferon α2b/ribavirin) alone) if "induction therapy" with Remicade is administered prior to initiation of pegylated -Interferon α2b/ribavirin therapy. It is predicted that the pro-inflammatory cytokine TNF-α will be suppressed to levels that would permit PegIntron plus ribavirin to be efficient in achieving greater virologic activity in the chronic HCV- infected person. Therefore, we propose to determine whether anti-TNF-α immunotherapy would be beneficial to the current standard of care therapy.

Objectives: The primary aims are to evaluate the effect of Remicade induction therapy on sustained virologic response (SVR) in treatment naïve, G1 HCV patients with high serum TNF-α and the immunomodulatory role of the combination therapy Remicade, PegIntron and Ribavirin. The secondary aims are to evaluate the safety of Remicade induction therapy in treatment naïve, G1HCV patients with high serum TNF-αg as well as to evaluate the effect of Remicade induction therapy on week 12 early virologic response (EVR) in treatment naïve, G1 HCV patients with high serum TNF-α.

Methods: Subjects must be 18 to 65 year of age (no sex and race criteria); HCV genotype 1 (including mixtures of subtypes of genotype 1); Naïve to interferon (any formulation) and ribavirin; Serum TNF-α $>$300 pg/mL.

This Canadian study will conclude the role of cytokine millieu in HCV resistence to current therapy.

CYTOKINES BASIC MECHANISMS.

FGL2 FIBROLEUKIN: A NOVEL CYTOKINE. Gary Levy, MD. Multi Organ Transplant Program, University Health Network, University of Toronto, Toronto, Ontario, Canada

Fgl2/fibroleukin was first cloned from cytotoxic T lymphocytes and sequence analysis showed that it shared significant homology to fibrinogen β and γ chains, and therefore was classified as a member of the fibrinogen superfamily. Studies have suggested that it is a type II

membrane glycoprotein expressed by reticuloendothelial cells and important in the pathogenesis of viral-induced fulminant hepatitis, cytokine-induced fetal loss syndrome, allo and xenograft rejection.

Fgl2 is constitutively expressed at low levels in T cells, endothelial cells, the intestinal mucosa and trophoblast cells. A cluster of cis DNA elements have been shown to account for core promoter activity that is essential for fgl2 constitutive expression. It has been shown that the nucleocapsid protein of both mouse hepatitis virus and hepatitis B virus can induce transcription of fgl2 through the liver enriched transcription factor hepatic nuclear factor-α (HNF4). Additionally cytokines have also been shown to induce expression of fgl2. Interferon-γ can induce macrophage induction of fgl2 whereas TNF-α can induce fgl2 in endothelial cells. The recent availability of an fgl2 knock out mouse have shown the relevance of fgl2 to the pathogenesis of experimental MHV-3 induced liver disease by the observation that fgl2 -/- mice are resistant to liver pathology and have increased survival.

Similar to other important cytokines including CD38, interleukin 1, tenascin and tissue factor, a soluble fgl2 has been shown to be generated by peripheral blood T cells. Soluble fgl2 binds primarily to antigen presenting cells including macrophages, dendritic cells and LPS stimulated B cells. Data now suggests that the fgl2 receptor is the low affinity receptor for immunoglobulin FcγrIIB, a member of the triggering receptor family expressed by myeloid cells (TREM). Binding of soluble fgl2 to FcγrIIB results impaired maturation of dendritic cells and subsequent inhibition of T cell and B cell effector function.

Studies to date, therefore have suggested that fgl2 is an important cytokine which may regulates the host response to viral disease both through its proinflammatory activity and ability to impair specific protective immune responses, and thus allow the virus to escape immune surveillance.

CYTOKINES AND LIVER FIBROGENESIS. G. Ramadori, MD. Department of Gastroenterology and Endocrinology, University of Göttingen, Göttingen, Germany

Hepatic fibrosis is the result of the wound- healing response of the liver to repeated injuries. After an acute liver damage, the liver has the capacity to fully recover. However if hepatic injury persists or is repeated, eventually liver regeneration fails finally leading to liver fibrosis. In all cases inflammation plays a crucial role. Liver inflammation may not be initiated by death of liver parenchymal cells but by liver resident and recruited inflammatory cells. The hepatocellular stress, induced by hepatotoxins (drugs, viruses, alcohol) may lead to activation of liver resident natural killer cells or macrophages. Proinflammatory cytokines like IFN-γ, followed by TNF-α are released very early by the NK- and/ or Kupffer- cells. They lead to changes in the expression of cell adhesion molecules on sinusoidal endothelial cells. These changes together with chemokines, also released by both parenchymal and non- parenchymal cells, allow the recruitment and sinusoidal transmigration of inflammatory cells. E.g. the CXC chemokine interleukin 8 (Il-8) is known as critical chemoattractant and activator for neutrophils, basophils and T cells. CXC is secreted by Kupffer cells, macrophages, and hepatocytes. Increased plasma, hepatic and monocyte levels of Il-8 are well documented in alcoholic liver disease and herein is suggested to play a key role in hepatic neutrophil infiltration. In addition the CXC chemokines macrophage inflammatory protein-2 (MIP-2) and cytokine induced neutrophil chemoattractant which are increased expressed in hepatocytes after endotoxin-induced liver injury are involved in the extravasacular recruitment of leukocytes. The increased expression of lymphocyte function- associated antigen-1 (LFA-1) on recruited mononuclear phagocytes and lymphocytes enables them to invade into the space of Disse, which precedes the death of hepatocytes. In addition to LFA-1 the a4b1 integrin may also be involved. Mediators released by resident and recruited leucocytes (IL-6, IFN-γ, TGF-β, TNF-α, IGF, free radicals, CCL21, CD40), hepatocytes (TGF-β, TNF-α, EGF, IGF) and biliary cells (TNF-α, Endothelin-1, PDGF) during liver injury modulate the behaviour of matrix-producing cells (activation of HSC and liver myofibroblasts). Acitvated HSC themselves produce monocyte chemoattractant protein-1 (MCP-1), angiotensin II or RANTES which stimulate matrix deposition and contribute to further recruitment of monocytes and macrophages. These cells in turn are involved in increased matrix deposition by the action of cytokines, especially TGF-β, PDGF and reactive oxygen intermediates/lipid peroxides. However they are also known to express death ligands strongly suggesting that death receptor mediated apoptosis may contribute to liver inflammation and fibrosis. CD95- agonists, for example, induce chemokine expression in the liver cells, promote neutrophil infiltration into the liver and stimulate liver fibrogenesis.

EGF MEDIATES PROTECTION AGAINST FAS-INDUCED APOPTOSIS. Marc Bilodeau MD. Centre de recherhce du CHUM, Université de Montréal, Montréal, Canada

Hepatocyte cell fate is strongly dependent upon a proper environment. This is afforded by the establishment of intercellular contacts among hepatocytes and non-parenchymal cells as well. Extracellular matrix of the liver, albeit present in small quantities in comparison to other tissues, is also important for the survival of hepatocytes. Humoral factors present in the blood as well as nutrients and proper level of oxygen are also essential for hepatocytes survival and for the maintenance of appropriate cell function. Among these extracellular factors, growth factors have been particularly studied because of their capacity to directly block signals afforded by death signals.

Our laboratory has been particularly interested in the capacity of Epidermal Growth Factor (EGF) to block the prominent death signal afforded by Fas receptor activation. Using primary cultures of mouse hepatocytes, we have determined the importance of the tyrosine kinase activity of the EGF receptor in transmitting the survival signal of this molecule. Furthermore, we have confirmed the EGF, similar to other liver growth factors, modifies the level of expression of BCl_2 family proteins. In particular, EGF increases the level of expression of the anti-apoptotic BCl-xL protein. Furthermore, our laboratory has established that EGF also decreases the level of expression of one of the prominent pro-apoptotic BCl_2 protein: Bid. Indeed, EGF rapidly decreases Bid expression at the protein and mRNA levels. Bid knock-out animals lose their sensitivity toward EGF stimulation. Finally, we have also been interested in the signal transmission pathways activated by EGF: both MAP kinase and PI-3 Kinase pathways are activated following stimulation by EGF. PI-3 kinase activity is essential for the survival signal afforded by EGF. Furthermore, we have identified that EGF increases oxidation of glutathione presumably via a small oxidative stress signal. This leads to a global decrease in glutathione and is associated with resistance to apoptotic stimulation. In conclusion, liver growth factors are potent anti-apoptotic agents that exert this activity at multiple different levels. They are probably essential for hepatocytes survival and well being.

IN VITRO AND IN VIVO MODELS FOR HEPATITIS B AND C. Shlomo Dagan. XTL Biopharmaceuticals Ltd

The lack of *in vitro* and small animal models that are suitable for evaluation of potential therapeutics severely hinders the development of new therapies for HBV and HCV. *In vitro* model systems for HBV infection include hepatocytes and hepatoma cells that are not suitable to study the viral life cycle due to very low levels of viral replication. There are few *in vitro* systems available to study HCV replication. The recently developed replicon system provides a better understanding of viral replication, but does not produce viral particles or infectious virions. More relevant systems based on the generation of HCV like particles and infectious HCV pseudo particles were generated. We have developed a cell-based system, which allows HCV infection, replication, emergence of infectious particles, and evaluation of potential therapeutics. HCV infection and replication was tested in modified human hepatoma cell lines. Cells were infected by incubation with HCV-positive serum. Infection was followed by RT-PCR of HCV-RNA and confirmed by in-situ hybridization. Viral replication was assessed by detection of (-) strand RNA in infected cells. Emergence of infectious particles was demonstrated by the ability of infected cells to infect naïve cells by cell-to-cell contact. *In vivo*, chimpanzees were the major model in the development of HBV and HCV vaccines, evaluation of safety and efficacy of human plasma derived antibodies, and evaluation of antivirals. Their limited availability, expense, endangered status, and the lack of chronic liver disease make them an impractical model system. Recently, we generated the Trimera® mouse model for HBV and HCV infection by using lethally irradiated normal mice, reconstituted with SCID mouse bone marrow cells, where human liver fragments infected *ex vivo* with HCV or HBV had been transplanted. Viremia in these Trimera® mice peaked at approximately day 18 after liver transplantation and an infection rate of 85% was reached. The usefulness of these models for evaluation and anti-HCV or anti-HBV agents was demonstrated by the ability of a small molecule to reduce viral loads in the Trimera® mice in a dose dependent manner. These *in vitro* and *in vivo* model systems provide powerful tools for evaluating efficacy of antivirals.

MECHANISM OF AUTOIMMUNE HEPATITIS AND HEPATOCYTE APOPTOSIS. F. Alvarez, MD.

Autoimmune responses and autoimmune diseases can be generated or aggravated by viral infections both in humans and experimental animals. The infection induces the activation of lymphocytes recognizing dominant and subdominant epitopes of the pathogen, as well as self peptides in a MHC context. This mechanism called "molecular mimicry" has already been proposed for the pathogenesis of autoimmune hepatitis (AIH). Generation of animal models based on clinical and experimental experience are giving new and broader insights into the relative importance of different pathogenic factors and therefore allow the design of more specific treatments.

Several animal models of immune system-mediated liver disease and "AIH" were described, but none of them appeared completely satisfactory in terms of specificity and chronicity. We have generated transgenic mice expressing the Lymphocyte Choriomeningitis Mouse Virus (LCMV)–Nucleoprotein (NP) in their hepatocytes. Five months after peripheral DNA immunization these mice developed an AIH, as shown by an increase in serum transaminase activity levels and an interface hepatitis at the liver histology. The inflammatory liver disease was the result of a CD4+–Th1 response. Cytotoxic T-lymphocytes activated against the neo-self antigen in the periphery migrated and proliferated in the liver. This model showed that breaking of peripheral immune tolerance to a liver specific protein can trigger an hepatocyte injury. This process occurs in the absence of antecedent of liver injury and inflammation.

Recently, we reproduced in mice a model of type 2 AIH by immunization with the human auto-antigenes CYP2D6/FTCD. Cross-species immunization was proved to be successful in producing this animal model of AIH. Mice with elevated serum transaminase levels showed a characteristic liver inflammatory infiltrate, constituted mainly of CD4+ lymphocytes, but also CD8+ and B lymphocytes. Mice displayed a Th1 phenotype of immune

response. When the same protocol was applied to three mouse strains (C57BL/6, 129, and Balb/c), only the C57BL/6 mice developed an AIH. These experiments showed the influence of MHC and non MHC genes in the triggering and development of liver autoimmunity.

Conclusions: These two animal models show that an AIH can be induced in naïve mice using DNA immunization to break peripheral immune tolerance. This break of tolerance against self-proteins induced by molecular mimicry is proof that foreign antigens can use the same mechanism to trigger on autoimmune process in a particular genetic background.

THE ACTIVATION OF INTERFERON AND INNATE IMMUNE PATHWAYS. Sid Balachandran, Raja Venkataraman, Emmanuel Thomas, Ayaz Majid and Glen N. Barber. Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, Florida, 33136, USA

Activation of host innate immune responses following virus infection is largely mediated by viral dsRNA, the mechanisms of which remain to be fully determined. We have recently reported that murine embryonic fibroblasts (MEFs) lacking the death adaptor molecule FADD are defective in double-stranded RNA (dsRNA)-activated antiviral gene expression, including Type I interferon (IFN), and thus predisposed to virus infection. The dsRNA signaling pathway incorporating FADD was found to be largely independent of Toll-like Receptor (TLR)-3, tumour-necrosis factor (TNF) receptor-associated factor 6 (TRAF6) and the dsRNA-dependent protein kinase, PKR, though obligated TBK1 activation of IRF3. The requirement for FADD in innate immune responses is evocative of the *imd* pathway in Drosophila, which involves an *imd*/dFADD complex that responds to bacteria infection by activating the transcription of anti-microbial genes. Accordingly, cells lacking the mammalian *imd* homologue, receptor interacting protein 1 (RIP) were also found to be defective in Type I IFN induction and antiviral activity in response to virus/dsRNA, similar to FADD deficient cells. This data indicate the existence of a key intracellular dsRNA/virus recognition pathway in mammalian cells, which is central for the induction Type I IFN and the activation of other important primary innate immune response genes.

INFLAMMATION AND MALIGNANCIES IN GASTRO-INTESTINAL TRACT

PATHOGENETIC MECHANISMS OF UPPER DIGESTIVE TRACT CANER IN ALCOHOLICS. H.K. Seitz, MD. Department of Medicine, Salem Medical Center Heidelberg, Germany

Chronic excessive alcohol consumption is the strongest risk factor for upper aerodigestive tract cancer (oral cavity, pharynx, hypopharynx, larynx, esophagus). A great number of epidemiological studies have demonstrated a correlation between alcohol ingestion on occurrence of cancer in these organs. These studies clearly show that the ingestion of all types of alcoholic beverages is associated with an increased cancer risk, that suggests that ethanol itself is the crucial compound that causes that effect. The exact mechanism of ethanol associated carcinogenesis has remained obscure because ethanol by itself, when given to animals is not carcinogenic. Multiple mechanisms are involved in the alcohol associated cancer development of the upper aerodigestive tract including the effect of acetaldehyde the first metabolite of ethanol oxidation, the induction of Cytochrome P4502E1 leading to the generation of reactive oxygen species and an enhanced procarcinogen activation, as well as nutritional deficiencies. Acetaldehyde is highly toxic mutagenic and carcinogenic. It interferes at many sites with DNA synthesis and repair. Recent and striking evidence of the causal role of acetaldehyde in alcohol-associated carcinogenesis derived from genetic linkage studies in alcoholics. Individuals who accumulate acetaldehyde due to polymorphism or mutation in the genes coding for enzymes responsible for acetaldehyde generation and detoxification have been shown to have an increased cancer risk. In Caucasians the ADH1C1 allele encodes for an ADH enzyme which produces 2.5 times more acetaldehyde than the corresponding allele ADH1C*2. We have studied the polymorphism of the ADH1C gene in a total of 818 patients with alcohol associated upper aerodigestive tract cancer and control patients and found that the ADH1C*1 allele frequency and rate of homozygosity was significantly associated with an increased risk for alcohol related cancer ($p < 0,001$). Homozygous individuals with the ADH1C*1 allele also have increased acetaldehyde levels in the saliva. In addition to the acetaldehyde which is produced by mucosal alcohol dehydrogenases, acetaldehyde can also be generated by oral bacteria. These bacterial flora is affected by smoking. Smoking itself results in increased acetaldehyde concentrations. Taking these data together, individuals who produce high amounts of acetaldehyde due to genetic predispositions, oral bacteria or smoking are on high risk to develop alcohol- associated upper aerodigestive tract cancer.

ALICAFORSEN ENEMA: ANTISENSE TO ICAM-1 IN PATIENTS WITH ULCERATIVE COLITIS AND CHRONIC UNREMITTING POUCHITIS. P.B. Miner MD, M.K. Wedel MD, E. Chuang, MD, R. Leong, MD. Clinical Research, Isis Pharmaceuticals, Carlsbad, California, USA

Intercellular adhesion molecule-1 (ICAM-1) plays a pivotal role in the recruitment of leukocytes into inflammatory sites and is a co-stimulatory signal for T cells. ICAM-1 is up-regulated in inflammatory bowel disease and has been shown to correlate with disease activity. Alicaforsen (ISIS 2302) is a specific antisense inhibitor of human ICAM-1. Five clinical studies were performed to evaluate

the potential role of alicaforsen enema in the treatment of patients with mild to moderate ulcerative colitis (UC) and chronic, unremitting pouchitis. An initial pilot, dose-escalation study of 40 patients with mild to moderate UC given nightly for 4 weeks showed that alicaforsen enema was safe and well tolerated, with a mean enema retention time >8 hours. The study demonstrated dose response in alicaforsen activity and durability, with statistically significant activity (from the Disease Activity Index scores) in the 120 and 240 mg/enema dose. Subjects in the 240 mg treatment group required no additional surgical or medical intervention during the 6-month follow-up period of the study. Safety and tolerability were again demonstrated in a single-site, open-label PK study of 15 patients with UC given 240 mg alicaforsen enema nightly for 6 weeks. Effective local tissue concentrations were achieved with minimal systemic exposure (<1% absorption) despite mucosal inflammation. Twelve of 15 patients improved and 9 achieved mucosal healing. This study validated the concept of alicaforsen enema use as topical treatment for a topical disease. A placebo-controlled, multi-center study of 112 patients with mild to moderate UC given 240 mg alicaforsen enema nightly for 6 weeks confirmed that the enema was safe and well tolerated. Disease improvement lasted at least 6 months, with results being more prominent in patients with moderate and pure left-sided disease. Alicaforsen enema also reduced the most troubling symptoms of UC; those of rectal bleeding and stool frequency.

An active-control, multi-center study of 159 patients with mild to moderate UC given either 120 or 240 mg alicaforsen nightly for 6 weeks compared alicaforsen to 4 g mesalamine enema, the current enema standard of care. This study showed that the enema was well tolerated and safe, with no drug-related serious adverse events. Alicaforsen 240 mg enema nightly was found to be equal to or more effective than mesalamine enema, and induced a longer duration of response. Finally, an open-label proof-of-concept study of 12 patients with chronic, unremitting pouchitis given alicaforsen enema nightly for 6 weeks showed that alicaforsen enema was safe and well tolerated in this condition as well. Eleven of 12 subjects responded with improvements in clinical symptoms, histology, pouchoscopy, and Pouchitis Disease Activity Index score, and 7 of 12 subjects achieved remission, with remission sustained for one year after treatment cessation in 4 of the 7. In conclusion, these Phase 2 studies in 251 alicaforsen enema-treated subjects demonstrate that alicaforsen enema is safe and well-tolerated, achieves effective local tissue concentrations with minimal systemic exposure, and provides a durable response. It is possible that alicaforsen may be modifying the natural history of these diseases; as such, this promising treatment requires further study.

TRAFICET-ENTM, A HIGHLY POTENT AND SELECTIVE ANTAGONIST OF CCR9, AMELIORATES EXPERIMENTAL ILEITIS AND COLITIS. Zheng Wei,* Linda Ertl,* Trageen Baumgart,* Werner Rubas,* Sok-Ying Hor,† Solomon Ungashe,* Satish Keshav,† Maureen Howard,* J. J. Kim Wright,* and Thomas Schall*. *ChemoCentryx Inc., Mountain View, CA USA; †Centre for gastroenterology, Royal Free Hospital, London, UK

Background: CCL25 (also TECK or thymus-expressed chemokine) is highly expressed in the small intestine. It stimulates chemotaxis by binding to its cognate receptor CCR9, which is expressed on gut-homing T cells co-expressing integrin a4i7. Almost all lymphocytes in the small intestine and approximately 20% in the large intestine are CCR9 positive, suggesting that this chemokine receptor and ligand pair is critically important in localizing lymphocytes within the gastrointestinal tract in health and disease.

Objectives: To determine if pharmacological blockade of CCR9 could ameliorate experimental inflammatory bowel disease.

Methods: Traficet-ENTM (CCX282) is a highly selective and potent (IC50 < 1 nM) small molecule inhibitor of CCR9-mediated chemotaxis, which was developed to achieve excellent in vivo pharmacokinetic and safety properties and oral bioavailability. CCX282 was administered by subcutaneous injection in two murine models of inflammatory bowel disease. The effect on inflammation in the small bowel was determined using a well-established model of ileal Crohn's disease, the TNF? ARE mouse, in which regulatory elements of the TNFa gene have been deleted. The effect on inflammation in the large intestine was determined using a model of ulcerative colitis caused by transgenic disruption of the MDR1a gene. An unrelated chemokine receptor antagonist and vehicle only were administered in parallel as controls.

Results: Blockade of CCR9 significantly ameliorated the severity of ileitis and colitis in the animal models, as determined by clinical and histopathological measurements. No adverse effects of CCX282 treatment were observed, and the control treatments were ineffective.

Conclusion: Blockade of CCR9-mediated lymphocyte recruitment has efficacy in animal models and the data raise the prospect of a safe and effective oral therapy, with a novel mode of action, for human inflammatory bowel diseases.

THE UTILITY OF THE ANTI-TNF ALPHA ANTIBODY, INFLIXIMAB IN THE TREATMENT OF INFLAMMATORY BOWEL DISEASE. M.I. Plotnick, MD. Centocor, Horsham, Pennsylvania, USA

TNF-α is a cytokine that plays a major role in driving acute and chronic inflammation. Therapies targeted to TNF-α such as monoclonal antibodies and TNF receptor-IgG Fc region fusion proteins, to varying degrees, have demonstrated efficacy in the treatment of a number of immune mediated inflammatory disorders. Infliximab is a chimeric IgG Kappa monoclonal antibody that binds the

soluble and cell membrane associated forms of TNF-α. Infliximab has proven utility in the treatment of Rheumatoid arthritis, Ankylosing Spondylitis and Crohn's disease. The mechanism of action of infliximab likely involves blocking of the interaction of TNF-α (soluble or membrane associated) with TNF receptor but other mechanisms may also be involved. The clinical effects of infliximab in inflammatory bowel diseases and potential mechanisms of action will be discussed.

SIGALING FOR INFLAMATION AND REPAIR IN PRIMARY BILIARY CIRRHOSIS AND PRIMARY SCLEROSING CHOLANGITIS. Manuela G. Neuman, PhD. University of Toronto, Toronto, ON, Canada

In primary biliary cirrhosis (PBC) and primary sclerosing cholangiatis (PSC) cholangiocytes and hepatocytes exhibit apoptosis. Tumor necrosis factor-α (TNF-α) and its receptors: B cell activating factor (BAFF) and a proliferating inducing ligand (APRIL) are involved in the regulation of the apoptotic and inflammatory responses.

Our **aim** was to assess: 1- the relationship between serum and tissue TNF-α and liver damage in PBC and PSC patients, 2- the role of BAFF and APRIL in severity of liver damage in PBC and PSC and 3- the immunomodulatory and antiapoptotic effect of ursodeoxycholic acid (UDCA) in these patients.

Methods: Serum levels of TNF-α, apoptosome 30, cytochrome C and caspase 3 activity were determined using ELISA in 180 PBC patients and 96 PSC patients. The liver biopsies of 30 PBC and 20 PSC patients were studied using electron microscopy and immunohistochemistry. BAFF and APRIL were detected by multiplexed protein quantification assay. Measurements were repeated at one year of treatment with UDCA at a dose of 13-15 mg/kg/d (90 PBC and 81 PSC patients) or 25-30 mg/kg/d (PSC 15 patients) or placebo (90 PBC). 560 healthy volunteers were used as controls. The ANOVA or Mann-Whitney test as were used to compare continuous variables among and between groups. Correlations were done using simple linear regression.

Results: Levels of TNF-α (pg/mL) in patients with PBC (920 ± 160) and PSC (1200 ± 180) were higher than in healthy controls (50 ± 15) ($p < 0.001$). TNF-α correlated significantly with more advanced histological activity index (HAI). Patients with HAI > 6 had significantly higher ($p = 0.001$) TNF-α (PBC 1420 ± 200 vs. 170 ± 15 pg/ml; PSC 1820 ± 200 vs. 220 ± 40). When compared to normal controls, caspase 3 activity, apoptosome and cytochrome C release were significantly higher in patients with PBC and PSC ($p < 0.001$). APRIL has significantly higher levels ($p < 0.05$) in PSC than in PBC patients, while BAFF levels do not differ. High levels of APRIL correlate positively with the number of Kupffer cells (CD68 antibodies) in PSC patients. APRIL and BAFF are significantly down ($p < 0.05$) and up-regulated ($p < 0.001$) by UDCA therapy. In PSC treated with a higher UDCA the levels of BAFF are significantly elevated ($p < 0.05$) vs. the patients treated with the lower dose. UDCA significantly lowered ($p < 0.05$) the levels of TNF-α and its receptors in sera and tissue, apoptosome, cytochrome C and caspase activity during the 2 years in PBC and 1 year in PSC patients when compare to their initial values. In **conclusion**, UDCA therapy modulates TNF-α and its receptors signaling for apoptosis in patients with PBC and PSC. This is the first report to show the role of B cell activation in PBC and PSC.

MUCOSAL PLASMACYTOID DC AND THE INDUCTION OF REGULATORY T CELLS. Joanne L. Viney. Amgen, Seattle, WA, USA

The gut is under constant onslaught from a wide range of antigens varying from potentially harmful pathogens to beneficial commensal organisms, yet the mucosal immune cells are competent at initiating appropriate immune responses without much difficulty. Dendritic cells (DC) play a critically important role in regulating the generation of either immunity or tolerance, although the precise mechanistic basis for how this regulation is controlled is not clear and is the topic of this presentation. We have identified a subpopulation of DC that function to promote the tolerogenic responses that may be required in the gut. These DC can differentiate naïve T cells into T cells that exhibit regulatory properties, and these DC can also enhance the suppressive efficacy of CD4+CD25+ T regulatory cells. We propose that these mucosal antigen presenting cells provide a mechanistic basis for contributing to homeostatic regulation in the gut.

CYTOKINES AND DISEASES OF THE LIVER

COMPLICATIONS OF HCV. Nir Hilzenrat, MD. McGill University, Montreal, Canada

Hepatitis C Virus (HCV) infection is a worldwide infection that causes chronic hepatitis. It leads to hepatic fibrosis and eventually, in 10–20% of the patients, to cirrhosis. Those patients may develop the known complications of liver cirrhosis, namely, ascites, esophageal variceal bleeding, splenomegaly and encephalopathy. Approximately 2–3% per year will develop hepatocellular carcinoma. In the United States chronic HCV is the single major reason for liver transplantation in adults.

HCV affects not only the liver but also non-hepatic tissues. Approximately 38% of the patients with HCV will show symptoms of at least one extrahepatic manifestation during their illness. However, the prevalence of clinically significant extrahepatic manifestation is relatively low. For some of these disorders their association with HCV infection is well established while for others it remains probable or weak. The spectrum of extrahepatic manifestations appears in the following systems: endocrine (thyroid and diabetes), haematological (mixed

cryoglubinemia vasculitis, Non-Hodgkin's lymphoma), renal (Glumerulonephritis), musculoskeletal (arthralgia), and dermatological (Porphyria Cutanea Tarda, Lichen Planus). The pathogenic mechanisms of those extrahepatic features in HCV are still unclear. However, a direct viral involvement, a triggering of the immune system, and a presence of circulating immune complexes in the disease suggest an immunological basis. Treatment with Interferon-α decreases the HCV viremia and improves the clinical signs.

LIVER TRANSPLANT THERAPIES, RESISTANCE AND RESERVOIRS. T. Shaw-Stiffel, MD, J. Bierenbaum, MD. University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Host genetic factors appear to play an important role in the development of hepatic fibrosis with recurrence of hepatitis C (HCV) post-liver transplantation. To date, TNF-alpha 308 and IL-10 polymorphisms have been correlated with a higher incidence of acute rejection with the –308A polymorphism in particular. However, this finding is contrasted with another study that looked at a different endpoint and did not show any significant statistical correlation with recurrent HCV and the same TNF polymorphism. Genotypes with high levels of IFN-gamma have shown a significantly lower frequency and severity of HCV recurrence than genotypes producing lower levels. IL-10 has shown an equivocal relationship with HCV recurrence post-liver transplant, and no relationship has been demonstrated with IL-6. Genotypes producing high levels of TGF-B1 have also been correlated with a significantly greater degree of fibrosis in general, although this has not been directly related with HCV recurrence post-liver transplantation. Co-stimulatory molecule (CTLA-4, CD28) polymorphisms may lead to poorer outcomes with the GG genotype of CTLA-4 but no differences with the CD28 polymorphisms have been identified. Chemokines MCP-1, RANTES, CCR5, CCR2, and CCR5 have shown no association with acute rejection or long-term graft survival, whereas the 1-3'A polymorphism of stromal derived factor-1 (SDF1) has been associated with a significantly higher mortality rate post-liver transplant. Lastly, carriage of the e4 allele variant of Apolioprotein E in the recipient, but not the donor, has been associated with better histological outcome in recurrent HCV. In summary, to date several specific gene polymorphisms have been identified as possible targets that might affect progression of liver fibrosis in recurrent HCV post-liver transplantation. Further studies by our group and others are currently in progress to clarify this key area further.

HEPATOCELLULAR CARCINOMA—VASCULAR ENDOTHELIAL GROWTH FACTOR AND ANGIOGENESIS. M. Sherman, T.P. Segerson, MD. The Toronto Health Network, University of Toronto and Bayer, Inc. Toronto ON Canada

A characteristic feature of HCC is hypervascularity and studies have shown that angiogenesis is implicated in survival and growth of this malignancy (1–6).

In cancer, VEGF is produced by tumor cells, but stimulates the proliferation of endothelial cells through specific tyrosine kinase receptors, flt-1 and flk/KDR (7–9). VEGF is also known as vascular permeability factor. VEGF is important in angiogenesis of HCCs, and the VEGF gene is reported to be transcribed, expressed and secreted by HCC cells (10). Overexpression of VEGF in HCC was shown by Northern blot and immunohistochemical studies[2]. Recently, several VEGF activity targeted compounds have been developed. Some are exclusively antiangiogenic, others include antiangiogenic activity (11). BAY43-9006 (sorafenib) has been studied as a single agent for the treatment of advanced HCC. Sorafenib is a novel, potent, orally available bi-aryl urea with dual anticancer functionality. A phase II study has been completed in advanced, inoperable HCC patients that showed a median TTP of 4.2 months and a median OS of 9.2 months. Other antiangiogenic compounds currently being investigated for the treatment of HCC will be discussed briefly.

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INTERFERON AND HEPATOCELLULAR CARCINOMA. Morris Sherman, MD. Liver Unit, University Health Network, University of Toronto, Toronto, Ontario, Canada

Interferon may be involved in several aspects of the pathogenesis and management of hepatocellular carcinoma. Three aspects will be discussed in this presentation

1. Is interferon involved in the pathogenesis of hepatocellular carcinoma?
2. Does interferon therapy prevent the development of HCC?
3. Can interferon be used in the treatment of hepatocellular carcinoma?

1. There is no evidence that the interferon pathways play any role in the pathogenesis of hepatocellular carcinoma. However, the activities of interferon have been investigated in Hepatoma cell lines. For example, there is evidence in PLC/PRF/5 cells that interferon exerts its anti-proliferative activity via suppression of the JAK STAT pathway, which in turn inhibited ERK and MEK. Whether this is important in the pathogenesis of HCC, or in prevention of the development of HCC is unknown.
2. There is considerable evidence of the effect that interferon therapy of hepatitis C on the development of HCC. Interferon therapy is associated with a reduction in the risk of HCC, although the effect is not great. The effect is seen in patients who respond to interferon, and to a lesser extent in patients who do not respond. Whether interferon works by directly inhibiting cellular proliferation, or whether it works indirectly by removing the stimulus for cell proliferation by eradicating or suppressing virus is not clear. The effect of interferon therapy on prevention of HCC in hepatitis B is not as clear, with different reports showing conflicting results.
3. There are several studies of the effect of interferon in combination with chemotherapeutic agents in the management of HCC. However, these are all phase 1 or phase 2 studies, so that it is impossible to fully assess the effect of the interferon. However, in a randomized controlled trial of interferon monotherapy there was no different between treated and control groups.

THERAPEUTIC STRATEGIES IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) -INFECTION AND CO-INFECTION HIV- VIRAL HEPATITITIS C (HCV). Curtis Cooper. University of Ottawa, Division of Infectious Diseases, The Ottawa Hospital, Ottawa, Ontario, Canada

The natural history of HCV infection is altered by co-infection with HIV. Plasma HCV RNA is increased, histological and clinical features of HCV liver disease progress more rapidly and mortality is increased. Although progression to AIDS may be accelerated in those with co-infection, this is predominantly explained by concurrent substance abuse, co-morbid illness, and poor socioeconomic status. There may be a small direct effect of HCV on HIV progression.

In most cases HAART represents the most beneficial therapeutic intervention for HIV-HCV co-infection. HAART often controls HIV disease, is generally associated with less toxicity than HCV interferon-based regimens, reduces hepatic inflammation, slows fibrosis progression, and creates an immunologic milieu which may optimize the effects of HCV drug therapy. Although careful monitoring of liver function is required clinically relevant hepatotoxicity occurs infrequently. Liver enzyme flares occurs in 5–10% of patients starting HAART but are generally asymptomatic and resolve spontaneously. HAART-induced cytokine milieu changes likely have a significant influence on the occurrence and severity of antiretroviral-related liver toxicity. Liver specific mortality is reduced in co-infected remaining on long-term HAART. Antiviral therapy for HCV achieves a SVR in a minority of HIV-HCV co-infected subjects and is associated with multiple toxicities. Although potentially organ and life saving, it should, in most cases, be reserved for those abstaining from alcohol and achieving HIV RNA suppression and immune restoration from HAART.

MOLECULAR EPIDEMIOLOGY OF INFECTIOUS DISEASE & HEALTH POLICY ON THE CYTOKINE BASED ANTI-INFLAMMATORY, ANTI-FIBROTIC TARGETS AND VACCINES

GENETIC DIVERSITY OF INACTIVATED HIV-1 FOR ELICITING NEUTRALIZING ANTIBODIES TO COMPLEMENT CTL INDUCED BY DNA VACCINATION. G. Vyas, MD. UCSF School of Medicine, San Francisco, California 94143-0100, USA

Background: Success of monoclonal vaccination against HBV provided a paradigm for HIV vaccine research. However, cloned viral DNA or proteins neither induce broadly neutralizing antibodies (NAB) against prevalent quasi-species of HIV-1 (pHIV) nor protect against the infection, despite vectored DNA inducing CTL responses that attenuate HIV infection. HIVIG shows *in vitro* NAB-pHIV and *in vivo* protection against infection in chimpanzees. Thus, priming with CTL-inducing vaccine/s, complemented by HIVIG-mimetic antibodies inducible with genetic diversity of inactivated HIV vaccine candidate (HIVACC), is a rational approach.

Methods: Viral genetic diversity, hydrolysis of host/viral nucleic acids, and native conformation of the envelope proteins (gp41/120) are key elements of HIVACC development. For this purpose NAT+/antibody-negative plasma from acute HIV infection was used as the source for pHIV. RPMI containing high levels of IL-2 was used in viral co-cultures with pooled CD4+ cell-substrate derived from "buffy coats" of transfusable blood. Cell-free supernatants from 10-day viral cultures were treated with (i) CD45-coated beads to remove microvesicles, (ii) protease-free DNAse-I and RNAse-A to hydrolyze free host and viral nucleic acids, and (iii) 3X wash through 300 kD Centricon to obtain purified virions. Pooled, purified virions, permeabilized by high hydrostatic pressure (HHP 50Kpsi) or 150/300/500 mM β-cyclodextrin (BCD) were inactivated with Benzonase to hydrolyze viral RNA. Viral culture and RT-PCR validated viral inactivation.

Results: Stock aliquots of pHIV produced ~132 nanograms of virion p24 per million cells. Typically, two

billion CD4+ cells derived from 4 buffy coats produced 264 micrograms of pHIV. Aliquots of purified virions were permeabilized by HHP or by BCD-extraction of cholesterol. Virion RNA and p24 were depleted but envelope proteins (gp120/gp41) were retained in the native lipid bilayer of permeabilized virions. HHP+Benzonase inactivated HIV-1 and gave negative results in culture and PCR tests. However, successive BCD-extractions of cholesterol and repeated Benzonase hydrolysis progressively decreased viral RNA but did not eliminate it completely.

Conclusions: It is practical to produce quantities of different pHIV isolates in pooled CD4+ primary cell substrate. Hydrolysis of host and viral DNA/RNA provides inactivated, genetically diverse, purified pHIV, with depleted capsid proteins but retaining both envelop proteins in lipid bilayer. Comparative immunogenicity in primates to induce NAB-pHIV will permit choice of a final protocol for preparing HIVACC. Ultimately, priming with CTL-inducing vectored DNA, followed by boosting with HIVACC, could be effective and safe for preventive vaccination, especially in Africa and Asia.

PEGYLATED INTERFERON PLUS RIBAVIRIN VERSUS NON-PEGYLATED INTERFERON PLUS RIBAVIRIN FOR CHRONIC HEPATITIS C: COCHRANE HEPATO-BILIARY GROUP SYSTEMATIC REVIEW. M. Simin,*† J. Brok,* D. Stimac,† L.L. Gluud,* C. Gluud*. *The Cochrane Hepato-Biliary Group, Copenhagen Trial Unit, Centre for Clinical Intervention Research, Dept. 71.02, H:S Righospitalet, Copenhagen University Hospital, Blegdamesvej 9, DK-2100 Copenhagen, Denmark and †Clinics of Internal Medicine- Gastroenterology, Clinical Hospital Centre of Rijeka, Kresimirova 42, Rijeka 51000 Croatia.

Background: Pegylated interferon plus ribavirin is recommended as the standard treatment for patients with chronic hepatitis C. We aimed to assess the beneficial and harmful effects of pegylated interferon plus ribavirin versus non-pegylated interferon plus ribavirin.

Methods: We searched The Cochrane Hepato-Biliary Group Controlled Trials Register, The Cochrane Central Register of Controlled Trials in The Cochrane Library, MEDLINE, EMBASE, LILACS, and SCI EXPANDED to March 2005 and contacted pharmaceutical companies and authors of relevant studies. We analysed the following outcomes: sustained virological response, liver-related morbidity plus all-cause mortality, histological response, quality of life, and adverse events.

Findings: We included 14 randomised clinical trials with 4762 participants. Pegylated interferon plus ribavirin had significantly beneficial effects on sustained virological response (RR 0.79, 95% CI 0.71 to 0.87), and histological inflammation (RR 0.38, 95% CI 0.34 to 0.43) compared with non-pegylated interferon plus ribavirin. There were no significant differences between the two interventions regarding liver-related morbidity plus all-cause mortality (OR 0.30, 95% CI 0.05 to 1.73), liver fibrosis (RR 1.00, 95% CI 0.94 to 1.07), or post-treatment quality of life (WMD 0.20, 95% CI −1.96 to 2.36). Pegylated interferon plus ribavirin was associated with significantly lower quality of life during treatment (WMD 1.50, 95% CI 0.32 to 2.68) and increases of adverse events: neutropenia (RR 2.12, 95% CI 1.72 to 2.61) and injection-site reaction (RR 1.66, 95% CI 1.47 to 1.87).

Interpretations: Pegylated interferon plus ribavirin significantly increase the proportion of patients with sustained virological response, but also the risk of some adverse events.

CYTOKINES SIDE EFFECTS. M. Voiculescu, MD. Department of Internal Medicine – Fundeni Hospital, Bucharest, Romania

The side effects of cytokines or cytokine blocking agent treatments are still largely unknown and currently under investigation. This is not surprising given the poor understanding of the complex relationship between cytokines and the relatively small number of cases. Despite their very low concentration levels and short range of action, cytokines are powerful "bullets" due to their synergism, pleiotropic effects, redundancy etc. Only 10 molecules of IL-1 induce T-cells to produce IL-2 or 50 molecules of IFN cause T cells to produce 3-5 oligoadenylate synthetase for arresting viral replication. Due to their pleiotropic effects more than one receptor is activated at the same time, each cytokine having multiple target cells and actions. A given effect is mediated by different cytokines due to their overlap activation. Cytokines also act synergically. The effects of two cytokines are greater than the sum of their parts, IL-1 or TNF being more potent together than either alone.

Cytokines are essential factors in the coordinated immune response and defense against infections and malignancies and are under tight, multilevel control. At DNA level, the great majority of cytokine genes are switched off and strictly coordinated due to their close positioning on the same long arm of chromosome 5. At the posttranscriptional level, the control is performed by the repeat of a common consensus actamer (UUAUUUAU) at the 3' untranslated end of mRNA which make RNA instable. There is also a post secretion control favored by the short half life of cytokines (minutes), soluble cytokine receptor, presence of receptor antagonist or cytokines with opposite actions. Finally, the control at the level of responding cells is performed by regulating the number or sensitivity of receptors or by feedback regulation. Despite highly regulated and tightly controlled cytokine network, administration of cytokines (IFN) or blocking agents of cyto- kines like anti TNF-α (Infliximab, Etanercept) or anti IL-1β (Anakinra) could induce adverse effects in some cases. Case series and anecdotal reports have a low level of evidence but they could provide important information and improve the treatment. Post marketing surveillance

has also identified new and important adverse effects, some life threatening. Controlled clinical studies about cytokine therapy failed to show a significant increase in the risk of vasculitis, malignancy or serious sepsis. Despite this, post-marketing surveillance programs on thousands of patients have identified an increased risk of severe infections, vasculitis and malignancy. The exact role of cytokines therapy in emergence of vasculitis or serious sepsis is difficult to know. Association does not necessary mean a cause-effect relationship. Many of those patients have co-morbidities, are very disabled and usually take corticosteroids and/or immunosuppressive treatment. This makes causal analysis difficult and raises serious doubts about the cytokines therapy role in infections or vasculitis emergency. In addition, case series or anecdotal reports are low grade evidences and do not have enough statistical power to prove an association between two events. In practice, even rare side effects could be life threatening and the specialist must prevent or treat this severe events. There is a big difference between working in an office with numbers, odds and relative risk and quickly taking a right decision in a particular case. Despite low statistical significance of post marketing data they provide useful data for reassessing exclusion criteria to minimizing the rare side effects. Exclusion criteria for anti TNF-α therapy are pregnancy or breast feeding, active infection, high risk of infection (various identified), malignancy or premalignancy. We found that some patients with family or personal history of autoimmune diseases have developed important side effects during IFN therapy. A 21 years old Caucasian female developed SLE with severe nephropathy (class IV WHO) after IFN therapy. Her father suffered from Rheumatoid Arthritis and she was diagnosed with Idiopathic Thrombocytopenic Purpura ten years ago. Another 52 years woman was diagnosed with autoimmune hemolytic anemia and acute renal failure after IFN therapy.

Conclusions: The post-marketing surveillance and periodically reassessing the exclusion criteria are important tools for minimizing the effects of rare side effects. Based on our clinical observations we believe that family or personal history of autoimmune diseases or chronic infections (tuberculosis etc) could be considered as exclusion criteria for cytokine therapy.

PROTEOMICS APPLICATIONS IN PHARMACEUTICAL RESEARCH–IDENTIFICATION OF RATIONAL BIOMARKERS FOR CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD). J.E. Bandow,* J. Sepulveda,‡ J.D. Baker,* C. Painter,* R. Hagler,§ K.A. Clark,* R. Leadley,* I. James,† I. Kilty,† R.A. VanBogelen*. *PGRD, Pfizer Inc., Ann Arbor, MI, USA; †PGRD, Pfizer Inc., Sandwich, UK; ‡currently: Lake Erie Center, University of Toledo, OH, USA; and §currently: Proteome Research Services, Ann Arbor, MI, USA

Whole cell proteome profiling has varied applications in pharmaceutical research. Early in drug discovery insights gained from proteomics can increase knowledge about the mechanism of action of compounds or reveal early signs of toxicity and thereby lead to attrition of compounds. During later stages of drug development proteomics can provide compound differentiation and increasingly proteomics profiling is being employed in the discovery of biomarkers of disease and of compound efficacy impacting clinical trials.

As an example we present a recent effort to identify rational disease biomarkers for COPD from human plasma that could aid in the selection of subjects for future clinical trials. An estimated 600 million people suffer from COPD worldwide. An effective COPD treatment that can restore lung function is not available and little is known on a molecular level about the cause or progression of the disease.

Plasma samples of 24 patients and 24 controls were depleted of twelve highly abundant proteins and separated on 2D-gels. Proteins were stained with Sypro Ruby and quantified using DELTA2D software. The quantitative data was statistically analyzed to identify protein spots with differential abundance in patients compared to controls and False Discovery Rates were used to control the number of false positives. A total of 68 protein spots were selected for identification by mass spectrometry. Several proteins could be linked to the disease, revealing interesting molecular insights into the physiology of the disease, among them proteins involved in the coagulation cascade and lipid metabolism. We were able to build multivariate discriminate models that differentiated COPD patients from controls. To further reduce complexity of a potential biomarker panel abundances of just two proteins were plotted against each other and reasonable separation of patient and control samples was achieved.

We were able to identify potential rational disease biomarkers and independent validation through analysis of additional plasma samples is underway.

This research was performed and funded by Pfizer Inc., Ann Arbor, MI, USA

PLANT DERIVED VACCINE ANTIGENS AND BIOPHARMACEUTICALS: EXPRESSION, FOLDING, ASSEMBLY, FUNCTIONALITY AND ORAL DELIVERY. Henry Daniell. Department of Molecular Biology and Microbiology, University of Central Florida; Biomolecular Science, Bldg #20, Room 336, Orlando, FL 32816-2364, USA (daniell@mail.ucf.edu)

Chloroplast genetic engineering offers a number of unique advantages, including high-level transgene expression, multi-gene engineering in a single transformation event, transgene containment via maternal inheritance, lack of gene silencing, position & pleiotropic effects, undesirable foreign DNA or antibiotic resistance genes.

Transgenes have been stably integrated and expressed via the chloroplast genome to produce large quantities of vaccine antigens and biopharmaceuticals. Hyper expression of vaccine antigens against cholera, tetanus, anthrax, plague or canine parvovirus (4–31% of total soluble protein) in transgenic chloroplasts (leaves) or non-green plastids (carrot) as well as the availability of antibiotic free selectable markers or the ability to excise selectable marker genes facilitate oral delivery. Hyper expression of several therapeutic proteins including human serum albumin (11.1% tsp), somatotropin (7% tsp), interferon-α (19% tsp), interferon-gamma (6% tsp), anti-microbial peptide (21.5% tsp) facilitates efficient and economic purification. Also, the presence of chaperones and enzymes in chloroplasts facilitate assembly of complex multi-subunit proteins and correct folding of human blood proteins with proper disulfide bonds. Functionality of chloroplast derived vaccine antigens and therapeutic proteins has been demonstrated by several assays including the macrophage lysis assay, GM1-ganglioside binding assay, protection of HeLA cells or human lung carcinoma cells against encephalomyocarditis virus, systemic immune response, protection against pathogen or toxin challenge, growth or inhibition of cell cultures. Purification of human proinsulin has been achieved using novel purification strategies (inverse temperature transition property) that do not require expensive column chromatography techniques. Thus transgenic chloroplasts are ideal bioreactors for production of functional human and animal therapeutic proteins in an environmentally friendly manner. Oral delivery of therapeutic proteins via plant cells eliminates expensive purification steps, cold storage & transportation and health professionals/facilities for sterile delivery. Recent developments in this field of research will be presented.

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SELECTED POSTERS

CURCUMIN RESTORES TUMOR-INDUCED IMMUNO-DEPLETION THROUGH CYTOKINE-DEPENDENT AND -INDEPENDENT PATHWAYS INVOLVING JAK-3/STAT-5 and NFκB. Gaurisankar Sa, Sankar Bhattachcaryya, Debaprasad Mandal, Tanya Das. Bose Institute, P-1/12 CIT Scheme VII M, Kolkata 700 054, India (gauri@boseinst.ernet.in)

Patients with advanced cancer exhibit multi faceted defects in their immune capacity, which are likely to contribute to an increased susceptibility to infections and disease progression. We have shown that curcumin, the active component of turmeric, inhibits tumor growth in vivo. Here, we report that tumor-induced immunosuppression involves depletion of both thymic $CD4^+CD8^+$ double positive, $CD4^+$ or $CD8^+$ effecter as well as loss of circulating $CD4^+/CD8^+$ cells. Administration of curcumin to tumor-bearing animals resulted in restoration of progenitor and effecter thymocytes as well as circulating T lymphocyte. A search for the molecular mechanism revealed that tumor burden increased the expression of the pro-apoptotic proteins Bax in thymocytes and peripheral! blood lymphocytes while decreasing significantly the level of pro-proliferative protein Bcl-2. Interestingly, curcumin down-regulated Bax level moderately, augmenting Bcl-2 expression in both these cells. As a result, Bcl-2/Bax ratio was increased and the immunocytes were protected from tumor-induced apoptosis. Further investigation indicates that curcumin restores Bcl2/Bax ratio via Jak-3/Stat-5-dependent pathway. Curcumin restored the expression of common γ chain receptor for cytokines in the immune system, indicating that curcumin restored cytokine induced Jak-3 signaling pathway. In addition to this pathway, we observed another ! very interesting aspect of cancer-induced immunosuppression, which involves the increased oxidative stress-mediated inhibition of nuclear translocation as well as activity of NFκB in thymus. There are some contradictory reports indicating the role of oxidative stress in NFκB activation. Our results indicate that curcumin could ameliorate tumor-induced oxidative stress and that may be the reason for the restoration of NFκB activity, and could also down regulate tumor-induced increase in TNF Receptor-1 expression. Thus restored NFκB activity and down regulated TNF Receptor-1 may be the reason behind curcumin-induced survival of thymocytes. Thus, unlike many other chemotherapeutic agents, curcumin is not only devoid of immunosuppressive effect but also acts as immuno-restorer in tumor-bearing host. These results, thus, raise the possibility of inclusion of curcumin in successful therapeutic regimen against cancer.

HEPATIC NEUTRAL ENDOPEPTIDASE (EC 3.4.24.11) EXPRESSION IN RAT LIVER CIRRHOSIS: A NEW TARGET TO TREAT PORTAL HYPERTENSION? G. Sansoè,*† M. Aragno,† F. Wong,* M. Rizzetto†. *Department of Medicine, Toronto General Hospital, Toronto, ON, Canada; †Department of Gastroenterology, University of Turin, Turin, Italy

In liver cirrhosis atrial natriuretic peptide (ANP) decreases intrahepatic vascular resistance, whilst endothelin-1 (ET-1) causes contraction of hepatic stellate cells and increases intrahepatic resistance to portal flow. The enzyme neutral endopeptidase (NEP) degrades ANP and bradykinin and generates ET-1 from big-endothelin. We determined the effects of NEP inhibition by candoxatrilat on hormonal status, liver function and arterial and portal pressures in rats with CCl_4–induced cirrhosis. Furthermore, NEP protein concentration and immuno-location were analyzed in normal and cirrhotic livers. Two groups of 7 controls received 1 ml 5% glucose solution alone or containing 10 mg/Kg b.w. candoxatrilat; three groups of 10 ascitic cirrhotic rats received placebo, 5 or 10 mg/Kg b.w. candoxatrilat, respectively. In cirrhotic rats 10 mg/Kg b.w. candoxatrilat significantly increased steady-state indocyanine green clearance (a parameter reflecting liver plasma flow) ($P < 0.01$), decreased portal pressure ($P < 0.01$), had no effect on arterial pressure and plasma renin activity but increased ANP systemic plasma levels ($P < 0.05$). Hepatic production of ET-1, as determined through the hormone plasma levels in hepatic vein, was also significantly reduced by candoxatrilat ($P < 0.03$). In the cytosol fraction of rat cirrhotic livers a 280% increase in NEP content was found ($P < 0.01$), chiefly localized in desmin-positive activated stellate cells in fibrous septa. In conclusion, candoxatrilat has few effects on systemic hemodynamics and hormonal status, but decreases portal pressure due to a reduction of liver ET-1 production and intrahepatic portal vascular resistance.

GENETIC MANIPULATION OF LIVER SINUSOIDAL ENDOTHELIAL CELLS. Yoshiyuki Takei, Kenichi Ikejima, Nobuyuki Enomoto, Atsushi Maruyama,* Nobuhiro Sato. Department of Gastroenterology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo, *Institute of Materials Chemistry and Engineering, Kyushu University, 6-10-1 Hakozaki, Higashi, Fukuoka, Japan
Keywords: sinusoidal endothelial cells, gene manipulation, PLL-g-HA

Altered gene expression of liver sinusoidal endothelial cells (SECs) is associated with cell injury and impaired immune response. SEC is a professional antigen presenting cell, inducing antigen-specific T-cell tolerance. Thus, the ability to modulate SEC functions may lead to a new modality to treat intractable liver diseases and achieve liver-specific immunotolerance. SECs possess unique receptors that recognize and internalize hyaluronic acid (HA). We utilized this characteristic of SECs and unique assembling properties of comb-type polycations with DNA in the development of a system for targeting foreign DNA to the SEC. A gene carrier system, comb-type polycations having HA side chains, was prepared by coupling a reduction end of HA oligomers to poly-L-lysine (PLL) in a 1:1 weight ratio. Turbidity and photon correlation spectroscopic measurements revealed that PLL-g-HA formed soluble nanoassociates with DNA of 100–200 nm in hydrodynamic diameter. To determine whether the PLL-g-HA/DNA complexes were recognized by SEC HA receptors in vivo, Wistar rats were injected intravenously via the tail vein with PLL-g-HA complexed to a β-galactosidase expression plasmid (pSV β-Gal) labeled with ^{32}P. One hour postinjection, more than 90% of the injected radioactivity remained in the liver. Administration of the PLL-g-HA complexed to an FITC-labeled DNA revealed that the carrier-DNA complex was distributed exclusively in SECs. The synergetic effect of HA glycocalyx to reduce non-specific interaction and to interact selectively with the HA receptors on SEC played an important role in the targeted gene delivery. Expression of β-galactosidase were detected in SECs when transfected with the PLL-g-HA/pSV β-Gal complex. The PLL-g-HA/DNA carrier system permitted targeted transfer of exogenous genes and DNA agents selectively to the SEC. We showed that the decoy technique in combination with PLL-g-HA effectively inhibits NK-kB translocation to the cell nucleus leading to suppression of TNFα-induced ICAM-1 expression in SEC. Furthermore, PLL-g-HA effectively stabilized DNA triplex formation: it diminished potassium inhibition of the purine motif triplex formation as well as pH-dependence of the pyrimidine motif triplex formation. Thus, the PLL-g-HA/DNA system may provide a novel strategy for manipulation of SEC functions.

FIBROSIS RATE A PREDICTOR OF RESPONSE TO ANTIVIRAL THERAPY IN CHRONIC VIRAL HEPATITIS C. M. Strain, MD, R. Strain, MD. Department of Gastroenterology, University of Medicine, Timisoara, Romania

Aim: To evaluate the relationship between the various factors and the sustained virologic response (SVR) to interferon (IFN)-based treatment in patients with chronic C hepatitis genotype 1b.

Material and methods: 202 HCV genotype 1b patients treated with IFN and Ribavirin were evaluated. 70 of them had an identifiable moment of infection, i.e. transfusion-hepatitis. All patients underwent a liver biopsy and the rate of fibrosis progression was calculated (the stage of liver fibrosis according to the Knodell modified score/duration of infection).

Results: The results for the 70 patients are summarized in the following table:

Demografic data	SR (15)	Non-responders (55)	Student t-Test
Age	47,2 ± 8,84	52,66 ± 9,72	**0,0537**
Knodell	9,46 ± 3,01	11,06 ± 3,42	0,1046
HAI	8,3 ± 2,81	8,9 ± 2,72	0,4546
Fibrosis	1,15 ± 0,98	2,16 ± 1,20	0,0891
Duration of infection	22,77 ± 8,46	22,47 ± 10,96	0,9221
Age at infection	24,46 ± 10,31	30,19 ± 11,51	0,0855
Fibrosis rate	0,058 ± 0,053	0,142 ± 0,141	**0,0274**

The median age of the patients was 47 years and 33% were male. The median rate of fibrosis progression was 0.127 units/year; 62% patients had F2–F4 fibrosis. After 48 weeks of therapy with IFN and ribavirin, 15 patients (28%) achieved an SVR. The rate of fibrosis was higher in non-responders (0,142 units/year) than in those with SR (0,058 units/year) ($P = 0.0274$). In order to realise a logistic multivariate analysis, patients were grouped according to the following criteria: age at therapy < versus ≥45 years, men versus women, fibrosis rate < versus ≥0,127 units/year, disease duration < versus > 22 years.

Logistic multivariate analysis of factors that may influence the response to antiviral therapy

Variabil	Regression coeficient	Standard error	Odds ratio 95% CI	P
Duration of infection <22 years	2,3444	1,0435	10,4268 0,7063-0,9579	**0,0247**
Severe Fibrosis F>1	1,0335	0,9809	2,8166 0,4743-16,7274	0,2546
Sex M	0,9459	0,8655	2,5750 0,4721-14,0440	0,2744
Age at infection	-0,073	0,0507	0,9296 0,8416-1,0267	0,1498
Fibrosis rate	-2,8004	1,2507	0,0608 0,0052-0,7054	**0,0252**
Age at therapy ≤ 45 years	-2,0258	1,5533	0,1319 0,0063-2,7694	0,1922

Conclusions: The rate of fibrosis progression is an independent predictor of SVR to IFN-based therapy in patients with chronic hepatitis C. This factor can be considered in prognostic models evaluating the chance of cure in patients infected with HCV treated with IFN-based therapies.

OLIGOVIA™, A NEW LOW MW DERIVATIVES OF CHITOSAN STIMULATES IL-10 PRODUCTION BY PERITONEAL MACROPHAGES AND DECREASES THE PRODUCTION OF PRO-INFLAMMATORY CYTOKINES AND PGE2. Alexia Monges,* Christian Bleau,* Isabelle Boucher,† Serge Brunet,† Lucie Lamontagne*. *Département des Sciences Biologiques. Université du Québec à Montréal; †ISM Biopolymer Inc, Granby, Québec, Canada

Chronic inflammatory bowel diseases such as Crohn's disease result from an imbalance in cytokines produced by intestinal cells under pressure of baterial flora. No oral treatment is available to control or cure such diseases. Chitin or chitosan-natural products derived from crustacean shells, are co-polymers of N-acetyl-D-glucosamine and D-glucosamine. Many immunomodulating properties were associated with high molecular weight derivatives from chitosan. However, low molecular weight chitosan derivatives express rather anti-inflammatory properties. Oligovia™ is a new commercial nutraceutical ingredient derived from a specific bacterial enzymatic digestion of chitosane-containing specific deacetylated oligomers of glucosamine. Previous *in vivo* studies revealed that this product can control, decrease or prevent inflammatory experimental disease in a mouse model following daily use by oral administration without any detectable toxic effects. In order to determine the mechanism involved in anti-inflammatory properties of the chitosan-derived oligomers, unactivated and activated intestinal macrophages and lymphocytes were treated with various concentrations of the Oligovia™ preparation and purified oligomers of glucosamine. The metabolic activity, the secretion of TNF-α , IL-1β, IL-6, IL-10, IL-12, PGE2 and NO and the Th1/Th2 imbalance were analyzed. Results showed that chitosan oligomers and Oligovia™ modulated differently and in a dose-dependent manner the production of TNF-α, IL-6, IL-10 and IL-12 by inactivated and LPS or PEP-activated macrophages. Thus, low increases of TNF-α were detected while IL-12 rather decreased in oligomer-treated unactivated and activated macrophages. IL-6 increased in oligomer-treated unactivated macrophages but decreased in activated macrophages. PGE-2 and NO decreased or were produced at lower levels in unactivated and activated macrophages. However, IL-10 were induced both in oligomer-treated inactivated and activated macrophages. The shift of IL-10/IL-12 ratio in oligomer-treated macrophages involved a decrease in the metabolic activity of lymphocytes favoring the inhibition of Th1 dependent inflammatory response. Taken together, these results indicate that Oligovia™ and specific oligomers of chitosane provide a new alternative to control or improve chronic inflammatory diseases without toxic or adverse effects.

LATEST TRENDS IN ALCOHOL ABUSE BIOMARKERS. Pamela Bean, PhD, MBA. Executive Director of Research, Rogers Memorial Hospital-Oconomowoc, WI; and Main Partner, Millennium Strategies, 418 N. Westfield Rd, Madison, WI 53717 (PamBean@charter.net)

For the last 20 years, Carbohydrate deficient transferrin (CDT) has been shown to be a valuable biomarker for identifying excessive alcohol consumption and for monitoring relapses during alcohol treatment. A new CDT test was granted FDA approval earlier this year and the three largest laboratories serving the life insurance industry in the U.S. and Canada are preparing to incorporate this test in their menus. This new assay quantifies CDT as a percent of total transferrin using capillary electrophoresis (CE) and it's called the CAPILLARYS™ CDT test. In the CAPILLARYS assay, separation occurs as the transferrin

and the CDT molecules in the serum sample migrate at different velocities through the capillary. The within-run and total precision values ranged between 2 and 18%; inter-day variation was less than 3%.

An important advantage of the new CDT test compared to the mini-column assay is its capacity to provide a visual pattern of the test results for each subject, which in turn translates into an increased specificity of the new assay. Interferences such as those previously encountered with genetic variants of transferrin and non-alcohol related liver conditions show different banding patterns than the ones seen in the alcohol abuser. In clinical terms, this feature not only contributes to the improved specificity of the new test but it also makes it possible to use CDT to screen and to confirm a positive result in the same run.

The new CDT test generates savings in terms of labor costs because it is a walk-away system with little technicians' involvement. The technician loads the samples in the capillary and comes back a couple hours later to get the results. The new method uses parallel electrophoresis capillaries for a throughput of 38 samples per hour and it requires only one pipetting step. After the sample is placed in the rack, the instrument reads the barcode and it processes the specimen without tech involvement. Even more, it analyzes all transferrin forms and does the calculations automatically. At the end it displays a chromatogram showing an image of all transferrin forms.

This presentation will describe how new advances in CDT testing are likely to overcome the challenges perceived today in regards to alcohol abuse biomarkers.

THE ETHIOPATHOLOGY OF THE HEPATOCELLULAR CARCINOMA IN MOROCCO AND THE GENETIC MECHANISM OF THE CARCINOGENESIS. S. Benjelloun,* A. Essaid,† R. Afifi,† E. Cordina,‡ M. Benazzouz,† Marchio,‡ S. Ezzikouri,* I. Chemin,§ C. Trépo,§ P. Pineau¶. *Laboratoire des hépatites virales, Institut Pasteur du Maroc- Casablanca, Maroc; †Service de Médecine C- CHU Ibn Sina- Rabat- Maroc; ‡Unité d'Organisation Nucléaire et Oncogénèse, Institut Pasteur Paris; and §Unité 271, INSERM, Lyon, France

Aim: To better understand the implication of HBV and HCV in the development of the HCC in Morocco, we chose to carry out a pilot study on 40 patients with HCC and 68 control subjects. The average age of the patients is 58.6 years. Serologic (AgHBs, anti-HBc, anti-HBs, anti-HCV and Ag delta) and molecular (standard and Supersensitive PCR for the HBV and HCV) were carried out. The HBV and HCV were genotyped. The existence or not of the pre-core mutant among the patients infected by the HBV was required. The distribution of the HCC according to sex, shows a significant gender difference [three times higher for men (72.5%) compared to the women (27.5%)]. 12.82% (5/39) of the patients were infected with HBV and 65.79% (25/38) with HCV. These results were significantly higher than those observed within the pilot population (2.94% and 1.47% respectively for the HBV and HCV). The majority of HCV subjects were genotype1b. Co-infection HBV/HCV was observed and only one patient had HCC. No patients infected with VHB presented co-infection by the virus delta. Among patients infected with HBV, the genotype carried out by PCR (area PréS1 and PréS2) follow-up of a RFLP showed the presence of the D2 genotype. Mutant precore were observed among these two patients. The research of sequences HBV and HCV, by techniques of sensitive ultra PCR, revealed the presence of sequence HBV among 5 patients among the 34 who are negative Ag HBs (14,70%). Sequences HCV were also found among three of the nine negative anti-HCV patients. The genetic study found specific changes in genes of the p53 and the b-catenine with a proportion of 36% and 7% respectively. A loss of the heterozygote was required on 9 chromosomal arms considered as the principal targets of the deletions in the cancer of the liver. The rates of deteriorations range between 5% (9) and 36% (17). The average fractional allelic loss (FAL, fractional allelic loss, the proportion of chromosomal arms with an imbalance for a given patient) was 31%.

In conclusion in Morocco there is a dominating role of HCV among the patients that reached HCC (65, 79%). The role of the HBV and the "occult" HBV appears as considerable (10/39 patients is 25,64%). Sequences of RNA of the HCV were also detected among 3 patients deprived of anti-HCV antibody. Further studies are necessary for the better apprehension the mechanisms implied in this phenomenon.

THE ROLE OF VEGF SIGNALING DURING OXIDATIVE STRESS. B. Das, R. Tsuchida, O. Morozova, H. Yeger, D. Malkin, S. Baruchel. Institute of Medical Science, University of of Toronto, Toronto, Ontario, Canada

Introduction: VEGF is a pro-angiogenic cytokine involved in vascular permeability and angiogenesis. VEGF mediated signaling induces survival and proliferation of endothelial cells. VEGF signaling may also act as a protective signaling in cells other than endothelial cells. We have recently shown that VEGF/Flt1 signaling serve as an autocrine survival to protect neuroblastoma tumor cells from the post-hypoxia oxidative stress. We also found that VEGF/Flt1 signaling protects osteosarcoma cells from cisplatin-induced oxidative stress. Here we further investigated the molecular mechanism of VEGF-mediated protective activity against oxidative stress.

Methods: Several normal as well as malignant cell lines having active VEGF signaling were exposed to oxidative stress induced by cisplatin treatment (2 μM) with or without treatment with a neutralizing antibody against VEGF. After 24hrs, GSH, GST, and SOD were measured from the cell lysates using standard kits (Cayman Inc. USA). Cellular toxicity was measured by Trypan blue and alamar blue assays.

Results: In all the cell lines tested, 2 μM cisplatin reduced GSH level by 20–30% depending upon cell lines. There was a modest increase of GST level (15–35%) whereas SOD level remain unaffected. The addition of anti-VEGF antibody resulted in further reduction of GSH levels and increased in cellular toxicity. In some cell lines, GST and SOD levels were also affected. For example, in the neuroblastoma cell line, SKNBE-2, where VEGF/Flt1 signaling is active, the combination of cisplatin and anti-VEGF antibody treatment resulted in the significant reduction of GST and GSH level (36% and 68% respectively; $p < 0.05$), whereas SOD level remained same. The cellular toxicity was increased by 18% ($p < 0.05$). Interestingly, anti-VEGF treatment alone did not influence the GSH, GST and SOD levels. In the osteosarcoma cell line, HOS, where VEGF/Flt1 signaling is active, the combination treatment resulted in the rapid decline of GSH, but GST and SOD level remained unaffected. The toxicity increased by 16% ($p < 0.05$). In the hepatocellular carcinoma cell line, HepG2, where VEGF/KDR signaling is active, the combination treatment reduced GSH and SOD levels significantly (35 ± 12% and 21 ± 9 respectively), whereas GST level was upregulated by 24 ± 12 ($p < 0.02$). In the WRL-68, a normal fetal hepatocyte cell line, the combination treatment resulted in the significant reduction GSH, GST and SOD levels. Interestingly, anti-VEGF treatment alone was sufficient to reduce GSH level in the WRL-68 cells. Most importantly, treatment with recombinant VEGF increased the GSH level significantly, and rescued the WRL-68 cells from the cisplatin-induced toxicity. However, addition of a MAPK, ERK 1,2 inhibitor U0126 reversed the VEGF-mediated rescue suggesting that MAPK, ERK1, 2 pathway may be involved in VEGF-mediated GSH upregulation.

Conclusion: Here we show that GSH is a target of VEGF signaling during oxidative stress in normal as well as malignant cell lines.

POSTERS: I–ANTIOXIDANTS.

ANTI-HANGOVER PROTECTS HEPATOCYTES FROM ETHANOL-INDUCED APOPTOSIS. G.G. Katz, M. G. Neuman. Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada

Recently in Canada, anti-hangover capsules are used to reduce the sensation of nausea and headache, in humans when taken together with alcoholic beverages or immediately after drinking. The product contains a combination of herbs e.g., 42.5 % Perilla frusescens and 42.5% Agatache rugosa. In addition it contains 5% taurine a non-essential amino acid. Since taurine is a component of both tauroursodeoxycholic and antihangover product we hypothesis that the anti-hangover it might act as an enhancer of the glutathione (GSH). We developed an experimental model of EtOH and TNF-induced hepatotoxicity using normal human hepatocytes. The objective of this study was to determine whether the PAT would attenuate caspase-3 activation and apoptosis, and the mechanism by which PAT may have beneficial effects. Normal human hepatocytes cells have been treated with either 120 mM EtOH or 30 pg/mL TNF/24 hours, to produce apoptosis. Apoptosis has been reduced significantly both by anti-TNF and caspase 3-inhibitor ($p < 0.001$). When employed at physiological concentrations (50 mM), PAT protected against caspase-3 activation and DNA fragmentation. Apoptosis was quantified both by ELISA and TUNEL. At the ultrastructural level, 120 mM EtOHinduced 36 ± 1.8% ($p < 0.001$) apoptosis vs. control. Two consecutive doses of 120 mM EtOH caused 55 ± 3% apoptosis ($p < 0.0001$ vs. control and $p < 0.001$ vs. previous dose). 50 mM PAT reduced apoptosis at only 12% in 1 dose and 22% after 2 consecutive doses. PAT also protected against intracellular glutathione depletion. In conclusion PAT inhibits caspase-3 activation and cell death, possibly by enhancing mitochondrial integrity.

SILYMARIN PREVENTS HEPG2 CELLS FROM PALMITATE INDUCED CELL DEATH AND INTERLEUKIN-8 PRODUCTION. Z. Song, PhD,* M. Song,* S. Urearte,* T. Chen,*† I.V. Deaciuc, PhD,* D. Lee,§¶ C.J. McClain, MD*†‡. *Department of Medicine; †Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, Kentucky; ‡Department of Veterans Affairs Medical Center, Louisville, Kentucky 40292; §Department of Psychiatry, Bio-Organic and Natural Products Laboratory, McLean Hospital/ Harvard Medical School, Belmont, MA. ¶Natural Pharmacia Interntional Inc., Belmont, MA 02478

Nonalcoholic steatohepatitis (NASH) is part of the spectrum of nonalcoholic fatty liver disease (NAFLD), a condition becoming increasingly recognized both in the United States and worldwide due to its prevalence in obesity, diabetes, and insulin resistance syndrome. Due to the fact that approximately 50% of NASH patients develop liver fibrosis, 15–30% develops cirrhosis, and 3% may progress to liver failure, there is an increasing need to recognize and understand the etiology and treatment of this condition. Emerging evidence suggests that elevated serum FFA levels play an etiologic role in the pathogenesis of NASH, and this has been postulated to be a critical link between obesity, insulin resistance and the risk of NASH. Silymarin is a flavonoid, extracted from the milk thistle Silibum marianum. Although the hepatoprotective properties of silymarin have been reported both from in vitro and in vivo studies, its possible role in preventing hepatotoxicity from FFA, specifically saturated FFA like palmitate, has never been investigated. Here we investigated the effects of supplementation of silymarin on palmitate induced cell death and cytokine (IL-8) production in hepatocytes. HepG2 cells, a human hepatoma cell line, were treated with palmitate in the absence or

presence of silymarin. Supernatants or cell lysates were collected at different time points. Cell death was measured by DNA fragmentation ELISA and caspase-3 activity assay. IL-8 production was assayed by measuring IL-8 release in the supernatants using an ELISA kit. We have found that pretreatment with silymarin protected HepG2 cells from palmitate induced cell death and the protection was independent on its antioxidant character. Moreover, palmitate caused increased IL-8 production from HepG2 cells and silymarin supplementation prevented the enhancement of IL-8 production by palmitate. Although caspase-3 inhibitor also attenuated palmitate induced cell death, it did not prevent palmitate induced IL-8 production, suggesting that prevention of IL-8 production by silymarin was cell death independent.

(Supported by VA, NIH R01 AA01762, NIH R01 AA 10496).

THERAPEUTIC EFFICACY OF THE ANTIOXIDANT PTCA AGAINST STEATOHEPATITIS IN A MODEL. Helieh S. Oz,* DVM, PhD; Craig J. McClain, MD,† Theresa S. Chen, PhD,‡ Willem de Villiers, MD, PhD*. *Digestive Diseases and Nutrition, University of Kentucky Medical Center, Lexington, KY; †Department of Medicine, Division of Digestive Health and Nutrition; ‡Department of Pharmacology/Toxicology, University of Louisville Medical School, Louisville, KY, Ohio State University, Department of Internal Medicine, Division of Digestive Health, OH, USA

Introduction: The pathophysiology of Nonalcoholic Steatohepatitis (NASH) is likely multifactorial and poorly defined. Currently there is no effective therapy available. Some factors involved in the pathogenesis are dysregulated cytokines and the increased reactive oxygen radical caused by the disruption of redox signaling and the control. The aim of the present study was to determine the potential effect of 2(RS)-n-propylthiazolidine-4(R)-carboxylic acid (PTCA), an antioxidant involved in the biosynthesis of glutathione (GSH), to attenuate the progression of steatohepatitis in a dietary rat model.

Material and Methods: Male rats were fed amino acid based diets (1) Methionine Choline sufficient (MCS), or (2) Methionine Choline deficient (MCD) diet. After 3 weeks the rats on MCD diet were further divided and gavage treated with (group A) PTCA; or (group B) given vehicle (MCD). Five weeks after initiation of the study animals were euthanatized. Blood and liver samples were assessed for the pathogenesis and the inflammatory gene expressions.

Results: Rats fed vehicle/MCD (deficient diet) had significant increases in liver enzymes, markers of hepatocyte injury, aspartate (AST: x5.8-fold) and alanine transaminase (ALT: x3.22-fold, MCD vs. MCS $p < 0.001$). However, PTCA therapy suppressed these abnormal enzyme activities to a normal level (MCD>>PTCA∼MCS). Rats on MCD diet developed severe liver pathology manifested with fatty degeneration, inflammation and necrosis (MCS vs. MCD $p < 0.001$) which was improved with therapy (PTCA vs. MCD $p < 0.05$). The endogenous liver antioxidants (SAMe and GSH) were significantly depleted in MCD rats but treatment improved these abnormalities. RT-PCR measurements showed a significant overexpression of inflammatory cytokines, IL-1β (7-fold), IL-6 (17-fold), TNF-α (6-fold) and TGF-β (3-fold) in MCD animals.

Concomitantly, genes involved in the tissue remodeling and fibrosis such as matrix metalloproteinases (MMP13 x4-fold, MMP9 x8.5-fold), collagen-α1 (9.2-fold) and suppressor of cytokines signaling 1 (SOCS1 x2.1-fold) were significantly upregulated in MCD rats. Administration of PTCA significantly down regulated these deleterious genes.

Conclusion: This data indicates the novel action of PTCA against liver injury in this model suggesting a possible application of this compound in NASH/NAFL patients.

HIGHLY EFFICIENT DELIVERY OF ANTI-TUMOR PEPTIDES INTO AGGRESSIVE MALIGNANT TUMORS USING THE PEPTIDE TRANSPORTER SYSTEM. Eisaku Kondo, Tadashi Yoshino. Department of Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmacology, Okayama, Japan

The development of efficient gene/gene product delivery system has recently gained great attention for advanced molecular researches such as gene function analyses and molecular-targeting therapeutic approaches. Here, we present a highly efficient peptide/protein delivery system using a novel peptide transporter. The transporter, Wr-T, raises the efficiency of peptide delivery more than 10 times in comparison to the previous methods, and it enables a cargo peptide/protein to penetrate into various kinds of cells including lymphocytes, epithelial cancer cells, neurons, and mesenchymal cells. For example, it is well known that molecular targeting of hematopoietic malignancies has been generally hindered by technological obstacles to gene delivery in the neoplastic cells, however, Wr-T-mediated transport of p16INK4a functional peptide dramatically inhibits growth of highly aggressive leukemia/lymphomas by up to 80% with a single administration, through restorable of p16 function.

As another noticeable characteristics of Wr-T, it successfully targets not only small oligopeptides but also various kinds of high molecular weight proteins up to a ∼500 kDa. Moreover, it enables simultaneous transport of multiple peptide/proteins into cells, which shows the possibility of multiple molecular-targeting system as well as providing a convenience in choosing protein/peptide as a cargo following the molecular feature of each tumor. The Wr-T system thus represents a powerful tool and highly

effective approach to cargo peptide/protein delivery, with the potential for both basic molecular study and substantially developing peptide/protein-based therapy for human cancer.

II–EPIDEMIOLOGY

HEPATOPROTECTIVE AND IMMUNOMODULATORY EFFECT OF KOBAVIT AND POLYOXIDONIY IN ENVIRONMENTAL-INDUCED TOXIC HEPATITIS LIVING IN KARAKALPAKSTAN. D.A. Musakhodjaeva, A.J. Koshkarov,* A.B. Kurbanov*. Institute of Immunology. Academy of Sciences of the Republic of Uzbekistan; *Nukus branch of the Tashkent Pediatric Medical Institute. Tashkent, Nukus, Karakalpakstan. Uzbekistan

Background: Under abrupt ecological conditions on a world scale, the health care of people and their safety should be a priority in medicine and society as a whole. Karakalpakstan is considered one of the most ecologically polluted regions in the world. Toxic elements (mainly pesticides) are transferred out from the dry bottom of the Aral Sea, where they were brought by water of Sirdarya and Amudarya. As a result of pesticide circulation in the environment the conditions have formed for their penetration and deposition in the human body. During the process of accumulation of pesticides in the body the products of their metabolism may be more toxic and induce disturbance of antitoxic liver function. It is well known that interrelations between hepatobiliary and the immune system in the body are very complex and varied.

Objective: We aimed to observe the role of Kobavit in repair of the toxic depleted immune function of the patient with toxic hepatitis.

Methods: Healthy control volunteers and patients with toxic hepatitis were studied one time. The toxic-induced hepatitis patients received 2 Kobavit tablets twice daily for a duration of 20 days, followed by 1 tablet twice daily for 2 months. In addition, Polyoxidoniy was used i.m. once per day for the first 10 days of treatment, followed by one time/week for 2 months.

Results: Therapy with Kobavit significantly changes the immune parameters in patient with toxic hepatitis.

Immune parameters	Control group (25)	Before treatment (36)	After 6 months (29)
$CD3^+$, %	47,9 ± 0,8	38,8 ± 1,9	45,9 ± 2,2**
$CD4^+$, %	30,1 ± 0,9	23,6 ± 1,4*	30,5 ± 1,7**
$CD8^+$, %	22,1 ± 0,9	32,5 ± 0,9	25,1 ± 0,6
$CD16^+$, %	17,5 ± 0,8	6,9 ± 0,7*	**15,9 ± 0,8****
$CD20^+$, %	28,1 ± 1,0	37,4 ± 1,4*	28,8 ± 1,3**
$CD25^+$, %	26,3 ± 0,9*	13,0 ± 0,4	**25,5 ± 1,6****
HLA-DR^+, %	28,5 ± 1,5	18,7 ± 1,2*	**28,9 ± 1,0****
$CD95^+$, %	25,1 ± 0,6	38,8 ± 1,4*	**27,3 ± 1,1****
IgG,—g/%	822 ± 24	597 ± 21*	900 ± 43**
IgA,—g/%	105 ± 4,7	175 ± 6,6*	147 ± 13
IgM,—g/%	83 ± 3,0	212 ± 8,7*	160 ± 14**

Conclusions: New views on the hepatitis pathogenesis induce necessity of choice of new approaches for their treatment such as antioxidants and polyvitamins to reduce liver inflammation and induce repair.

PARAMETERS OF IMMUNE STATUS IN PATIENTS WITH TOXIC HEPATITIS LIVING IN KARAKALPAKSTAN. D.A. Musakhodjaeva, A.J. Koshkarov,* A.B. Kurbanov*. Institute of Immunology. Academy of Sciences of the Republic of Uzbekistan; *Nukus branch of the Tashkent Pediatric Medical Institute. Tashkent, Nukus, Karakalpakstan. Uzbekistan

Background: Interrelationships between humans and their environment are often target issues in biology and medicine. Mankind actively changes his environment, and he himself has become the object of these changes. Karakalpakstan is ecologically unfavorable. Under the effect of negative environmental factors, pathological changes occur resulting in appearance of new forms of ecological diseases. The harmful substances in the environment accumulate in the hepatobiliary system. The liver and immune system are an integrated immunochemical functional system for homeostasis regulation. Formation of secondary immune deficiency associated with chronic toxic hepatitis is related to development of inflammatory processes, dystrophy, destruction and hepatocyte cytolysis in the hepatic tissue.

Objective: This investigation aimed to study the state of immune systems in patients with chronic toxic hepatitis living in the Karakalpakstan.

Methods: We studied 36 patients with chronic toxic hepatitis admitted for in-patient treatment. The content of lymphocytes in the peripheral blood was determined using monoclonal antibodies ("Sorbent-service," Moscow) to the markers $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$, $CD20^+$, $CD25^+$, $CD95^+$. Immunoglobulins were also studied. IgA, IgG,and IgM using immunodiffusion.

Results: The investigations performed showed reliable reduction of number of $CD3^+$-, $CD4^+$ and $CD8^+$cells and immunoregulatory index–CD4/CD8 during increase in expression of activating markers ($P < 0,001$). A study of humoral immunity revealed reliable increase in numbers of $CD20^+$ lymphocytes, and levels of IgA and IgM were reduced in comparison with the control group ($P < 0,01$). Marked tendency to increase was noted in content of $CD16^+$ cells. During liver decompensation autoantibodies to IgG were found. This complex formed due to high immunologic activity of cells localized in the deep layers of liver tissue. Increased proliferation of these cells occurs due to exceeded content of glycogen, environmental pH change and presence of ektropion, providing development of a pathological state in the connective tissues. Interleukin-1, proteoglycans, neutral proteinase and toxic metabolites excreted by cells finally destroy liver tissue.

Conclusion: The liver has a wide spectrum of specific antigens and participates in the synthesis of immunoglob-

ulins, complement components, inactivation of foreign antigens of different specificity. It is natural to suggest that liver damage may be accompanied by changes of body immune activity. In turn, changes of immunocompetent cell functional state give significant effects on the development of pathological processes in the liver. Formation of secondary immunodeficiency associated with chronic hepatitis is connected with the development of inflammatory processes, dystrophy, destruction and hepatocyte cytolysis in the hepatic tissue.

ORGANIZATIONAL RESPONSE OF US UNIVERSITIES TO INTERNATIONAL AIDS RESEARCH IN AFRICA. Andreea Voinea-Griffin, MBA. University of Alabama School of Medicine, Birmingham, AL, USA

Background: AIDS research in Africa led this trend, fueled by the magnitude of the crisis, availability of research subjects, lack of healthcare systems able to sustain such a public health burden and growing funds from public and private organizations.

Objective: This study examines the organizational responses of US universities to address ethical, regulatory and administrative challenges posed by this international research growth and relationship between policies and structures conducted by the academic institutions.

Methods: Universities were selected based on the amount of AIDS research conducted in the US and abroad with a particular emphasis on research in Africa. The following data were gathered on a convenience sample of 24 of these US Universities: the existence of a specific IRB review/units for international projects, the existence and type of policies/procedures developed by Universities to address aspects specific to international research, the existence of offices/officials responsible for international research and the type of organizational structures used abroad to conduct research. Website searches and a follow-up semi-structured interview were used to determine if there are similar approaches to international research in the sample. Interviews were targeted to 2–3 officials in each University: one from the research compliance office, from the research administration office and from the director of the AIDS research center within the University. At least one official from 20 Universities responded to the survey. 3 Universities restrict access to policies/compliance programs to University employees.

Results: On average, the Universities in this group conduct AIDS research in 8 foreign countries, with a mean of 4 African countries. While only 15 academic institutions had NIH-funded Centers For AIDS Research (CFARs), another 8 universities had designated AIDS Centers. 17 Universities receive Fogarty AIDS International Training and Research Program funds, 5 are recipients of President's Emergency Plan For AIDS Relief funds, and 5 were awarded Bill and Melissa Gates Foundation grants for AIDS research between 2003 and 2005.

There is a great variability on the Universities' approach to international AIDS research. Only 4 of 23 Universities centralized the information in a comprehensive international research policy. Universities identified 15 different areas needing regulations specific to international research. Based upon this identification each composite policy score was given (mean 4; range 1–9). The number of organizational structures created within the university and abroad for the pursuit of international research received a structural score (mean 2; range 1–6). A negative correlation between the quantity of research (as measured by the total NIH ranking in 2003) and the presence of a comprehensive policy ($r = -0.20$), the composite policy score ($r = -0.19$) and the composite structural score ($r = -0.34$).

Conclusions: Most academic institutions appear to rely on the same structures and policies for domestic and international research. If specific policies/amendments about international research existed, they were rarely centralized in a unified document. There was a lack of consistency between Universities on which topics were addressed by specific policies, not directly related to how research-intensive an organization was. A more systematic/best practices approach to international research may improve administrative efficiency and decrease ethical risks posed by conducting research in a foreign environment.

INDUCTION OF APOPTOSIS IN PAPILLARY THYROID CARCINOMA CELLS BY TUMOR NECROSIS FACTOR-A AND CYCLOHEXIMIDE IS PROTECTED BY HEME OXYGENASE-1. G.G. Chen, Z.M. Liu, A.C. Vlantis, C.A. van Hasselt. Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong SAR, China

Both heme oxygenase-1 (HO-1) and $p21^{WAF1/Cip1}$ (p21) are involved in the pathogenesis of human cancer and their functions are closely associated with apoptosis. However, how these two molecules regulate apoptosis in human thyroid cancer is unknown. In this study, we investigated how HO-1 and p21 were regulated in thyroid cancer cell line KAT5 (thyroid papillary carcinoma cell). The KAT5 cells were treated with hemin or cadmium to induce HO-1. The result showed that HO-1 protein was significantly induced by hemin or cadmium in KAT5 cells. Following the HO-1 expression, p21 level was also markedly induced. The increased expression of HO-1 and p21 confers KAT5 cells growth arrest in G_0/G_1 phase of cell cycle. The cells with increased HO-1 and p21 showed obviously resistant to apoptotic stimuli (tumor necrosis factor α and cycloheximide). The levels of HO-1 and p21 induced were significantly inhibited by p38 mitogen-activated protein kinase (p38 MAPK) inhibitor (SB203580) and extracellular-regulated kinase (ERK) inhibitor (PD098059). Parallel to decreased HO-1 and p21 expression, the kinase inhibitors also significantly attenuated the resistance of the cells to

apoptosis. The elevated HO-1 and p21 was further found to be associated with increase activity of the nuclear NF-κB and the inhibition of NF-κB led to the block of their induction. In conclusion, we demonstrate that the resistance to apoptosis in thyroid cancer cells with elevated HO-1 and p21 is involved in a p38 MAPK and ERK-mediated pathway and that this pathway is sensitive to the inhibition of NF-κB.

ASSOCIATION BETWEEN SMALLPOX VACCINATION AND HEPATITIS C ANTIBODY POSITIVE SEROLOGY IN PAKISTANI VOLUNTEERS. M. Aslam, J. Aslam, B.D. Mitchell, K.M. Munir. Punjab Hepatitis Research Center, 31/10A Abu Bakr Block, New Gardentown, Lahore, Pakistan

Goals: To determine whether the smallpox vaccination program has significantly contributed to the widespread prevalence of hepatitis C infection in Pakistan.

Background: Hepatitis C virus has become a worldwide pandemic and has especially devastated developing nations such as Pakistan. There continues to be an increase in fatalities due to hepatitis C-related cirrhosis in Pakistan.

Study: We studied 523 volunteers in the city of Lahore to determine whether the smallpox vaccination program, which ran from 1964 to 1982 in Pakistan, may be responsible for the national surge in hepatitis C viral infection, perhaps due to repetitive use of vaccination devices without proper sterilization or to contaminated vaccine contents.

Results: There was a significantly higher likelihood of hepatitis C antibody seroprevalence in individuals vaccinated for smallpox versus non-vaccinated individuals (21.0% vs. 4.6%, $p < 0.001$, age-adjusted odds ratio = 3.39 [95% confidence interval: 1.36–8.46]). Subjects with positive hepatitis C serology were also more likely to have a history of transfusions (19.2% vs. 9.0%, $p = 0.01$), but hepatitis C viral infection was not significantly associated with a history of surgery or dental procedures. Following adjustment for age, sex, and history of other conditions, including transfusion, the association between prior smallpox vaccination and hepatitis C antibody seroprevalence remained strong and highly significant (multivariate adjusted odds ratio = 6.11 [95% confidence interval: 2.58–14.51]).

Conclusion: These results suggest that the widespread prevalence of hepatitis C infection in Pakistan may be an unintended consequence of the country's smallpox vaccination program, and that blood transfusion is also a significant risk factor.

Keywords: Hepatitis C, Pakistan, smallpox vaccination.

CYTOKINE UP REGULATION PREDICTS DISEASE SEVERITY AND MORTALITY IN HIV/AIDS ZAMBIAN PATIENTS WITH CHRONIC DIARRHEA. I. Zulu,*† G. Hassan,† L. Njobvu,* P. Kelly*†. *Departments of Medicine , University of Zambia School of Medicine, Lusaka, Zambia; †Centre for Adult and Paediatric Gastroenterology, Bart's & The London, School of Medicine, London, UK

Introduction: The HIV pandemic remains a huge public health problem in Sub-Saharan Africa, despite recent gains in reduction of prevalence in some countries and introduction of antiretroviral therapy. The proportion of people receiving life saving antiretroviral therapy remains low. Opportunistic infections such as chronic diarrhea are still common. Understanding of factors such as cytokine expression that are associated with severity of HIV related diarrheal diseases and mortality may lead to development of treatment strategies that will slow down mortality in patients in HIV/AIDS patients presenting with opportunistic gastrointestinal infections . There is currently not much information on cytokine expression in HIV related intestinal infections in Africa and our results brings out such desired information necessary for formulating treatment strategies.

Aim: To determine cytokine and acute phase reactants expression and their association with disease severity and mortality in persons with AIDS related diarrhea in Sub Saharan Africa.

Methods: Serum samples from 105 HIV positive people with and without diarrhea were analyzed for tumor necrosis factor (TNF)-α receptor, Interleukin-6 (IL-6), Interleukin-12 (IL-12), Macrophage Inhibitory Factor (MIF), Interferon-γ (IFN-γpgnd C-reactive protein (CRP). CD4+ cell counts measurements were also performed. In order to study the associations with disease severity and mortality, the study cohort was divided in 5 groups; 1) HIV negative participants who served as controls, 2) asymptomatic HIV positive participants, 3) HIV positive diarrhea participants with CD+ cell count above 200 cells/μl, 4) HIV positive diarrhea participants with CD4 counts below 200 and 5) HIV positive patients with diarrhea who died during follow up.

Results: The TNF-α, CRP and MIF concentrations corresponded with disease severity and were highest in patients who died. Lowest concentrations were in the HIV negative group. TNF-α concentration was significantly different between the 5 different groups ($p = 0.0001$) and between group 5 and the rest of the groups ($p = 0.0001$). Similarly, CRP concentration was significantly different between the 5 groups ($p = 0.0001$) and between group 5 and the rest of the groups ($p = 0.0001$) while, MIF concentration was significantly different between the 5 groups ($p = 0.0001$) and between group 5 and the rest of the groups ($p = 0.03$). IL-6 concentration was significantly different between the 5 groups ($p = 0.001$) and between group 5 and the rest of the groups. However, concentration in group 3 was higher than the rest of the groups. IFN-γ concentration was significantly different between groups ($p < 0.001$) but the pattern was hard to interpret with highest values in groups 2 and 5. There was no correlation between IL-12 with diseases severity.

Conclusion: TNF-α receptor, CRP and MIF up regulation corresponded with disease severity, therefore, could be markers of disease severity and predictors of mortality in Zambian HIV/AIDS patients with diarrhea.

III–GASTROINTESTINAL TRACT

***IN VITRO* MODULATION OF PERIPHERAL MONONUCLEAR BLOOD CELL CYTOKINE EXPRESSION BY THE ANTI-TNFα ANTIBODY INFLIXIMAB.** D. Raddatz, MD, H. Ho, MD, S. Yeruva, MD, G. Ramadori, MD. Department of Gastroenterology and Endocrinology, University of Göttingen, Göttingen, Germany

Introduction: Cytokines play an important role in the pathogenesis of inflammatory bowel disease (IBD) and tumor necrosis factor α (TNF) seems to the most relevant of them. The anti-TNFα antibody, Infliximab (INFL) is effective in many patients with Crohn's Disease (CD). The mechanisms of action of anti-TNFα antibodies are incompletely understood and seem to go beyond pure TNFα neutralisation. In order to better understand the mechanisms of action of anti-TNFα antibodies we aimed to elucidate the effect of INFL on cytokine regulation *in vitro*.

Methods: The influence of INFL on phythemagglutinin (PHA) stimulated mRNA expression of IL-2, IFNγ and TNFα in human peripheral mononuclear blood cells (PBMNC) of four healthy volunteers was measured by quantitative RT-PCR in time course experiments lasting up to 28 hours. PHA was added at baseline and after 20 hours. TNFα protein levels were determined by ELISA.

Results: PHA stimulation was followed by a time dependent increase of all cytokine mRNAs measured. INFL led to a decrease of PHA stimulated T-cell cytokine (IL-2, IFNγ) mRNA expression in all individuals, which was most pronounced after PHA restimulation. TNFα mRNA expression was largely unaffected by IFNL treatment. On the protein level, we could show that TNFα was absent in all INFL treated cultures by ELISA. We could exclude that cells were killed by INFL, since restimultion with PHA 20 hours after the primary stimulation was possible.

Discussion: INFL downregulated the PHA induced mRNA expression of the T-cell cytokines IL-2 and IFNγ in all four individuals, suggesting that the inhibition of T-cell activation might be one major mechanism of INFL action. This effect is not likely to depend on inhibition of TNFα *denovo* synthesis, since TNFα mRNA concentrations remained mostly uninfluenced.

The persistent inhibition of IL-2 and IFNγ after recurrent stimulation, a condition mimicking the situation in chronic inflammation, may be the basis for the sustained immunosuppression observed *in vivo*.

QUANTITATIVE GENE EXPRESSION OF CYTOKINES IN PERIPHERAL BLOOD LEUKOCYTES STIMULATED *IN VITRO* AND IN MUCOSAL BIOPSIES FROM IBD PATIENTS. F. Moriconi, D. Raddatz, S. Yeruva, J. Dudas, B. Meraikib, G. Ramadori. Department of Gastroenterology and Endocrinology, University Clinic, Robert-Koch-Straße 40, 37075 Göttingen, Germany

Background: Cytokines are critical modulators of physiological and pathological immune responses. Quantification of cytokine production is a valuable adjunct to standard immunologic assays in defining several pathologic processes, like inflammatory bowel diseases (IBD) but also rejection reactions after organ transplantation. Quantitative measurement of cytokine mRNA in activated PBLs but also in inflamed tissue may be of importance in the monitoring of immunosuppressive therapy.

Methods: The time kinetics of mRNAs for interleukin-1β, IL-6, TNF-α, (acute phase cytokines) and interferon-γ, IL-2, IL-4, IL-10, IL-12α, IL-12β, IL-15, (T-cell related cytokines), were analyzed by RT-PCR following *in vitro* PHA-stimulation of human peripheral blood leukocytes (PBLs) from five healthy donors. Specific RNAs of IL-1β, IL-6 and β-actin were also evaluated by Northern blot analysis. Cytokine-proteins (IL-1β TNF-α, IL-6, IL-2) and Caspase-1 were measured in the supernatants by enzyme-linked immunosorbent assay (ELISA) and by biosynthetic labeling, immunoprecipitation and SDS-PAGE analysis (IL-1β, IL-6) as well. Biopsy samples of the colon were obtained from 10 patients with established IBD (UC or CD) and from control patients; in the case of control patients indications were evaluation of diarrhea, screening for neoplasia, constipation and anaemia. Cytokine mRNA expression from tissue samples was measured by quantitative real time PCR.

Results: In stimulated PBLs, for most of the cytokines, induction of gene expression started immediately after treatment with phytohemagglutinin (PHA). The peak of stimulation was reached after 4 hours in the case of acute phase cytokines and after 8 to12 hours in case of T-cell cytokines (IL-4, IL-10, IL-12α). In activated PBLs and in inflamed tissue acute phase cytokines were by far more abundantly expressed compared to T-cell-related cytokines at the RNA level. The RNA up-regulation *in vitro* was confirmed at the protein level. IL-6 was the cytokine which was most markedly induced in activated PBLs and in inflamed mucosa of the colon (1110 and 45 fold increase respectively). Remarkable levels of IL-1β and TNF-α were detected in normal colon mucosa. However, in Crohn's disease patients an increase in IL-1β and TNF-α expression of 2.2 and 1.7 fold compared to the control, respectively, was observed. In Ulcerative colitis patients an increase in IL-1β and TNF-α expression of $\sim$10 and 4.8 fold compared to the control, respectively, was shown. The quantitative relationship between the single cytokines (acute phase cytokines vs. T-lymphocyte cytokines) observed *in vitro* mimics that observed in the inflamed mucosa of patients with inflammatory bowel diseases.

Conclusions: Our results, obtained both *in vitro* and from human samples, show that macrophage-derived cytokines are more abundant in the early phase of PBLs PHA-stimulation and in inflamed mucosa compared to so called T-cell cytokines. Detection of significant levels of IL-1β and TNF-α in the control colon mucosa can be interpreted as a basal activation of the mucosal immune system. The *in vitro* model of PHA-stimulated PBLs may be useful for studying compounds for the treatment of IBD.

ABNORMALITIES OF MAST CELLS DISTRIBUTION AND PEPTIDERGIC INNERVATION OF THE GUT WALL IN CROHN'S DISEASE. RELEVANCE OF NEURO-IMMUNE INTERACTION TO INTESTINAL INFLAMMATION AND TREATMENT. I. Stoyanova. Department of Anatomy, Faculty of Medicine, Thracian University, P.O. Box 1025, BG-6010 Stara Zagora, Bulgaria (stoyanovai@yahoo.co.uk)

Background: Regulatory neuropeptides and must cells play an important role in motility, secretion, and immune and inflammatory response in the gastrointestinal tract.

Objectives: This study aimed to investigate the chemical coding of the enteric nervous system (ENS), as well as the distributional pattern of mast cells in the distal ileum of patients with Crohn's disease (CD).

Methods: Immunocytochemical techniques to localize substance P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide (VIP), and the mast cells marker enzyme – tryptase were applied on specimens from patients with CD. Significant alterations in the neuronal structures innervating the gut were observed in all layers of the ileum with CD.

Results: The number of SP-, VIP-, and CGRP-expressing neurons was higher than in the controls. In addition, there was a chaotic display of nerve fibres containing the neuroactive substances SP, VIP and CGRP with high frequency of enlarge varicosities in the circular muscle layer and in the submucosal and the serosa layer. The number of tryptase-positive mast cells was significantly increased in CD. Such a dramatic increase in neuronal fibers expressing SP, VIP, and CGRP, as well as in the number of mast cells, indicates that they may contribute to impaired gut motility as observed in Crohn's ileitis.

Conclusions: These findings suggest that mast cell activation and nerve-immune interactions may play a significant role in the pathophysiology of the intestinal inflammation in CD. Moreover, it raises the possibility for development of new pharmacotherapeutic approach to CD.

APOPTOTIC IMBALANCE OF INFILTRATING LYMPHOCYTES BETWEEN TUMOR AND NON-TUMOR TISSUE IN THE DEVELOPMENT OF COLORECTAL CANCER. G.G. Chen,*† J.FY Lee,* U.P.F. Chan,* H. Xu,* R.Y.C. Yiu,* K.L. Leung*. *Department of Surgery; †Sir Y.K. Pao Center for Cancer, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong

Immunological cells play an important role in neoplastic development. Among immunological cells, lymphocytes exert a diversity of mechanisms against tumor growth. In solid tumors, the presence of tumor infiltrating lymphocytes, natural killer cells and macrophages, provides an effective antitumor response. The number of tumor infiltrating lymphocytes in resected tumor specimens negatively correlates with the size of tumor but positively with survival. The mechanism leading to the dysfunction of infiltrating lymphocytes in the tumor remains unclear. The aim of the study was to investigate whether apoptosis contributed to the loss of infiltrating lymphocytes and the inactivation of their antitumor functions in colorectal cancer. We first demonstrated a significant increase in apoptosis in infiltrating lymphocytes of colorectal cancer tissues, compared to those in non-cancerous tissues from the same patient. Furthermore, our result suggested that disturbance of apoptosis resulted from an imbalance of decreased antiapoptotic molecules and increased proapoptotic ones, reflected by reduction of Bcl-2 level and elevation of Bax level. The shift of balance of Bcl-2 and Bax in favor of the latter accelerated the activity of caspase-3, as Bcl-2 expression led to functional inhibition of caspase-3 while Bax promoted caspase-3 activity. Therefore, caspase-3 could be considered as an executer of apoptosis in Bcl-2 and Bax pathways. In line with the role of caspase-3 in apoptosis caused by a decrease in Bcl-2 and an increase in Bax, the expression of caspase-3 was found to be significantly elevated in infiltrating mononuclear cells of colorectal cancer but not those in non-cancerous tissues. The apoptosis or the activity of caspase-3 may also be associated with inducible nitric oxide synthase (iNOS), whose level increased in the present study. The inhibition of nitric oxide production may revoke immunosuppression and benefit progressively growing malignant tumors. Therefore, increase in iNOS expression in infiltrating mononuclear cells is thought to down-regulate the immune response against colorectal cancer cells and thus may contribute to the loss of lymphocyte functions in the tumor. In conclusion, this study reveals that apoptotic imbalance occurs in infiltrating lymphocytes between tumor and non-tumor tissues. This imbalance may attenuate the function of infiltrating lymphocytes in the tumor tissue and thus contribute to the development of colorectal cancer.

ACTIVITY OF CHITOSAN ON THE EPITHELIUM AND INDUCTIVE SITES OF THE INTESTINAL MUCOSA. C. Porporatto, M. Canali, S.G. Correa. Inmunología. CIBICI (CONICET). Facultad de Ciencias Químicas. UNC. Argentina

Intestinal epithelial cells (IEC) are key components of the mucosal immune system acting as lining barrier, sensing antigens or sampling macromolecules. IEC participate actively in response to luminal stimuli through the

secretion of cytokines and chemokines and the expression of pro-inflammatory genes. Chitosan is a mucoadhesive polysaccharide able to increase the intestinal permeability, to deliver antigens, to adjuvate mucosal vaccines and to induce anti-inflammatory cytokines such as IL-10. To assess the activity of chitosan at the epithelial and inductive sites we fed rats 200 μl of acetic acid (diluent group) or 1, 3 or 5 mg of chitosan and 16 h later we purified IEC and mononuclear cells from Peyer's patches (PP) and mesenteric lymph nodes. The mean fluorescence intensity of CD54 expression and the percentage of pSTAT-3 positive IEC (flow cytometry) were similar in diluent or chitosan fed groups. The NO/arginase balance (colorimetric method) showed an increment in the arginase pathway in a dose dependent manner: 104.9 (diluent), 146.8 (1 mg), 201.7 (3 mg) and 295.2 (5 mg) mU/10 6 cells ($p < 0.05$) without changes in the release of NO ($p =$ NS vs. diluent). The expression of the secretory trefoil factor 3 (TFF3) assessed by RT-PCR diminished ($p < 0.05$) after chitosan feeding. The expression of TNF-α ($p < 0.05$) and the chemokine MCP-1 ($p < 0.05$) was up-regulated in IEC in a dose dependent manner after the feeding, without changes in IL-12 or IL-8 mRNAs ($p =$ NS) (RT-PCR). In PP, chitosan triggered a significant increment in IL-4 ($p < 0.05$) and a reduction in TNF-α ($p < 0.05$) mRNA expression, without modifying IL-12 mRNA expression (RT-PCR). In MLN, the polysaccharide induced increments in the percentage of OX62+ and IL-10+ cells. A trend in the increment of OX62+IL-10+ cells was observed (flow cytometry). Chitosan up-regulates the expression of chemokines and cytokines that participate in cell recruitment while settles a non-inflammatory environment at the inductive sites.

THE EFFECT OF HELICOBACTER BILIS INFECTION IN HUMAN BILE DUCT CANCER CELL LINE. S. Takayama, MD, H. Takahashi, MD, Y. Matsuo, MD, Y. Okada, MD, T. Manabe, MD. Department of Gastroenterological Surgery of Nagoya City Univ., Nagoya, Japan

Background: *Helicobacter pylori* (*H. pylori*) infection is associated with chronic atrophic gastritis, peptic ulcer, and gastricmalignancies. But other Helicobacter species are not known well yet. In mice, *Helicobacter hepaticus* is closely relation to hepatoma and hepatocellular carcinoma. Even in human, there are many reports that helicobacter species are found commonly in extragastric organs by polymerase chain reaction (PCR). In biliary tracts, there are reports that *H. bilis (Helicobacter bilis*), which is isolated from bile of mice and cause of chronic active hepatitis, may be associated with biliary malignancies of human.

Aim: We investigated the effect of *H. bilis* infection in human bile duct cancer cell line (HuCCT-1).

Methods: We co-cultured HuCCT-1 with *H. bilis* which were purchased from ATCC (American Type Culture Collection). The concentration of VEGF (Vascular Endothelial Growth Factor) in the conditioned medium was measured using ELISA (Enzyme-Linked Immunosorbent Assay) kit. To investigate the effect of *H. bilis* in HuCCT1 regarding HUVECs (human umbilical vein endothelial cells) tube formation, HuCCT-1, HUVECs, and fibroblasts were co-cultured using a double-chamber method in 24-well plates with and without conditioned medium. Furthermore, HuCCT-1 cell line, which was transfected NF-κB luciferase vector, the activity of NF-κB was measured by Dual Luciferase Reporter Assay.

Result: VEGF level, Angiogenesis, and NF-κB activity in H.bilis infected cell line were higher than control.

Conclusion: The infection of *H. bilis* in human bile duct cancer cell line activates NF-κB, which stimulates production of VEGF then lead to enhancement of angiogenesis. Those pathways are quite similar to which of *H. pylori* infection for gastric cancer cell line. The detail of this mechanism is still unknown, but *H. bilis* infection may play an important role for biliary tract malignancies.

IV–LIVER: TRANSPLANT

CYTOKINE POLYMORPHISMS AND LIVER TRANSPLANT OUTCOMES. J. Bierenbaum, MD, T. Shaw-Stiffel, MD, A. Steele, RN, M. Romkes, PhD, R. Branch, MD, A. Marcos, MD. University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Background: Liver fibrosis is the result of a complex series of events involving multiple genes and cell signals. Gene polymorphisms are now being applied to predict fibrosis progression in liver transplant recipients with recurrent hepatitis C virus (HCV) infection. Scientifically, this is more difficult to analyze since genetic profiling is required in the recipient as well as in the donor. To date, tumor necrosis factor (TNF)-alpha 308 and interleukin (IL)-10 polymorphisms have been linked to a higher incidence of acute rejection mostly with the −308A polymorphism, although in a more recent study, no statistically significant correlation was found between the severity of recurrent HCV post-liver transplantation and the same TNF polymorphism. On the other hand, genotypes with high levels of interferon (IFN)-gamma have been linked to less severe HCV recurrence. IL-10 has demonstrated an equivocal relationship with HCV recurrence, whereas no relationship has been shown with IL-6. Genotypes producing high levels of transforming growth factor (TGF)-B1 have shown a significantly greater degree of fibrosis. However, this did not appear related to HCV recurrence. Co-stimulatory molecule (CTLA-4, CD28) polymorphisms suggest poorer outcomes with the GG genotype of CTLA-4, but no differences with the CD28 polymorphisms. The chemokines MCP-1, RANTES, CCR5, CCR2, and CCR5 have not been linked with acute rejection or long-term graft

survival. However, the 1–3′A polymorphism of stromal derived factor-1 (SDF1) has been associated with a significantly higher mortality rate post-liver transplantation. The e4 allele variant of Apolipoprotein E in the recipient, but not the donor, was associated in one study with improved histological outcome in recurrent HCV.

Methods: We will perform genetic testing on explanted livers for some or all of the following targeted genes: TGF-B1 (Codon 10 or Codon 25 A->G), Angiotensinogen (-6 G->A, M235T), Angiotensin II Type 1 Receptor (A1166C), TNF-alpha (−308 G->A), HFE (845 G->A) and ApoE (e4 allele), in an attempt to correlate this with the post-liver transplant outcomes in recurrent HCV, specifically patient and graft survival. This should help to improve risk stratification, ultimately leading to better transplant outcomes.

ASSESSMENT OF FUNCTIONAL IMMUNITY DURING TREATMENT OF CHRONIC HEPATITIS C INFECTION WITH PEGYLATED INTERFERON AND RIBAVIRIN. P. Schmeltzer, MD, T. Shaw-Stiffel, MD, A. Steele, RN, A. Zeevi, MD. University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Background: The current standard treatment of chronic hepatitis C viral (HCV) infection calls for 12 to 48 weeks of pegylated interferon and ribavirin, based on viral genotype. Quantitation of serum HCV RNA is usually performed after 3 or 6 months of therapy. Due to the side effects and cost of HCV treatment, it would be beneficial to assess response more accurately during therapy. The Immuknow^{TM} (Cylex, Inc) quantifies cell-mediated immunity by measuring ATP production by CD4+ lymphocytes. Similarly, the T Cell Memory Assay^{TM} (Cylex, Inc) can measure ATP production by CD3+ T cells. Until now, these assays have been studied primarily in solid organ transplant recipients on immunosuppression in an effort to determine global immune response. We are now looking at this test in patients chronically infected with HCV during combination therapy with pegylated interferon and ribavirin.

Methods: 15 consecutive patients with chronic hepatitis C (high viral load genotype 1) who are candidates for treatment with pegylated interferon and ribavirin will be eligible for this study. All patients will be screened for HIV as well as other causes of liver disease. Baseline CD3+, CD4+, and CD8+ counts and functional immunity will be determined prior to treatment. Thereafter, blood will be drawn for the Cylex assays at specified intervals after the start and finish of combination treatment.

Results: We hypothesize that functional immunity, measured by the Cylex assay, will increase after starting pegylated interferon and ribavirin. Furthermore, those patients who demonstrate an early immune response measured via the Cylex assays may have a better chance of achieving a sustained virologic response. We expect to show a direct correlation between an early increase in functional immunity and viral clearance.

IV–LIVER: NONALCOHOLIC STEATOHEPATITIS

CITOKYNES, ENDOTHELIAL DYSFUNCTION AND NONALCOHOLIC STEATOHEPATITIS. L. Miele, A. Santoliquido,* C. Di Campli,* M.L. Gabrieli, A. Di Giorgio,* M.A. Zocco,* A. Forgione, A.P. Hernandez, V. Vero, A. Gallo, C. Cefalo, A. Lupascu,* R. Flore,* P. Tondi,* P. Pola,* G. Gasbarrini, A. Gasbarrini,* A. Grieco. Departments of *Internal Medicine and †Angiology, Catholic University, Roma, Italy

Introduction: Nonalcoholic fatty steatohepatitis (NASH) is considered a clinical and histological feature of of metabolic syndrome. NASH is associated with increase of blood pressure, but data on endothelial function in this condition are lacking.

Aims and Methods: To assess the systemic endothelial function and soluble adhesion molecules in NASH. Foe this aim, 17 patients with histological proven NASH (Age: 35 ± 7; BMI: 26.3 ± 3.1 Kg/m2) were consecutively enrolled. The endothelial dysfunction at the brachial artery was performed according to the standard protocol. The flow-mediated dilation (FMD) was expressed as percent change in diameter after reactive hyperemia relative to the baseline value. The serum levels of sE-Selectin, sVCAM, and sICAM and VEGF were measured by ELISA. A group of 15 healthy subjects matched by sex and age were enrolled as control group.

Results: The mean FMD in patients with NASH was significantly reduced compared to FMD of healthy controls (4.1 ± 10.48 vs 16.7 ± 9.7; $p < 0.001$). Endothelial independent vasodilation was similar between the two groups (18.89 ± 6.46 vs 18.57 ± 7.4, NS). There was no significant difference in serum levels of VEGF, sE-Selectin, ICAM1 and VICAM between the two groups.

Conclusion: For the first time we report the result of endothelial dysfunction in NASH. As demonstrated in other inflammatory chronic diseases, endothelial dysfunction could be an independent risk factor for cardiovascular disease. These data support the presence of systemic endothelial dysfunction in patients with NASH.

TIMPs, MMPs AND FIBROGENESIS IN NONALCOHOLIC STEATOHEPATITIS (NASH). L. Miele, A. Forgione, P. Di Rocco, V. Vero, M.L. Gabrieli, A. Gallo, C. Cefalo, G. L. Rapaccini, G. Gasbarrini, A. Grieco. Department of Internal Medicine, Catholic University, Rome, Italy

Introduction: NASH has increasingly been recognised as an important and common form of liver disease over the past 20 years. The strong correlation between the development of fibrosis and the necroinflammatory activity

in the liver is well known and implies a role for inflammatory mediators in fibrogenesis. Non-invasive approaches to assess the presence of liver fibrosis would be helpful to confirm and to quantify the presence of fibrosis.

Aims and Methods: The aim was to characterize the fibrosis network in patients with NASH. We assayed TGF beta, metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and laminin in subjects with histologically proven NASH, compared to patients with HCV chronic active hepatitis and patients with well compensated cirrhosis. We studied 19 patients with histological diagnosis of NASH (age: 37 ± 9 yrs, M/F:17/2), 23 patients with chronic active hepatitis (CAH) (age: 50 ± 9 yrs, M/F:15/8) before antiviral treatment and 9 patients with well compensated cirrhosis (age 55 ± 8 yrs, M/F:6/3, Child A-B). Eight volunteers (age: 31 ± 8 yrs, M/F:5/3) served as healthy controls well matched for age, gender and BMI . The fibrosis (Knodell score) was minimal in NASH patients (range 0–1) and mild-severe in CAH (range 1–3).

Results:

	Healthy	NASH	CAH	Cirrhosis
ALT (IU/L)	46 ± 19.7	69.8 ± 44.02	96.77 ± 35.92	60 ± 51
AST (IU/L)	29.5 ± 5.1	39.16 ± 6.4	62.01 ± 22.86	50 ± 43
TGF-beta (ng/ml)	12.15 ± 10.03	49.83 ± 18.34*	34.21 ± 22.52*	193.7 ± 181.1*
MMP-1 (ng/ml)	3.68 ± 3.63	$6.5 \pm 5{,}06$	5.5 ± 15.5	2.21 ± 1.6
MMP-2 (ng/ml)	1264 ± 109	1157 ± 380	1465 ± 399	1445 ± 153
TIMP-1 (ng/ml)	277 ± 48	422 ± 155	1575 ± 394	660.6 ± 328.2
TIMP-2 (ng/ml)	67.07 ± 10.9	149.2 ± 91	113 ± 105.6	170 ± 79
Laminin (ng/ml)	undetactable	141.61 ± 27	124.35 ± 26	150.11 ± 20

*$p < 0.001$ (NASH vs Healthy; CAH vs Healthy; Cirrhosis vs Healthy, CAH, NASH)

Conclusions: TGF beta is a profibrogenic cytokine that regulates cell growth, and extracellular matrix metabolism. The TGF levels could be used as a marker of fibrosis. Circulating levels of MMPs and TIMPs have been shown to correlate with the development of inflammation and fibrosis. TIMP-1 levels correlates significantly to inflammation rather than fibrosis, MMP-2 levels increase with the amount of fibrosis. In our series we found in NASH and CAH patients similar significant increase of TGF beta levels, while cirrothics show significantly higher levels if compared to NASH and CAH; TIMP-1 levels increases from normal liver to chronic active hepatitis with a decrease in chirrostics. The significant increase of TGF beta and TIMPs levels in NASH when compared to healthy controls may represent the progression of fibrosis in these patients; the decrease of TIMP-1 levels in cirrothics might represent the early stage of cirrhosis or a low grade of inflammation in our series. Patients with NASH, CAH and cirrhosis show similar levels of laminin suggesting that in NASH fibrogenetic events and fibrosis have already started despite histological features. While laminin is an extracellular matrix protein reflecting deposition of fibrosis, our data point out serum TGF-beta and TIMPs as index of "continuous" fibrogenic and inflammation activity in NASH. Their profile may be a useful tool to assess progression of disease.

FAMILIAL NON-ALCOHOLIC STEATOHEPATITIS. Manuela G. Neuman, PhD, Marius Brown, MD, Ross G. Cameron, MD, Laurence M. Blendis, MD, Steve Malnick, MD. Departments of Pharmacology & Laboratory Medicine, University of Toronto and In Vitro Toxicology Laboratory, Toronto, Canada, Liver Institute, Rabin Medical Center, Beilinson Campus, Petah Tikva, Israel, Division of Gastroenterology & Department of Medicine, Kaplan Hospital, Hebrew University, Rehovot, Israel and Rabin Medical Center, Tel Aviv, Israel

Background and Aims: Non-alcoholic steatohepatitis (NASH) has been associated with progressive changes such as fibrosis, which becomes part of cirrhosis. We aimed to examine hepatocytes and perisinusoidal cells in liver biopsies of 2 families (3 males and 2 females) with non-cirrhotic and cirrhotic NASH to determine what changes are occurring at different stages of this disease during a period of 2–5 years since diagnosis. Body mass index was over 32.

Methods: In this study, hepatocytes, stellate cells and Kupffer cells were analyzed using light and electron microscopy, and immunohistochemistry with specific anti-macrophage antibody staining of liver biopsies.

Results: There was a three-fold increase in relative numbers of Kupffer cells (perisinusoidal macrophages) in the older sister with NASH compared to livers of the younger siblings. The lipid droplets were more numerous and larger in the hepatocytes and the collagen deposits were 5 times larger compared to the siblings. The second family, the older brother had cirrhosis, minimal inflammation and mild fatty infiltration while the younger had fatty infiltration with no inflammation or fibrosis. After 2 years period all the patients progresses in the severity of their disease. The special finding in livers of patients with NASH was accumulation of groups of perisinusoidal macrophages, which was not associated with focal necrosis.

Conclusion: Perisinusoidal macrophages appear to accumulate in NASH. It is possible that collections of sinusoidal macrophages are a response to chronic portal endotoxemia or bacteremia. The persistent activation of these macrophages could lead to the chronic release of cytokines and contribute to chronic inflammation, fibrosis and cirrhosis.

IV–LIVER: DRUG ADVERSE REACTIONS

UTILITY OF PATCH TESTING IN PATIENTS WITH ANTICONVULSANT-INDUCED HYPERSENSITIVITY SYNDROME. Simon Nigen, MD, Lori E. Shapiro, MD, Sandra R. Knowles, PhD., Neil H. Shear, MD, Manuela G. Neuman, PhD. Service de Dermatologie, Département de médecine, Hôpital Maisonneuve-Rosemont, Université de Montréal, Montréal, Québec; Divisions of Dermatology, Clinical Pharmacology and Drug Safety Clinic, Departments of Medicine and

Pharmacology, Sunnybrook and Women's College Health Sciences Centre, University of Toronto, Toronto, Ontario, Canada

Background: Drug hypersensitivity syndrome (DHS) is a severe idiosyncratic reaction that is a major concern in clinical practice. It is a potentially life threatening syndrome, characterized by a triad of fever, skin eruption and internal organ involvement. Drugs most commonly associated with DHS include sulfonamide antibiotics, allopurinol and anticonvulsants. Previous studies evaluating the utility of patch testing in anticonvulsant hypersensitivity syndrome are limited and provide inconsistent results. Cytokines are known to be involved in the mechanism of DSH.

Objective: Our aim was to evaluate the utility of patch testing as an ancillary diagnostic tool in DHS and their cross-reactivity and compare it with the present already standardize test for laboratory. The cytokine net-work in these patients based on serum levels was evaluated.

Method: Seventeen patients with a documented anticonvulsant-induced hypersensitivity syndrome and with a positive lymphocyte toxicity assay (LTA), which is the actual gold standard for laboratory diagnosis of DHS to an anticonvulsant underwent patch testing to each of carbamazepine, phenytoin, phenobarbital and lamotrigine. Drugs were tested diluted 1% and 10% in both petrolatum and Phlojel base. Serum cytokines were measured by ELISA. Levels are given (TNF-α, IL-6 pg/mL and Fas ng/mL) as mean $\pm$ SE. The Wilcoxon rank sum compared differences between groups. Differences among samples in time in the same group were determined using analysis of variance.

Results: Among the 17 patients who were patch tested, 4 (24%) had a positive test. In 2 cases, patch test with phenytoin was positive in Phlojel base but negative in the petrolatum base. One patient with a carbamazepine-induced hypersensitivity syndrome had a positive test to carbamazepine as well as with phenobarbital. Also, one patient with phenytoin-induced hypersensitivity syndrome had a positive test to phenytoin and to phenobarbital drug. No side effects associated with patch testing were reported. LTA test showed a 99% sensitivity and 98% specificity with a positive predictive value of 89%. TNF α (133 ± 3) were significantly higher in LTA positive than LTA negative (40 ± 6) ($p < 0.001$). Baseline Fas levels were lower in LTAs negative (2.3 ± 0.2) than LTAs positive (5.4 ± 0.4) ($p < 0.05$). IL 6 was not significantly different when compare LTAs negative to the LTApositive. No differences have been observed in cytokine values between LTA positive patients to phenytoin, phenobarbital or carbamazepine.

Conclusion: Patch tests with anticonvulsants are safe but of limited value in investigating hypersensitivity syndrome reactions. Cross reactivity with other anticonvulsants may be detected by patch tests. LTA is a very sensitive diagnostic test in patients with DHS to anticonvulsants. Cytokine environment may play a role in DSH.

DETERMINATION OF ZONISAMIDE HYPERSENSITIVITY WITH A LYMPHOCYTE TOXICITY ASSAY. M.G. Neuman, PhD, L. Cohen, MD. *In Vitro* Toxicology Laboratory, Department of Pharmacology, University of Toronto, Toronto, ON, Canada

Background: Hypersensitivity syndrome reactions (HSRs) to antiepileptic drugs (AED) and sulfonamide antibiotics (SMX) are a major concern in clinical practice. HSRs are associated with rash, fever and organ involvement culminating with toxic epidermal necrolysis (TEN), which has a mortality of 20-60%. Zonisamide is a broad spectrum antiepileptic that contains a sulfonamide moiety in its chemical structure.

Objectives: Correlation of Lymphocyte Toxicity Assay (LTA) positive responses and toxicity levels to AEDs or SMX with Zonisamide and to determine the cytokine network in these patients.

Methods: Forty patients were evaluated; ten patients on AEDs with TEN and 10 with HSR to AEDs as well as twelve patients on SMXs with TEN and 8 on SMXs with HSR. Controls consisted of 20 patients on AEDs without HSRs and 20 without SMX-HSRs. HSRs patients had manifested fever, cutaneous eruptions $\pm$ organ involvement within 8 weeks of exposure to lamotrigine, carbamazepine, phenytoin, phenobarbital or sulphametaxazole. The mitochondrial enzyme (succinate dehydrogenase) activity as a measure of cell viability was used. LTA showing toxicity higher than 12.5% was considered positive. The Wilcoxon rank sum test was used to compare differences between groups. Tumor necrosis factor alpha in serum and M30 (mitochondrial apoptotic marker) were employed to measure serum TNF levels and apoptotic score.

Results:

1. A perfect correlation between the LTA positive and the diagnosis in TENs ($r = 0.89$) and HSRs ($r = 0.80$).
2. No correlation was found between the AED-control, AED-HSR, and AED-TEN compared to Z-LTA.
3. Only one of 12 SMX-TEN was co-positive with Zonisamide with a lower toxicity [(SMX-LTA 38.8% vs. Zonisamide 22% ($p < 0.05$)]. TNF-alpha value in the patient that presented *in vitro* cross-reactivity to SMX and Zonisamide was 4 times higher than the patients that were positive to SMX but negative to Zonisomide.
4. Apoptotic index in all the LTA-SMX positive was significantly higher when compare with LTA-SMX negative.

Conclusions:

1. No correlation is found between Zonisamide HSR and other AEDs.

2. There is a weak correlation in patients with known sensitivity to SMX and Zonisamide.

INTERFERON–ALPHA AND RIBAVIRIN THERAPY COMPLICATED WITH AUTOIMMUNE HEMOLYTIC ANEMIA AND ACUTE RENAL FAILURE. E. Rusu, MD, D. Micu, MD, E. Buzatu, MD, M. Voiculescu, MD. Centre of Internal Medicine and Nephrology, Fundeni Clinical Institute, Bucharest, Romania

We present the case of 52 years old Caucasian female patient with chronic hepatitis C treated with standard interferon (INF) alpha and ribavirin. The periodic control analysis have detected anemia (hemoglobin level between 8 and 9 g/dl), mild thrombocytopenia, without any adjustment of the treatment doses. Seven months later the patient developed asthenia, vomiting, diarrhea, elevated blood pressure, normal urine output. Patient was referred to our clinic with anemia and acute renal failure. Assessment of anemia found hemoglobin 6.6 g/dl, hyperbilirubinemia (total bilirubin 5.4 mg/dl with direct bilirubin 2.6 mg/dl), macrocytes on the peripheral blood smear, high level of reticulocytes (6.5%), positive direct and indirect Coombs test, so autoimmune hemolytic anem! ia related to antiviral therapy was considered, by exclusion of other causes. Renal evaluation attested serum creatinine 10.5 mg/dl and blood urea 370 mg/dl, mild proteinuria (0.7 g/day), microscopic hematuria and normal kidney appearance on ultrasound examination. Our diagnosis was acute tubulointerstitial nephropathy with acute renal failure. We decided to stop INF-alpha and ribavirin therapy. Immediate management consisted of corticoid therapy with favorable clinical and biological response. After two months of corticoid therapy, the patient is in very good condition, with mild arterial hypertension, persistent polyuria, without anemia or kidney failure (creatinine 0.6 mg/dl), and mild hepatocytolytic syndrome (ALT 1.5 X N). INF-alpha was associated with autoimmune hemolytic anemia with warm and cold antibodies and acute renal failure. The pathogenic role for INF-alpha is suggested by the close temporal relationship and improvement of clinical and laboratory abnormalities after drug withdrawal. Patients treated with INF-alpha must have close hematological and renal surveillance during, and several months after, the therapy has stopped.

PROLONGED TREATMENT WITH INTERFERON α AND PEGINTERFERON INDUCES RHEUMATOID ARTHRITIS SYNDROME AND ERYTHEMA NODOSUM. C. Ionescu, MD,* M. Voiculescu, MD,* C. Ursaciuc, MD,† G. Ismail, MD,* L. Micu, MD,* N. Caceaune, MD*. *Department of Internal Medicine, Fundeni Clinical Institute; †Immunology Laboratory, V. Babes Institute Bucharest, Romania

Interferons (INF) are a family of natural cytokines that share a common ability to interfere with viral replication and immunoregulation. We present the case of a 40-year-old Caucasian female with chronic hepatitis C (CHC), genotype 1b treated with INF-α and Ribavirin for 48 weeks. In the 45 weeks of treatment she developed symmetric peripheral inflammatory arthralgia associated with pain, edema, and sensibility. Blood tests defined nonspecific inflammatory syndrome (ESR = 48mm/1h, fibrinogen = 580mg/dl, moderate polyclonal hypergammaglobulinemie, IgG prevalent) and the presence of ANA in 1/256 titer. Rheumatoid factor was absent. There was an imbalance in T lymphocytes population with low Th/Ts (1.2, normal = 1.5–3) due to an increase of Ts (34%, normal = 15–25%). The HLA map showed the presence of DR3 and DR4 and the absence of B8. The rheumatoid arthritis syndrome (RA) disappeared after interrupting INF therapy under analgesic and non-steroidal antiinflammatory drugs. 6 months after therapy ANA was negative. Two years later, because CHC relapsed, the patient restarted PegIFN and Ribavirin therapy. After 10 months of treatment she developed a similar RA associated with tender, red bumps on the shins of both legs. Titers of ANA were elevated and there was an imbalance in T cells (Ts = 33%, Th/Ts = 1.2). Biopsy of the cutaneous lesion diagnosed erythema nodosum. Antibiotics and corticosteroid therapy for 10–14 days and non-steroidal antiinflammatory drugs were used until the end of antiviral therapy. After cessation of INF therapy the symptomatology rapidly regressed. The follow-up after for two years showed no symptoms of RA and no sign of erythema nodosum. We conclude that INF-α and PegINF induced reversible immune changes such as RA and erithema nodosum. The presence of a HLA DR3 and DR4 profile, as in our patient, is a favorable circumstance of INF-α induced autoimmunity due to a lymphocyte subpopulation imbalance. Retreatment with PegINF in patients with autoimmune disorders induced by INF-α caused more pronounced immune disease.

INFECTIOUS COMPLICATION RATE DURING INTERFERON-BASED HCV THERAPY ARE NOT RELATED TO NEUTROPENIA. S. Al-Bedwawi, MD, C. Lee, MD, D.E. Garber, RN, C.L. Cooper, MD. University of Ottawa, Division of Infectious Diseases, The Ottawa Hospital, Ottawa, Ontario, Canada

Background: Infectious complications of interferon-based HCV therapy are not well described. The correlation to interferon-induced neutropenia is unclear.

Methods: All recipients of interferon-based HCV therapy followed at The Ottawa Hospital Viral Hepatitis Clinic between June 2000 and May 2005 were identified from a SPSS 11.0 clinical database containing patient characteristics and laboratory data. All infectious complications identified by staff or reported by patient during the period of interferon exposure and one month after were identified by charts review. No patients received G-CSF.

Results: 195 patients received 211 courses of therapy (5684 person-weeks of therapy). 67 infectious complications occurred in 57 patients (1.18 infections/100 person-weeks of therapy). The median time to infection was 17 weeks of therapy (quartiles: 7, 17, 22 weeks). Infections included: 40% respiratory, 16% cutaneous, 15% oral cavity; 12% genitourinary, 12% gastrointestinal. 15% of infections were fungal (thrush-6, cutaneous-4) in nature. Four (6%) were considered severe adverse events (i.e. hospitalization and/or discontinuation of therapy). Age, sex, race, HIV status, stage and grade of biopsy, type of interferon, and use of erythropoetin were not correlated with infection rate by Cox regression analysis. Neutrophil counts declined from a baseline mean of 3800 cells/μL (SD 1700) to a nadir of 1900 cells/μL (SD 1100) by week 8 of therapy. The total, fungal and bacterial infection rates did not correlate with nadir neutrophil count or size of decline from baseline. The total infection rate was not greater for those with nadir counts $<$1000 cells/μL (0.83/100 person-weeks of therapy) or $<$750 cells/μL (0.71/100 person-weeks).

Conclusion: Neutrophil count is not correlated with infection rate in recipients of interferon-based HCV therapy. Interferon dose reduction and/or G-CSF dosing in those with neutropenia is not supported by this analysis.

CYTOKINE NETWORK IN IBUPROFEN-INDUCED HEPATOTOXICITY. Manuela Neuman,*† Lawrence Cohen,‡ Manuel Gomez,§ Joel Fish,§ Neil Shear,¶ Michael Nicar**. *In Vitro* *Toxicology Laboratory, †Div. Biochemistry & Genetics, ‡Gastroenterology, §the Ross Tilley Burn Centre; ¶Dermatology of the Sunnybrook & Women's College Health Sciences Centre, Toronto, ON, Canada and **Baylor University Medical Center, Dallas, TX USA

Background: Hypersensitivity syndrome reactions (HSRs) to drugs are a major concern in clinical practice. HSRs are associated with rash, fever and organ involvement culminating with liver failure leading to transplant or Steven-Johnson-Syndrome (SJS) and toxic epidermal necrolysis (TEN), which has a mortality of 20–60%.

Objectives: Correlation of Lymphocyte Toxicity Assay (LTA) positive responses and toxicity levels to Ibuprofen with the level of tumor necrosis factor alpha (TNF-α), Interleukin 6, Fas and RANTES in blood.

Methods: 17 suspected HSR to IBF were studied. HSR patients had fever, cutaneous eruptions and organ involvement within 8 weeks of exposure to Controls consisted of 17 volunteers that took IBF and did not have any adverse reaction to the drug. HSRs patients had manifested fever, cutaneous eruptions $\pm$ organ involvement within 8 weeks of exposure to IBF. 3 of them have been diagnosed with SJS and 1 with TEN. The mitochondrial enzyme (succinate dehydrogenase) activity as a measure of cell viability was used. LTA showing toxicity higher than 15% was considered positive. The Wilcoxon rank sum test was used to compare differences between groups.

Results:

1. A perfect correlation between the LTA positive and the diagnosis in SJS and TENs ($r = 0.89$) and HSRs ($r = 0.80$).
2. A good correlation between HSR positive and levels of RANTES and TNF-α. A negative correlation was found between the HSR-IBF positive and the levels of IL6.
3. The highest level of Fas was observed in the person that underwent liver transplant as a result of the hepatotoxic interaction of a combination of halothane and ibuprofen.

Conclusions:

1. LTA is a good ancillary diagnostic tool for HSR to IBF.
2. HSR may be a secondary phenomenon to a cytokine imbalance that leads to higher apoptotic procesis.

SIGNALING FOR HYPERSENSITIVITY SYNDROME. Manuela Neuman,*† Lori Shapiro,†¶ Izabella Malkiewicz,* Masud Taeri,* Lawrence Cohen,‡ Manuel Gomez,§ Joel Fish,§ Neil Shear¶. *In Vitro* *Toxicology Laboratory, †Div. Clinical Pharmacology, ‡Gastroenterology, §the Ross Tilley Burn Centre, and ¶Dermatology of the Sunnybrook & Women's College Health Sciences Centre, Toronto, ON, Canada

Background: Toxic epidermal necrolysis (TEN) is a potentially life threatening disease that is often associated with prior exposure to aromatic antiepileptic drugs (ACs) and sulfamethoxazole (SMX). We have used an in-vitro lymphocyte toxicity assay (LTA) to investigate systemic hypersensitivity reactions (HSRs) to these drugs and applied the same technology to investigate TEN.

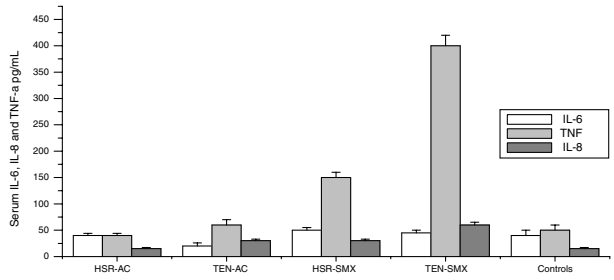
Objectives: 1-Validation of an in-vitro LTA for assessing TEN from ACs or SMX; and 2-Identification of specific immuno-responses.

Methods: Twelve patients with TEN (6 associated with ACs and 6 associated with SMX), and 178 patients with HSRs (88 suspected to ACs, and 90 suspected to SMX) were studied. HSR patients had fever, cutaneous eruptions and organ involvement within 8 weeks of exposure to ACs (carbamazepine, phenytoin or phenobarbital) or SMX. Both TEN and HSR patients were tested at least 2 months after the adverse event, therefore not during the acute situation. Mitochondrial succinate dehydrogenase activity was used to measure cell viability. LTA showing lymphocyte toxicity higher than 12.5% was considered positive. Differences in serum levels of interleukin 6 (IL-6), interleukin 8 (IL 8), and tumor necrosis factor alpha (TNF-α) among groups were determined by analysis of variance and Wilcoxon rank sum test. The χ^2 test

or Fisher's exact test was used to compare frequency data between groups, when appropriate.

Results: A high correlation was observed between a positive LTA and the clinical diagnosis in TEN patients ($r = 0.99$). TEN- ACs patients had lower IL-6 than controls ($p < 0.05$), and other groups (figure). TEN-SMX patients had the highest serum levels of TNF-α compared with HSR-SMX patients ($p < 0.05$) and TEN- ACs patients ($p < 0.001$). IL 8 was significantly higher in TEN-SMX patients than controls ($p < 0.05$).

Conclusions: The LTA technique appears to be an accurate diagnostic marker for TEN. The differences in chemokine and cytokine profiles may represent differences in the immunopathogenesis of TEN vs. HSR or may reflect changes in different underlying diseases. However, the indications for treatment in HSR and TEN patients was similar.



LONG-TERM NEPHROTIC SYNDROME, IDIOPATHIC THROMBOCYTOPENIC PURPURA AND SYSTEMIC LUPUS ERYTHEMATOUS INDUCED BY INTERFERON-α. M. Voiculescu, MD,* C. Ionescu, MD,* C. Ursaciuc, PhD,† G. Ismail, MD,* L. Micu, MD*. *Department of Internal Medicine, Fundeni Clinical Institute; †Immunology Laboratory, V. Babes Institute, Bucharest, Romania

Interferon-α (INF) has multiple side effects, some of them severe, which may require interruption of treatment. We report the case of a 21-year-old Caucasian female patient with chronic hepatitis C treated with INF. From the medical history of the patient we remarked the presence of ITP during childhood, without signs of SLE at that time. Six months after starting INF therapy the patient/s condition progressively worsened, with moderate fever, facial rash, migratory polyarthralgia, palpebral and ankle oedema, purpura, hemorrhagic gums and recurrent epistaxis. Blood tests showed severe thrombocytopenia ($20 - 52 \times 10^9$/dl) and nephrotic syndrome (proteinuria = 5.2 g/day, hypoalbuminemia = 2.3 g/dl). Immunological tests showed homogenous, spotted and ring-shaped FAN, LE cells++, and low C_3. HLA typing showed DR3 presence. There was an imbalance in T lymphocytes population with low Th/Ts (1.1, normal = 1.5–3) due to an increase of Ts (38%, normal = 15–25%). SLE diagnosis was established based on 5 from 11 ARA SLE diagnostic criteria with idiopathic thrombocytopenia purpura (ITP) and lupus nephropathy. The renal biopsy proved type IV WHO lupus membranous nephritis.

Thrombocytopenic purpura and nephrotic syndrome were remitted after INF interruption and corticosteroid therapy. Two years later only the nephrotic syndrome relapsed and after 9 years is still present. The PCR HCV level is undetectable.

In our case INF-α probably induced immune changes unmasking SLE in a HLA DR3 positive young woman. The presence of ITP during childhood is an early sign of autoimmunity aggravated by INF-α and by the presence of an imbalance in the T lymphocyte population. Patients treated with INF-α must have close immunological, hematological and renal surveillance during and after INF therapy. History of autoimmunity is an aggravating circumstance.

IV–LIVER: FIBROGENESIS, PRIMARY BILIARY CIRRHOSIS, PRIMARY SCLEROSING CHOLANGITIS AND HEPATOCELLULAR CARCINOMA

ACTIVIN SECRETED FROM HEPATOCYTES ACTIVATES HSCS AND PLAYS A KEY ROLE IN LIVER FIBROSIS. N. Kitamura, MD,*† T. Azuma, MD,† K. Tomita, MD,† S. Inokuchi, MD,† T. Nishimura, MD,† H. Nagata, MD,† T. Hibi, MD,† H. Ishii, MD†. *Hino Municipal Hospital; †Keio University School of Medicine, Japan

Background: Hepatic fibrogenesis represents wound-healing responses to chronic liver injury which includes hepatocellular damages. Molecular mechanisms by which hepatocellular damages lead to subsequent TGF-β gecretion from the nonparenchymal cells remain quite unknown. We have herein reported that the recombinant follistatin blocks hepatic fibrogenesis induced by dimethylnitrosamine (DMN) in rats.

Methods: HSCs were primary cultured with various concentrations of activin and TGF-β. Activin, TGF-β and collagen I mRNA expressions were measured by RT-PCR. Wistar male rats were injected intraperitoneally with dimethylnitrosamine (DMN) and intravenously with saline or follistatin three times a week for three weeks. Tissue sections were stained with collagen IV, α-smooth muscle actin, fibronectin, TGF-β. In situ hybridization was performed for the detection of activin. Activin and TGF-β mRNA expressions were measured by RT-PCR. Liver tissues were obtained 0, 12, 24, 36, 48, 60, 72 hrs and 7 days after single DMN-injection. Hepatocytes, stellate and Kupffer cells were isolated and analyzed for activin and TGF-β mRNA expression.

Results: 50% of control rats died whereas none of follistatin-treated rats died. In follistatin-treated rats; the serum hyaluronic acid, AST and ALT levels were significantly reduced, the expression of TGF-β, Collagen IV

and α SMA were reduced. Activin expression was observed at maximum level in hepatocytes 12hrs after DMN treatment. TGF-β expression was strikingly increased in stellate and Kupffer cells 24–48 hrs after DMN administration but was not detectable in hepatocytes. Exogeneous follistatin decreased activin and TGF-β expression from non-parenchymal cells. Activin expression from hepatocytes was prior to non-parenchymal cells and was not altered by follistatin treatment.

Conclusions: Hepatocytes exposed to the reagent turned out to secrete activin prior to the secretion and overexpression of TGF-β in HSCs. The blockade of activin by exogenous follistatin effectively inhibited proliferation and activation of HSCs, downregulated extracellular matrix production, and consequently attenuated liver fibrogenesis, suggesting that activin derived from hepatocytes accounts for a crucial initiator of HSC activation. These results not only suggest that hepatocytes utilize activin to trigger TGF-β-mediated fibrogenic responses of HSCs but also shed light on a possibility that recombinant follistatin serves as a potentially therapeutic stratagem that can target hepatocellular activin to modulate hepatic fibrogenesis.

IMMUNOMODULATORY EFFECT OF URSODEOXYCHOLIC ACID IN PRIMARY BILIARY CIRRHOSIS. M.G. NEUMAN, PhD, P. COLIN, PhD, P. ANGULO, MD, K.D. LINDOR MD. Department of Pharmacology, University of Toronto, Toronto, Ontario, Axcanpharma Inc. Mt. St. Hilaire, PQ, Canada; & Mayo Clinic and Foundation, Rochester, MN, USA

In primary biliary cirrhosis (PBC) liver cells exhibit both necrosis and apoptosis. Tumor necrosis factor-α (TNF-α) may signal for apoptosis via caspase activation.

Our **aim** was to assess: 1- the relationship between serum and tissue TNF-α as caspase activator and liver damage in PBC patients, 2- the effect of ursodeoxycholic acid (UDCA) on inhibiting (TNF-α) signaling for apoptosis in these patients.

Serum levels of TNF-α, and caspase 3 activity were determined using ELISA in 150 PBC patients and 320 healthy volunteers. The ANOVA or Mann-Whitney test as were used to compare continuous variables among and between groups.

Levels of TNF-α in PBC were significantly higher than in controls ($p < 0.001$). TNF-α correlated significantly with more advanced histological activity index (HAI). Patients with cirrhosis and HAI > 6 had significantly higher TNF-α (720 ± 160 vs. 170 ± 15 pg/ml, $p = 0.001$). When compared to controls, caspase 3 activity was significantly higher in PBC ($p < 0.001$). UDCA significantly lowered the levels of TNF in sera and tissue and caspase activity during the 2 years period in PBC patients when compare to their initial values. This phenomenon was paralleled by an improvement of HAI and lower apoptotic activity.

In conclusion, UDCA therapy inhibits TNF-α signaling for apoptosis by reducing caspase activity in patients with PBC.

HEPATOCELLULAR CARCINOMA DEVELOPMENT IN PATIENTS WITH LIVER CIRRHOSIS DUE TO HCV GENOTYPE 1B INFECTION. M. Strain, MD, R. Strain, MD. Department of Gastroenterology, University of Medicine Timisoara, Romania

Aim: To evaluate the evolution of patients with compensated liver cirrhosis due to hepatitis C virus during 5 years of follow-up.

Material and Methods: Between 1992 and 2002, we realized a retrospective/prospective study (not cohort) on patients with chronic hepatitis C, with no signs of decompensated liver cirrhosis or hepatocellular carcinoma. All patients underwent liver biopsy and the liver specimen was assessed by a single pathologist, using the modified Knodell score, in 441 patients out of 452 (97,5%). All patients have been tested for HCV-RNA by RT-PCR. All patients were HCV-RNA positive, genotype 1b. Thirty (7%) patients had a F4 fibrosis score–"histological" compensated cirrhosis. These patients did not receive any antiviral treatment (mono- or combination therapy). During a 5 years of follow-up period, four patients died due to HCC. Mortality in our study group was very low–only 2%.

Annual incidence of HCC and survival in patients with compensated "histological" liver cirrhosis (F4).

Study	Own	Serfaty L, *et al.*, 1998	Hu K, Tong M, *et al.*, 1999	Fattovich G, *et al.*, 1997
Number of patients	30	112	112	384
Follow-up (years)	5	3,3	4,5	5
HCC (%/year)	2,66%	3,3	2,3	1,4
5-year Survival	87,7%	84	83	91

Conclusions: In our experience, patients with compesated liver cirrhosis HCC incidence was low 2.66%/year, with a very good survival rate–88%.

Acknowledgment: Detections of HCV-RNA have been performed by RT-PCR at the Dept. of Gastroenterology and Endocrinology, Georg August University Göttingen, Germany, with the kind support of Prof. Giuliano Ramadori, head of departement.

II–VIRAL HEPATITIS

CORRELATION BETWEEN HAI SCORE AND HBeAg IN CHRONIC HEPATITIS B. Dr. M.A. Mahtab, Dr. S. Rahman. Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

Viral hepatitis is a commonly encountered liver disease in Bangladesh occurring sporadically throughout the year. Hepatitis B virus (HBV) is quite common here. We frequently encounter patients with acute viral B hepatitis, chronic viral hepatitis B, HBV related cirrhosis of liver

and its complications and hepatocellular carcinoma due to HBV in our clinical practice. It has been estimated that HBV is responsible for 10–35% cases of acute viral hepatitis, 35.7% cases of acute liver failure, 33.3–40.5% cases of chronic hepatitis and 46.8% cases of hepatocellular carcinoma in Bangladesh.

In our study we included 80 patients with chronic viral B hepatitis (CHB). The patients were HBsAg positive for over 6 months, HBV DNA positive by hybridization technique and had raised serum ALT within three times the upper normal level. HBeAg was positive in 52 patients and in 28 patients it was negative.

We did per-cutaneous liver biopsies of all the patients. None of the patients had any significant complications, except the complaint of mild upper right abdominal and right shoulder tip pain in a few pateints. The patients were discharged after observation for 48 hours. Histological Activity Index (HAI) scoring was done.

Results in HBeAg positive CHB show that the grade (i.e. necro-inflammatory score) was between 1–3 in 4 patients, between 4–8 in 36 patients, between 9–12 in 10 patients and 2 patients had score between 13–18. In HBeAg negative CHB, these figures are 3, 15, 7 and 3 respectively.

7.69% HBeAg positive CHB patients included in this study had minimal chronic hepatitis, 69.23% had mild chronic hepatitis and 19.23% had moderate chronic hepatitis while severe chronic hepatitis was present in 3.85% patients. In HBeAg negative CHB patients, these figures were 10.71%, 53.57%, 25% and 10.71% respectively.

The study shows that HBeAg positive CHB patients have higher percentage of mild chronic hepatitis while moderate and severe chronic hepatitis are more likely in HBeAg negative CHB patients.

CORE AND NS-4 PROTEIN SPECIFICITY OF MONOCLONAL IMMUNOGLOBULINS OF PATIENTS INFECTED WITH HEPATITIS C VIRUS. E. Bigot-Corbel,* M, Gassin,† I. Corre-Buscail,‡ D. Le Carrer,§ O. Delaroche,* S. Hermouet,‡¶. *Laboratoire de Biochimie Générale, CHU Nantes, France; †Laboratoire de Virologie, CHU Nantes, France; ‡INSERM U601, Institut de Biologie, Nantes, France; §Laboratoire de Biochimie Spécialisée, CHU Nantes, France; ¶Laboratoire d'Hématologie, CHU Nantes, France

A minority of patients infected with hepatitis C virus(HCV) develop a monoclonal Ig, the specificity of which is usually unknown. We previously reported a case of hepatitis C virus (HCV) infection followed by plasma cell leukemia where plasma blasts were infected with HCV (genotype 1a) and the monoclonal immunoglobulin (Ig) G kappa (IgGκ) they produced was directed against the core protein of the virus, suggesting that HCV infection led to plasma cell proliferation and transformation.

The aim of this study was to investigate the specificity of monoclonal Igs developed by HCV-infected patients. For a period of 2 years, 1400patients presenting with monoclonal Ig were systematically tested for HCV infection by qRT-PCR: 10 patients with monoclonal Ig were found HCV-positive. Serum monoclonal Ig were typed by immunofixation as IgGκ (5/10 patients), IgG lambda (λ) (2/10), IgM kappa (1/10), IgAκ (1/10) or IgAλ (1/10). Monoclonal Igs were separated by charge from polyclonal Igs and βglobulins on agarose gels; gel bands corresponding to each fraction were carefully cut and proteins were eluted. Purity of each protein fraction was analysed by immunofixation and isoelectrofocusing; purity of the monoclonal Ig fraction was confirmed for 7 patients. For 3 patients, presence of polyclonal Ig prevented complete purification of the monoclonal Ig (1 IgGκ, 2 IgGλ). The 7 purified monoclonal Igs were subjected to a recombinant immunoblot assay (RIBA III, Ortho Diagnostic Systems) which detects Igs directed against HCV non-structural proteins NS-3, NS-4, NS-5 and fragment C22-3 of the core protein. One monoclonal Ig did not recognise HCV; 4 recognized HCV core protein and 2 recognized HCV NS-4 protein. Viral genotypes of patients with monoclonal Ig directed against HCV core were 2k or 2f; one patient with monoclonal IgAκ directed against HCV core was diagnosed with multiple myeloma.

In conclusion, for 87.5% (7/8) of the "HCV+ monoclonal Ig+" patients studied, the monoclonal Ig was directed against HCV, typically the core protein (71.4% or 5/7 patients) and, to a lesser degree, NS-4 protein (28.6% or 2/7 patients). A switch from poly- or oligo-clonal to monoclonal Ig anti-core response during HCV infection could distinguish patients with increased risk of plasma cell malignancy.

CORRELATION BETWEEN TH1/TH2 CYTOKINES PRODUCTION AND PROLIFERATE ACTIVITY OF PERIPHERAL BLOOD MONONUCLEARS IN PATIENTS WITH VIRAL HEPATITIS B AND C. L.P. Titov, MD, U.V. Tarasiuk, MD, D.A. Charnashey, MD, L.S. Zhmurovskaya, MD. Research Institute for Epidemiology & Microbiology (Minsk, Belarus)

Introduction: It is known that protective immune response in viral hepatitis depends on the Th1/Th2 balance. The investigations of Th cells function regulation are of importance for the development of immunocorrection methods in patients with hepatitis B and C.

The aim of the study was the evaluation of IFN-gamma and IL4 production and proliferate activity of peripheral blood mononuclears (PBM) of viral hepatitis patients depending on the diseases form.

Material and Methods: 34 patients with hepatitis B and C (10 – acute hepatitis B (AHB), 8 – chronic hepatitis B (CHB), 5 – acute hepatitis C (AHC), 11 – chronic hepatitis C (CHC)) were examined. Control group included 10 healthy persons. IFN-gamma and IL4 production was evaluated in short term PBM culture (24 h).

PHA-P (10 mkg/ml) was used as stimulator. Basic cytokine production was assessed in culture without stimulators. The cytokines concentration in supernatants was measured by ELISA (Vector-best, Russia). Proliferate activity of PBM was evaluated in whole blood culture (72 h) with (10 mg/m) or without PHA-P. The results were measured by count of blast-transformed lymphocytes. Statistic analysis of data was performed by commonly used methods.

Results: PBM of patients with hepatitis B and C had decreased IFN-gamma production compared to healthy controls. The decrease was especially noted for AHC and CHC (26,8 $\pm$ 2,36 and 24,42 $\pm$ 5,15 pg/ml respectively; $P < 0,05$). IL4 secretion was increased, particularly in CHB and CHC. Proliferate activity of PBM in hepatitis B and C was decreased as well. Correlation analysis revealed strong positive correlation between PBM proliferate activity and IFN-gamma production ($r = 1$, $P < 0,05$) in AHB. In chronic hepatitis B, PBM proliferate activity correlated to IL4 secretion ($r = 0,82$, $P > 0,05$). But in viral hepatitis C strong negative correlation between PBM proliferate activity and cytokine (IL4 and IFN-gamma) production was found. The correlations found reflects the peculiarities of an immune response formation in viral hepatitis B and C. When activated by PHA PBM of patients with AHB produce significant amount of IFN-gamma, which testifies for cellular immune response formation and good disease prognosis. For some patients Th2 response dominates (their PBM produce IL4 when activated). It leads to chronic disease development. In chronic hepatitis C activation of PBM causes the suppression of both Th1 and Th2 cytokines production. This may indicate on regulatory immune cells activity which prevents effective immune response formation and causes HCV infection persistence.

Conclusion: Viral hepatitis B and C associated with decreased basic production of IFN-gamma and increased secretion of IL4 by PBM in short-term culture. Proliferate activity of PBM in hepatitis B and C was decreased as well. Investigation of correlation between proliferate activity and Th1/Th2 cytokines production by PBM may be useful for monitoring of viral hepatitis and for prognosis of its outcome.

VIRAL HEPATITIS IS MODULATED BY THE IMBALANCE IN NK/NK-T CELLS AND IN CYTOKINES INVOLVED IN THE IMMUNE TOLERANCE IN LIVER. A. Jacques,* C. Bleau,* J-P Martin,† Lucie Lamontagne, PhD*. *Département des Sciences Biologiques, Université du Québec à Montréal, Montréal, Québec, Canada and †Institut Pasteur, Strasbourg, France

The liver is an organ with high numbers of NK and NK-T cells involved in immune tolerance and in virus defence. The immune tolerance in the liver depends on an imbalance between suppressive cytokines, such as IL-4, IL-10 and TGF-β, and inflammatory cytokines. The immunosuppressive cytokines can be secreted by Kupffer cells and endothelial cells. It is known that NK-T cells may produce immunosuppressive cytokines and NK cells produce IFN-γ. NK and NK-T cells are involved in the first defence line against hepatotropic viruses such as HBV and HCV. To study the virus-mediated disorders in the regulation of immune activation and tolerance in liver, the natural infection of mice with a hepatotropic virus, the mouse hepatitis virus (MHV), may serve as an excellent animal model. MHV virus can infect many hepatic cells, such as hepatocytes, Kupffer cells and endothelial cells. We have reported a NK cell depletion in liver from infected mice due to a viral-mediated apoptosis. In order to clarify the role of Kuppfer and endothelial cells in the dysregulation of tolerance mechanisms of acute viral hepatitis, we have determined the levels of inflammatory and suppresssive cytokines as well as NK and NK-T cell subsets in liver from C57BL/6 mice infected with several MHV viral variants expressing different pathogenicity levels. The pathogenic properties of these virus variants reflect their tropisms for Kupffer and endothelial cells. At various times of post infection, livers and spleens were collected and TNF-α, IL-1β, IL-4, IL-6, IL-10, IL-12, IL-18, TGF-β and PGE2 were quantified by ELISA tests. Intrahepatic mononuclear cells were isolated, phenotyped by triple immunolabellings and NK and NK-T subsets, analyzed by cytofluorometry. Results revealed a transient higher recruitment of NK cells in liver, but not in the spleen, from highly pathogenic L2-MHV3 or 51.6-MHV3 variants than in livers from attenuated CL12- and YAC-MHV3 variants. However, NK-T cells did not increase in liver from mice infected with pathogenic variants. Recruited NK cells became apoptotic in the liver. Moreover, NK cell recruitment remained lower when the virus variant could not infect endothelial cells. However, suppressive cytokines decreased in liver from highly pathogenic L2- and 51.6-MHV3 variants while they increased in liver of mice infected with low pathogenic viruses. The decrease of suppressive cytokines was positively correlated with the viral tropisms of viruses for Kupffer cells. These results suggest that the intrahepatic increase of NK cells in acute hepatitis, leading to a higher NK/NK-T cell ratio, is under the control of endothelial cells, and that the decrease of cytokines involved in the tolerance depends on the viral permissivity of Kupffer cells.

This research was supported by a NSERC grant from the Government of Canada.

IMPLICATION OF TGF-β ON *TRYPANOSOMA CRUZI* INFECTION. M.C. Waghabi,* L. Mendonça Lima,* W. Degrave,* M. de Nazaré Soeiro,† M. Keramidas,‡ S. Bailly,‡ J-J Feige,‡ T.C. Araújo-Jorge†. *Laboratory of Functional Genomic and Bioinformatics, Department of Biochemistry and Molecular Biology, Institute Oswaldo Cruz, †Laboratory of Cell Biology,

Department of Ultra Structure and Cell Biology, Institute Oswaldo Cruz, ‡INSERM EMI 0105, Department of Molecular and Structural Biology, Commissariat à l'Energie Atomique, Grenoble, France

Fibrosis is one of the most important and significant characteristics of the Chronic Chagasic Cardiomyopathy (CCC) and the anti-inflammatory cytokine, TGF-β, plays a central role in the development of fibrosis by regulating both the synthesis and the degradation steps of the extracellular matrix. In the present work, we studied the possible role of TGF-β as an inductor/ regulator agent of the chagasic cardiac remodeling, especially fibrosis. Our data regarding the chronic chagasic patients indicated a clear association between TGF-β and fibrosis development since higher levels of circulating TGF-β were noted in patients presenting intense fibrosis and increased TGF-β activity in the heart tissues. Furthermore, we found important alterations in the pattern of Cx43 distribution in both heart cells from chagasic patients and within cardiomyocytes cell cultures incubated with exogenous TGF-β, which could be related to the altered impulse conduction in the heart and developmental of chagasic arrhythmias. As TGF-β has been concerned in the invasion of host cells by *T. cruzi*, we next studied the parasite ability to activate host TGF-β. We observed that infective forms of *T. cruzi* are able to activate latent TGF-β in a dose-response manner being also temperature-dependent. This capacity on activating TGF-β was noted by live parasites and by total or cytosolic parasite extracts, probably by a protein or lipid factor. Moreover, we observed that amastigote forms of *T. cruzi* were able (i) to uptake host TGF-β, (ii) to stock it during their intracellular proliferation and (iii) to possibly use it as a signaling mediator to trigger differentiation into trypomastigotes, completing its intracellular cycle. In a general context, the *T. cruzi* ability to accumulate and activate TGF-β could be related to different mechanisms directly or indirectly involved in the development of Chagas disease. Our data suggest that the presence of TGF-β in the heart can collaborate in the fibrosis formation and arrhythmias reported during the chagasic cardiomyopathy besides contributing for the maintenance of the parasite load in the host tissues.

Grant support: Inserm-Fiocruz research program, FAPERJ/FIOCRUZ, IOC, CNPq, FAPERJ, CEA and INSERM.

PREVALENCE OF HEPATITIS B AND C VIRUSES IN DIALYSED PATIENTS IN SOUTH-WESTERN ROMANIA. M. Strain MD,* M. Patrascu, MD,† R. Strain, MD*. *Department of Gastroenterology, UMF Timisoara; †County Hospital Drobeta Turnu-Severin

Aim: Because previous studies demonstrated the very high prevalence and incidence of HCV infection in chronic dialysed patients, we tried to determine what is the current status of HBV and HCV infection in this high-risk group of patients in Drobeta Turnu-Severin County Hospital.

Materials and Methods: We prospectively followed 144 chronic dialysed patients–74 F, 70 M, mean age ($\pm$SD) 60,22 $\pm$ 12,09 years. Mean follow-up was 43,08 $\pm$ 29,47 months. In all patients the following markers have been determined: anti-HCV (ELISA 3), HBsAg and transaminases at initiation of the dialysis, and at 6 month afterwards.

Results: 101 patients (70%) out of 144 presented anti-HCV antibodies, reflecting a vey high prevalence of this infection, in comparison with the accepted prevalence in general population in Romania – 5%. 35 patients were anti-HCV positive before initiation of the dialysis. 66 patients aquired the HCV infection during the dialysis, after a mean period of 25,4 $\pm$ 17 months, and a mean follow-up period of 55,3 $\pm$ 34,8 months. The HCV incidence is–in this group of patients–14%/year. Moreover, 11 patietns–9 F, 2 M–demonstrated also HBV coinfection, being HBsAg positive. 4 patients had the HBV infection before the dyalisis, 7 patients seroconverted to HBsAg+ during dialysis, after a mean period of 25,57 $\pm$ 20,02 months, with a follow-up period of cu o perioada de 26,7 $\pm$ 18,9 months. Finally, the HBV infection prevalence in whole group was 7,63%, with a annual incidence of 3,14%.

Conclusions: The high prevalence and incidence of HBV and–much more important–of HCV infections underscores once again the total lack of effective measures in preventing the nosocomial infections in dialysis patients.

PREDICTORS FOR SUSTAINED RESPONSE IN VIRAL HEPATITIS C. M.G. Neuman, PhD, A. Sujena, Z. Ben-Ari, H. Schmilovitz-Weiss, P. Marcellin, J.P. Benhamou, M. Bourliere, G. Katz, C. Trepo. University of Toronto, Toronto, Canada; Rabin Medical Center, Petach Tikva, Israel; Hopital Beaujon, Clichy, Hopital Saint Joseph, Marseille and Hopital Dieux, Lyon, France

Tumor necrosis factor-α (TNF-α) and its receptors: B cell activating factor (BAFF) and a proliferating inducing ligand (APRIL) are involved in the regulation of the inflammatory responses. We aim to assess: 1- the role of TNF-α and its receptors in severity of liver damage in chronic hepatitic C (HCV) and his value as a predictor for sustain response to therapy; 2- the antiapoptotic and immunomodulatory effect of therapy in these patients. Serum levels of APRIL, BAFF, TNF-α, apoptosome 30 (AP), cytochrome C (cyt C) and caspase 3 (CAP) activity were measured in 1080 HCV patients. The liver biopsies of 120 HCV patients were studied using electron microscopy and immunohistochemistry. APRIL and BAFF were detected by multiplexed protein quantification assay. Measurements were repeated at the end of weight-based regimen of pegylated interferon alpha 2 b (PegIFN) (1.5 ug/kg/wk) in combination with ribavirin,

800–1400 mg/day (415 patients) and ribavirin only in 20 patients. 560 healthy volunteers were used as controls. The ANOVA or Mann-Whitney test as were used to compare variables among and between groups. Levels of TNF-α (pg/mL) in patients (520 ± 160) were higher than in controls (50 ± 15) ($p < 0.001$). TNF-α correlated significantly with more advanced histological activity index (HAI). Patients with inflammation >6 and cirrhosis had significantly higher TNF-α (720 ± 100 vs. 120 ± 15 pg/ml). When compared to controls, AP, CAP activity, and cyt C release were significantly higher in patients ($p < 0.001$). APRIL has significantly higher levels ($p < 0.05$) in high HAI versus the low HAI, while BAFF levels are not significantly different between the 2 entities. High levels of APRIL correlate positively with the number of Kupffer cells (CD68 antibodies). PegIFN + ribavirin lowered significantly the levels of TNF-α, APRIL, AP, and cyt C when compare to their initial values. This trend was paralleled by a lower HAI and lesser apoptotic activity. In patients treated with ribavirin there is a significant decrease in CAP activity, AP and cyt C. Only low serum TNF alpha values 320 ± 15 pg/ml) at the base line were predictors for sustain response to therapy. In conclusion, in HCV-induced inflammation both B and T lymphocytes play a role. PegIFN inhibits TNF-α signaling for apoptosis by down-regulating its receptors, while ribavirin lowers the mitochondrial damage.

SERUM TGF-BETA1 AS A RELIABLE INDEX OF FIBROGENESIS IN ASYMPTOMATIC HCV CARRIERS. A. Forgione, L. Miele, P. Di Rocco, B. Alfei, V. Vero, M. L. Gabrieli, A. Gallo, C. Cefalo, R. Castellacci*, C. Puoti*, G.L. Rapaccini, G. Gasbarrini, A. Grieco. Department of Internal Medicine. Università Cattolica del Sacro Cuore. Roma. Italy and *Department of Gastroenterology. Ospedale di Albano Laziale-Genzano. Roma. Italy

Background: HCV screening programs has led to identification of so called "asymptomatic" carriers (AC). Despite this term, recent papers show minimal or mild inflammatory changes in the liver and the possible evolution to fibrosis. The gold standard method to assess the severity of the disease is liver biopsy, but the role in asymptomatic patients is under debate.

Aim: To assess the utility of serum TGF beta in the evaluation of liver damage in asymptomatic carriers.

Methods: We enrolled 9 AC (age 51.6 ± 8.9 yrs M/F:2/7), 23 "naïve" patients with chronic active hepatitis (CAH) (age: 50 ± 9 yrs, M/F:15/8) and 9 patients with well compensated cirrhosis (age 55 ± 8 yrs, M/F:6/3, Child A-B). Eight volunteers (age: 31 ± 8 yrs, M/F:5/3) served as healthy controls well matched for age, gender. All patient underwent liver function tests, HCV-RNA, liver biopsy and assay of serum TGF-beta (ELISA kit, Amersham Pharmacia, UK).

Results:

Parameters	Healthy	AC	CAH	Cirrhosis
ALT (IU/L)	46 + 19.7	29 ± 7	96.77 ± 35.92	60 ± 51
AST (IU/L)	29.5 ± 5.1	26 ± 5	62.01 ± 22.86	50 ± 43
TGF-beta (ng/ml)	12.15 ± 10.03	36.75 ± 31.9*	34.21 ± 22.52*	193.70 ± 181.11*
*$p < 0.001$ (AC vs Healthy; CAH vs Healthy; Cirrhosis vs Healthy, CAH, AC)				

Conclusions: Natural history of AC is far from being clarified. Performing liver biopsy, nevertheless provides accurate information, is still extremely controversial in AC, in which a mild histological damage may progress to more severe fibrosis. Our data point out serum TGF beta as an useful tool to assess fibrosis progression in AC.

CORRELATION OF SICAM-1 AND SVCAM-1 LEVEL WITH BIOCHEMICAL, HISTOLOGICAL AND VIRAL FINDINGS IN CHRONIC HEPATITIS C AFTER INTERFERON-A + RIBAVIRIN THERAPY. Corina Radu, Doru Dejica, Grigorescu Mircea, Zaharie Teodor, Daniela Neculoiu, Dana Damian. 3rd Medical Clinic, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj – Napoca, Romania

Intercellular cell adhesion molecules-1 (ICAM-1) and vascular cell adhesion molecules-1 (VCAM-1) are expressed in a high quantity on hepatocytes and at the level of endothelium cells from sinusoidal vessels in the liver tissue of patients with chronic hepatitis C. The soluble forms of these molecules sICAM-1 and sVCAM-1 can be determined in the serum of patients through the immunoenzymatic technique (ELISA). The aim of the study was to analyse the base level of these molecules and the changes induced through the combined treatment of interferon-α (IFN- α) and ribavirin (Rib).

Materials and Methods: Twenty patients suffering from viral chronic hepatitis C were studied: ten patients responded completely to antiviral treatment and ten patients showed no response at the end of the treatment. At the end of the therapy patients were placed under biochemical observation for a further six months. The serum concentration of sICAM-1 and sVCAM-1 was measured using ELISA assay at the beginning and after six months of treatment IFN 3MU (three times a week) associated with Rib.

Results: Statistically, a significant correlation was observed between the values of sICAM-1 pretreatment and the level of viremia, γ glutamiltranspeptidase (GGT) but without correlation to the alanin amino transferase (ALT) level. The value of sICAM-1 was significantly higher in patients who had fibrosis score F: 3–4. After the treatment, the serum concentration of sICAM-1 dropped significantly in patients with sustained biochemical response in comparison to patients who had an unsustained response or had no response whatsoever. A significant correlation between the sVCAM-1 pre-treatment value and the level of viremia, GGT, ALT was not established.

Conclusions: The level of sICAM-1 could be a useful parameter in the observation of the disease history of

patients with viral chronic hepatitis C treated with IFN- α and Rib.

Key Words: chronic hepatitis C, the intercellular adhesion molecule 1 (sICAM-1), the vascular adhesion molecule 1 (sVCAM-1).

DOUBLE-STRANDED RNA STIMULATION OF HUMAN LIVER PROGENITOR CELLS LEADS TO COMPLETE INHIBITION OF HCV REPLICATION THROUGH SECRETED COMPONENT(S). R. Parent, A.-L. Morand, L. Saccucci, C. Trepo, M.-A. Petit. INSERM Unit 271, 151 Cours Albert Thomas, 69424 Lyon Cedex 03, France

We recently reported that HepaRG cells constitute the first described human hepatic bipotent progenitor cell line (Parent, *et al.*, Gastroenterol 2004). Despite their unique properties and contrary to Huh-7 cells, the HepaRG cells were not competent for efficient hepatitis C virus (HCV) replication in the replicon systems. To understand such a finding, we investigated the functionality of type I interferon (IFN)-alpha and TLR3-IRF3 signaling pathways in HepaRG cells in comparison with Huh-7 cells. First, Jak-STAT and PKR activation after cell treatments with IFN-alpha were studied. Second, the induction of the double-stranded RNA-stimulated TLR3-IRF3 pathway in response to its synthetic ligand, poly(I:C), was examined. Our results showed an activation of the Jak-STAT pathway both in HepaRG and Huh-7 cells at high doses of IFN-alpha. In response to poly(I:C), the TLR3- IRF3 signaling pathway was found fully functional in HepaRG cells, in contrast to Huh-7 cells, and its activation specifically resulted in an exarcebated secretion of IFN-inducible protein-10 (IP-10/CXCL10) and interleukin-8 besides IFN-beta. Supernatant from poly(I:C)- stimulated HepaRG cells also exerted a complete inhibitory effect on HCV replication in replicon-harboring Huh-7 cells. In conclusion, this study gives evidence that following double-stranded RNA-stimulation liver progenitor HepaRG cells exert a dramatic antiviral activity against HCV replicons, which could contribute to their non-permissiveness to HCV replication after transfection with HCV RNA replicons or transcripts.

HYPEREXPRESSION OF PROLACTIN RECEPTOR IN TWO TYPES OF RAT LIVER CARCINOMA CELL LINES AFTER HEPATIC TRANSPLANTATION. T.Y. Ostroukhova,* A.V. Kulikov,* O.A. Kurashova,* O.V. Smirnova*. *Laboratory of Endocrinology, Faculty of Biology, M.V. Lomonosov Moscow State University, Vorob'ev Hills, Moscow, 119992, Russian Federation

Prolactin (Prl) is a hormone/cytokine responsible for the coordination of wide range of biological processes. In the liver cells Prl is involved in the regulation of many functions, including hepatocytes proliferation. The short form of PrlR does not activate STAT proteins. The present work was performed to study the intensity of PrlR expression and its dependence on sex in intrahepatic grafts of Mucous Cancer (MC-1, RS-1) and Hepatoma 27 (H27) cell lines, representing transplantable cancer cell lines, derived from rat cholangiocytes and hepatocytes. PrlR expression was analyzed with indirect immunocytochemical technique with quantitative computer analysis of imaging. In normal cholangiocytes PrlR-positive staining was seen in cellular membranes, cytoplasm, and nuclei with low, sex independent intensity. In tumor cholangiocytes after hepatic transplantation sharp elevation of PrlR-specific staining, mainly in nuclei, was observed in both sexes, with more strong intensity in females, being accompanied with much more pronounced vacuolization of tumor cells in females, as compared with males. Compartmentalization of PrlR in H27 intrahepatic grafts was the same as in hepatocytes of intact animals (cytoplasm and membrane staining). In females enhancement of PrlR expression was revealed in intrahepatic grafts of H27 as compared with hepatocytes of control females. Alternatively, in males PrlR expression in intrahepatic grafts of H27was lower than in control male hepatocytes. Presence of intrahepatic MC-1 or H27 grafts was shown to induce shifts in the level and compartmentalization of PrlR in hepatocytes both around tumor graft and in another hepatic lobe.

We concluded that hyperexpression of PrlR both in tumor cholangiocytes and tumor hepatocytes may be associated with tumor promoting action of Prl, which is more pronounced in females.