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Inflammation Research

Symposia Abstracts

New Technologies, Approaches and Models in Inflammation Research

Co-chairpersons: **Lawrence de Garavilla** (Janssen)
Alison O'Mahony (Bioseek LLC)

The theme for this Symposium "From Molecules to Models to Market: New Strategies in Drug Discovery". Our four speakers will present talks on emerging technologies that propel the discovery of new therapies, reduce the attrition rates and costs of development pipelines and help deliver safer and more efficacious compounds to the clinic. Starting with discussion of innovative strategies for discovering novel compounds and determining target selectivity (MOLECULES), through tracking their impact on signaling and disease biology in complex models (MODELS) to approaches used to assess compound efficacy and safety in vivo for progression to the clinic (MARKET).

SA01

KINOBEADS^{TM} AS AN ENABLING TECHNOLOGY FOR THE DISCOVERY OF NOVEL, HIGHLY SELECTIVE KINASE INHIBITORS

*Oliver Rausch**

Cellzome, Cambridge, UK

Abstract not received.

SA02

DYNAMIC PROTEOMICS: A NEW TOOL FOR BIOMARKER AND TARGET DISCOVERY

*Scott Turner**

Kinemed, Emeryville, USA

Abstract not received.

SA03

HUMANIZED MICE - A BRIDGE TO THE CLINIC

*Leonard D. Shultz**

The Jackson Laboratory, Bar Harbor, USA

Abstract not received.

SA04

IMAGING LUNG INFLAMMATION WITH POSITRON EMISSION TOMOGRAPHY

*Delphine Chen**

Washington University, Washington, USA

Abstract not received.

The Underappreciated Immune Cells: Eosinophils, Mast Cells, Basophils, and Platelets

Guest Symposium—Sponsored by the Society for Leukocyte Biology

Co-Chairs: **William Westlin** (Avila Therapeutics)
Kimberly Dyer (NIAID, NIH)

Our understanding and appreciation of the role of eosinophils, mast cells, basophils, and platelets in inflammation is changing. These effector cells have largely been thought to contribute to the negative sequelae associated with inflammatory and allergic diseases but recently it has been shown these cell types may play a more positive role in development and in innate and adaptive immunity. Similarly, these effector cells may play a role in immune activation, hypersensitivity, and the control of pathophysiologic processes. The contributions to TH2 inflammation by modulation of CD4 and dendritic cell function, potential role as antigen presenting cells and the expression and secretion of many proinflammatory mediators that can initiate and modulate immune responses will be explored in-depth. The Society for Leukocyte Biology is honored to present this symposium that highlights the roles of these underappreciated immune cells in inflammation.

SA05

EVIDENCE OF POSITIVE AND NEGATIVE REGULATION OF INFLAMMATION BY MAST CELLS

*Stephen Galli**

Stanford, USA

Abstract not received.

SA06

EOSINOPHILS IN INFLAMMATION

*Simon Hogan**

University of Cincinnati, Cincinnati, USA

Abstract not received.

SA07

DO BASOPHILS INITIATE OR MODULATE TH2 RESPONSES? EVIDENCE IN HUMAN ALLERGIC DISEASE

*John Schroeder**

Johns Hopkins University, Baltimore, USA

Abstract not received.

SA08

PLATELETS: SIGNALING CELLS IN THE IMMUNE CONTINUUM

*Andrew Weyrich**

University of Utah, Salt Lake City, USA

Abstract not received.

Inflammation and Fibrosis

Co-Chairpersons: **Lynne Murray** (MedImmune Ltd.)
Thomas A. Wynn (NIAID, NIH)

SA09

IMMUNOLOGIC ROLE OF SEMAPHORIN 7A IN TGFβ1-INDUCED LUNG FIBROSIS

*Erica Herzog**

Yale University, New Haven, USA

Abstract not received.

SA10

SYSTEMIC ANTI-FIBROTIC EFFECTS OF PIRFENIDONE IN PRECLINICAL STUDIES AND CLINICAL ACTIVITY IN IDIOPATHIC PULMONARY FIBROSIS (IPF)

Karl Kossen, CJ Schaefer, WZ Bradford, and SD Seiwert*

InterMune, Brisbane, USA

IPF is a chronic interstitial lung disease characterized by unremitting deposition of extracellular matrix and loss of lung function. Pirfenidone is an orally active small molecule that demonstrates systemic anti-fibrotic effects in animal models and is approved for the treatment of IPF in Japan and the European Union. Here we summarize the preclinical and clinical studies that establish the benefit of pirfenidone treatment. A series of preclinical studies demonstrate the anti-fibrotic activity of pirfenidone in animal models of fibrosis in the lung, liver, heart, and kidney. The clinical benefit of pirfenidone has been evaluated in four randomized, double-blind, placebo-controlled studies including two InterMune-sponsored multinational studies (the CAPACITY studies). The collective data from the CAPACITY studies demonstrate a favorable treatment effect on a number of clinically meaningful endpoints, including forced vital capacity, progression-free survival, and 6-min walk test distance. Additionally, an independent meta-analysis of all three completed phase III trials defined a statistically significant improvement in progression-free survival time.

SA11

BONE MARROW-DERIVED MYELOID CELLS RESOLVE PULMONARY FIBROSIS THROUGH A TRAIL-DEPENDENT MECHANISM

Cory M. Hogaboam, D.M. Habiel, U.B. Ismailoglu, A.P. Moreira*

University of Michigan Medical School, Ann Arbor, USA

The role of bone marrow-derived myeloid cells in pulmonary fibrosis remains controversial. The aim of the present study was to explore the role of myeloid cells expressing both CD11b and GR1 antigens in a bleomycin-induced model of pulmonary fibrosis. These myeloid cells were most abundant in the lung during the resolution phase at day 42 after bleomycin challenge. Immuno-depletion of $CD11b^{+}GR1^{+}$ cells prevented the resolution of fibrosis whereas the adoptive transfer of FACS-sorted $CD11b^{+}GR1^{+}$ cells accelerated the resolution of fibrosis in this model. Although these cells markedly inhibited the expression of profibrotic chemokines such as CCL17 and CCL22, the primary mode of action of these cells appeared to be directed through their expression of the pro-apoptotic factor TNF-related apoptosis-inducing ligand (TRAIL or CD253). Both in vitro and in vivo experiments confirmed that the presence of TRAIL was necessary for the anti-fibrotic effects of $CD11b^{+}GR1^{+}$ myeloid cells. Thus, the $CD11b^{+}GR1^{+}$ bone marrow-derived myeloid cells promote the resolution of experimental fibrosis in the lung.

SA12

DEVELOPING EFFECTIVE THERAPEUTICS FOR FIBROTIC DISEASE

*Thomas A. Wynn**

NIAID, NIH, Bethesda, USA

Abstract not received.

Novel Pharmacological Approaches for IBD

Co-Chairpersons: **Lisa Schopf** (Kala Pharmaceuticals)
Leo R. Fitzpatrick (Penn State College of Medicine)

Over the past decade, new scientific data have emerged, as related to our understanding of inflammatory bowel disease (IBD). Despite this emergence of new knowledge related to the pathogenesis of IBD, novel therapeutic approaches are still needed. Therefore, this session will focus on some of the novel pharmacological approaches that have emerged during the past decade. One approach that will be covered is the use of probiotics with diverse mechanisms of action, which may impact current therapeutic paradigms for IBD. In addition, melanin-concentrating hormone receptor antagonists, immunomodulatory functions by butyrophilin superfamily molecules, as well as modulation of TH-17 T-lymphocytes, will be discussed during the session. Some of these approaches could evolve as breakthrough pharmacological approaches for IBD.

SA13

PROBIOTICS AND IBD

*Karen Madsen**

University of Alberta, Edmonton, Canada

Abstract not received.

SA14

MELANIN CONCENTRATING HORMONE (MCH) AND INTESTINAL INFLAMMATION

*Efi Kokkotou**

Harvard Medical School, Boston, USA

Abstract not received.

SA15

IMMUNE REGULATION BY THE BUTYROPHILIN FAMILY

*Heather Arnett**

Amgen, Thousand Oaks, USA

The butyrophilin superfamily has structural homology to the B7 family of costimulatory molecules and represents an emerging family of immunoregulatory molecules. Within the butyrophilins, most is currently known about the interactions of butyrophilin-like 2 (BTNL2) with the immune system. Genetic polymorphisms leading to a truncation in BTNL2 have been associated with predisposition to many human diseases associated with aberrant inflammation, including inflammatory bowel disease. In function, BTNL2 protein is able to alter T cell responsiveness, suppressing activating signals through CD3. In addition to its direct suppressive effects, BTNL2 also modifies B7/CD28 signaling to promote expression of Foxp3, a transcription factor necessary for regulatory T cell development and function. Immunophenotyping and expression profiling reveal that BTNL2-induced Treg share many properties with natural Treg, and in vivo they suppress enteritis induced by effector T cells. Identification of novel pathways capable of attenuating inflammation at the mucosal surface could guide the development of new therapies that take advantage of enhancing natural suppressive mechanisms in IBD.

SA16

THE ROLE OF IL-22 IN MUCOSAL IMMUNITY

*Wenjun Ouyang**

Genentech, San Francisco, USA

Abstract not received.

Kinases as Targets: Past, Present & Future

Chairperson: **John O'Shea** (NIAMS, NIH)

SA17

TBA

*John O'Shea**

NIAMS, NIH, Bethesda, USA

Abstract not received.

SA18

TBA

*James D. Clark**

Pfizer, Cambridge, USA

Abstract not received.

SA19

DUAL INHIBITION OF p38 KINASE ACTIVATION AND ACTIVITY PROVIDES EFFICACY IN TREATMENT OF RHEUMATOID ARTHRITIS

*Gary L. Schieven**

Bristol-Myers Squibb, Princeton, USA

Although p38 kinase has been an intense focus of drug discovery, p38 inhibitors have shown little or only transient efficacy in rheumatoid arthritis (RA) clinical trials. This tachyphylaxis suggests development of resistance in patients. We report that BMS-582949 is a dual action p38 kinase inhibitor, blocking p38 activation in cells in addition to p38 kinase activity. By contrast, the three compounds reported to give tachyphylaxis did not block p38 activation. Cells treated with diverse p38 inhibitors showed changes in gene expression expected to drive p38 activation more strongly, suggesting a resistance mechanism that activation blockade could avoid. In a 28-day clinical study a daily dose of 300 mg BMS-582949 gave similar inhibition of Hsp27 phosphorylation and LPS-induced TNF-alpha production in the blood of subjects after the first and last doses, demonstrating durable inhibition of p38 signaling that was not overcome by any resistance mechanism. Treatment with BMS-582949 in combination with methotrexate for 12 weeks in a RA clinical trial was well tolerated and demonstrated rapid and durable efficacy that correlated with trough exposures and baseline CRP. These results suggest that dual action p38 inhibitors may overcome resistance mechanisms causing tachyphylaxis, and merit further investigation for the treatment of inflammatory disease.

Novel Therapeutics

Chairperson: **Larry Burgess** (Array BioPharma)

Over the past two decades, significant advances have been made in controlling rheumatoid arthritis (RA) and other autoimmune diseases by targeting specific and crucial pathways and mediators such as TNF-α. As a consequence, today's drug discovery and development efforts have been challenged to meet the needs of more severe and non-responding patient populations by applying innovative patient selection strategies coupled with novel therapeutics that address uncontrolled aspects of autoimmune diseases while maintaining or improving overall safety profiles. In this session, recent clinical results from emerging anti-inflammatory strategies will be presented including neutralizing IL-13 in a selected asthmatic population, antagonizing GM-CSF signaling in RA and selectively inhibiting JAK1 as a potentially safer approach versus pan-JAK inhibition for RA.

SA20

CAM3001: AN ANTI-GM-CSFR FOR RHEUMATOID ARTHRITIS

*Tomas Mustelin**

MedImmune, Gaithersburg, USA

Abstract not received.

SA21

PERSONALIZED MEDICINE IN ASTHMA: CO-DEVELOPMENT OF AN IL-13 INHIBITOR AND COMPANION DIAGNOSTIC

*Joseph Arron**

Genentech, San Francisco, USA

Abstract not received.

SA22

GLPG0634 SHOWS EFFICACY & SAFETY IN A RHEUMATOID ARTHRITIS PHASE II STUDY

*Frédéric Vanhoutte**

Galapagos, Mechelen, Belgium

Abstract not received.

Mini-symposia and Poster Session Abstracts

A100

RHYTHMS OF PRO-INFLAMMATORY CYTOKINES IN SALIVA

LG. Araujo[1], E. Reinhardt[1], L. Lemos[1], CRC. Moreno[1], PACM Fernandes[2], RP Markus[2], FM. Fischer[1]*

[1]Public Health Faculty, USP, Brazil; [2]Biosciences Institute, USP, Brazil

Salivary information may significantly facilitate immunotherapies. The aim of this study was to investigate the presence of circadian rhythm IL-1β, IL-6, and TNF-α in saliva. In a group of 11 clinically symptom free males (mean age 23.9 $\pm$ 1.7 years), saliva samples were obtained each 4 h, during three consecutive days. Individual cosinor was performed to investigate a rhythm of 24 and 12-h period. Statistically significant rhythm of melatonin ($p < 0.05$) was used as inclusion criteria to the populational cosinor analysis ($n = 7$). A significant 24-h rhythm was observed for cortisol (acrophase (Φ) at 10:41 h, MESOR (Me) = 1.13 pg/ml; amplitude (A) = 0.38 pg/ml; $p = 0.001$), and melatonin (Φ at 1:56 h, Me = 12,9 pg/ml; A = 10,82 pg/ml; $p = 0.01$) The cytokines TNF-α, IL-1β, and IL-6 peaked at 6:29, 6:02 and 5:16 h, respectively, but not reached significance for the 24-h period. A biphasic pattern was found only for IL-6 with a second peak at 2:50 h ($p = 0.02$). There was a detectable concentration of cytokines in saliva. Nevertheless, the lack of statistically significance suggests there is a need to conduct further studies to confirm rhythmic patterns of cytokines in saliva.

A101

SRC-HOMOLOGY DOMAIN CONTAINING PHOSPHATASE-1 (SHP-1) INHIBITION BY SODIUM STIBOGLUCONATE (SSG) AUGMENTS T CELL RESPONSE IN IMMUNODEPRESSED HEALTHY GERIATRIC SUBJECTS AND TRAUMA PATIENTS

Gautam Bandyopadhyay, Paul Bankey, Carol Miller-Graziano*

Department of Surgery, University of Rochester Medical Center, Rochester, NY, USA

Elderly subjects (age 65+) have increased infection risks and constitute the majority of septic patients in intensive care units. Depressed T cell function is linked to geriatric immuno-senescence. We found both healthy elderly subjects and trauma patients (Pts) developed similar T cell defects with increasing severity in elderly trauma Pts. Further, like trauma Pts' T cells, healthy elderly subjects' depressed T cells had significantly increased co-inhibitory receptors and subsequently elevated basal SHP-1 activation levels [pSHP-1 median MFI 568 in elderly vs. 388 in young]. We hypothesized that inhibition of hyper-activated SHP-1 would restore T cell response in both elderly and Pts, reducing their post-injury infection susceptibility. **Method:** Isolated T cells from healthy elderly and immuno-depressed Pts were pre-treated with 10 μg/ml SSG for 30 min, and then stimulated with αCD3 antibody to assess T cell proliferation. **Result and Conclusion:** SHP-1 inhibition by a clinically relevant inhibitor SSG partially restored both elderly and trauma patients T cell responses [51 and 58 % boost, respectively (median)].

A102

PRECISION CUT LUNG SLICES: A NOVEL METHOD TO STUDY RESPIRATORY DISEASES

Carla Bauer[1,], Javad Golji[1], Kristen Lambert[2], Donavan Cheng[1], Mario Giron[1], John Allard[1], Holly Hilton[1], Hans Bitter[1], Martin Stämpfli[2], and Christopher Stevenson[1]*

[1]pRED, Hoffmann-La Roche, Nutley, NJ, USA; [2]McMaster Immunology Research Center, McMaster University, Hamilton, Canada

Bacterial infections and smoking have been linked to exacerbations of respiratory diseases including severe asthma and chronic obstructive pulmonary disease. To this end, responses to TLR agonists and live pathogens were studied in precision cut lung slices (PCLS) generated from room air and smoke exposed mice. Principal component analysis revealed that live *S. pneumoniae* ex vivo stimulation of PCLS led to a distinct response and a greater number of differentially expressed genes when compared to the responses elicited by TLR agonists or *H. influenzae*. These data indicate that it is likely more appropriate to use murine pathogens (i.e. *S. pneumoniae*) as opposed to human pathogens (i.e. *H. influenzae*) or TLR agonists to model infection-induced changes in the airways of mice. Furthermore, these data highlight the strength of this culture technique in evaluating potential targets that may be linked to disease (or exacerbation) susceptibility.

A103

LATENT INFECTION WITH γ MURINE HERPES VIRUS AUGMENTS THE BLEOMYCIN-INDUCED LUNG FIBROSIS RESPONSE

Carla Bauer, Paul Harris, Ruoqi Peng, Martine Loubeau, Leena Chen, Lorena Renteria, Jonathan Phillips, and Christopher Stevenson*

pRED, Inflammation DTA, Hoffmann-La Roche, Nutley, NJ, USA

Idiopathic pulmonary fibrosis (IPF) is characterized by progressive and persistent lung scarring that is thought to be driven by injurious agents through a series of molecular mechanisms that are not well defined. Previous studies in IPF patients have correlated the presence of the human herpes viruses, raising the question as to whether latent herpes virus infection contributes to fibrotic responses following an injurious event. To this end, C57BL/6J mice were infected with 10^5 PFU of a γ murine herpes virus (MHV). Forty-nine days post-infection, mice were instilled with 2 U/kg of bleomycin or saline. We demonstrate that long-term latent infection with γMHV augments the response to bleomycin in the lungs of mice. Furthermore, this response is characterized by an increased lymphocytic infiltrate in γMHV-infected bleomycin-treated animals when compared to the relevant controls. Our model provides evidence that a latent γMHV infection acts as a cofactor in the development of fibrosis in the lungs of mice and will better inform of the mechanisms driving the fibrotic response.

A104

MOLECULAR CHARACTERIZATION OF A γ MURINE HERPES VIRUS INFECTION IN C57BL/6 MICE

Carla Bauer, Donavan Cheng, Ruoqi Peng, Paul Harris, Gaurav Tyagi, Leena Chen, Lorena Renteria, Hans Bitter, and Christopher Stevenson*

pRED, Inflammation DTA, Hoffmann-La Roche, Nutley, NJ, USA

Endogenous herpes viruses have been suggested to be an initiating source of injury in the lungs of patients with idiopathic pulmonary fibrosis (IPF). γ murine herpes virus (MHV)-68, much like the human herpes virus does not cause any overt symptoms in immuno-competent individuals upon lung infection. We hypothesized that although little symptomology is associated with infection; a pro-fibrotic environment is established in the lung following intranasal infection with MHV-68. To this end, C57BL/6 mice were instilled with either a control preparation or infected with 10^5 PFU of MHV-68. 2, 6, 24 h and 4, 7, 14, 21, 35, 49, 56, and 70 days post-infection lungs were harvested and the fibrotic response to injury by MHV was assessed. Although a very weak fibrotic response is observed in the lung of MHV-infected animals, the immune environment of the lung is arguably altered to a pro-fibrotic state. To evaluate this possibility further, microarray analysis was performed. The outcomes of these studies have identified novel pathways that may underlie IPF pathogenesis.

A105

IMAGING CUTANEOUS INFECTION USING ICG-LOADED MONOCYTES

JM Christensen, Y Chen, GA Brat, KJ Buretta, DS Cooney, G Brandacher, K Johnson, WPA Lee, X Li, JM Sacks*

Johns Hopkins SOM, 600 N Wolfe St., Baltimore, MD, 21287, USA

Distinguishing between infection and sterile inflammation is subjective, but assessment of variation in leukocytic infiltration might be a more objective approach. IV-injected monocytes loaded with indocyanine green (ICG) dye can be observed non-invasively to investigate cutaneous inflammation. RAW 264.7 mouse monocytes were coincubated with ICG. Chemotaxis of loaded cells in response to monocyte chemotactic protein-1 assessed using a microplate assay remained above baseline. Labeled cells were injected systemically into mice with induced sterile inflammation or infection of the lateral hind limb. Whole-animal near-infrared angiography revealed distinct local fluorescence at the inoculation site, which peaked 4–6 h later and returned to baseline by 12 h. Microscopic examination of inoculation-area tissue showed punctate areas of near-IR fluorescence, consistent with the presence of ICG-loaded cells. Development of a cutaneous imaging modality without ionizing radiation may lead to tools for bedside diagnosis of many pathological of conditions including cellulitis and surgical site infections.

A106

THE IN VITRO EFFECTS OF NEUROPEPTIDES AND BICYCLIC MONOTERPENE DIOL ON THE INFLAMMATORY PATHWAY USING PRIMARY HUMAN KERATINOCYTES

Catherine Ding[1], Donald Collins[2], Nadine Pernodet[2], Daniel Yarosh[2], Alan R. Shalita[1] and Wei-Li Lee[1]*

[1]Department of Dermatology, SUNY-Downstate Medical Center, Brooklyn NY, USA; [2]BioResearch Division, Estée Lauder Companies, Melville, NY, USA

Substance P (SP) has been suggested to be a critical player and modulator of itch in atopic dermatitis (AD) via its receptor NK-1. IL-31, a T cell cytokine, has also been shown to induce pruritis in those with AD via its main receptor IL-31RA, while the downstream pathway of the receptor remains unclear. Previous data showed that bicyclic monoterpene diols (BMTd) and SP both upregulate iNOS levels and are also known to increase skin vascular temperature. The objective of this study is to further examine the possible roles and relationship of BMTd and neuropeptides (NP) in inflammatory processes using human primary keratinocytes (NHEK). NHEKs were treated with BMTd; and with SP, and calcitonin-gene related protein (CGRP) with or without BMTd for 48 h. Cytoplasmic levels of NK-1, IL-31RA, and NF-κB p105 were assessed using Western blot and NIH Image J. The levels of LTB4, IL-1α, IL-1β, IL-8, and TNF-α were analyzed via ELISA. Our preliminary data indicated that BMTd caused a ten-fold decrease in the measurable density of IL-31RA, with similar decreases seen in NK-1 and NFκB p105 levels. Interestingly, when treated with BMTd and moderate doses of NPs, its effects are returned to baseline. There were notable increases in IL-1α, IL-1β, and TNF-α levels of 25, 50 and 45 %, respectively, when treated with the combination of BMTd and SP. We observed similar changes in IL-8 and LTB4 when treated with the combination of CGRP+BMTd. The data suggests that BMTd may play a role in modulating cytokine levels in the skin by working in conjunction with the NPs present in skin. BMTd showed a specific modulating effect on the activation of the NFκB transcription pathway, which has been associated with the pathogenesis of many inflammatory diseases including AD. We conclude that BMTd may be a therapeutic molecule that might have an anti-pruritic and anti-inflammatory effect by modifying the inflammatory signal pathway related to its effect on NK-1, NFκB or IL-31RA. These findings pave the way for future applications of BMTd as a modulator of the inflammatory response.

A107

TREGITOPE MECHANISM OF ACTION IN TOLERANCE INDUCTION

*L.P. Cousens[1], L. Levitz[1], R. Tassone[1], T. Messitt[1], W. Martin[1], and A.S. De Groot[1,2]**

EpiVax, Inc.[1]; University of Rhode Island[2], Providence, RI, USA

Tregitopes are T cell epitopes naturally contained in immunoglobulins that bind to multiple MHC Class II alleles and induce regulatory T cell (Treg) responses. Harnessing tolerogenic effects of Tregitopes provides a novel means to suppress unwanted immune responses and maintain antigen-specific tolerance, thus changing treatment paradigms in autoimmune diseases and transplant rejection, among other inflammatory conditions. We have demonstrated that Tregitopes suppress unwanted immune responses in vitro and vivo. Based on evidence presented here, the mechanism by which Tregitopes induce tolerance is proposed to be as follows: (1) APC present Tregitopes to nTreg, (2) nTreg proliferate and produce IL-10, (3) nTreg provide tolerogenic feedback signals to APC, modulating the APC phenotype, and (4) nTreg and tolerogenic APC together suppress antigen-specific T cell responses. Tregitopes are currently being developed as a therapeutic strategy for autoimmune diseases, as a tolerance-inducing adjunct to protein drugs, and as a tolerogenic feature integrated into protein therapeutics.

A108

TREGITOPES FOR TOLERANCE INDUCTION IN AUTOIMMUNE DISEASES

A.S. De Groot[1,2,], L.P. Cousens[1], N. Najafian[3], F. Mingozzi[4], W. Elyaman[3], B. Mazer[5], S. Khoury[3], Y. Su[6], D.W. Scott[6], and W. Martin[1]*

[1]EpiVax, Inc., Providence, RI; [2]University of Rhode Island, Providence, RI; [3]Brigham and Women's Hospital, Boston, MA; [4]Children's Hospital of Philadelphia, Philadelphia, PA; [5]McGill University Health Center, Montreal, Canada, and [6]Uniformed Services University, Bethesda, MD, USA

Modulation of T cell responses may contribute to the design of new approaches for the treatment of inflammatory diseases. Tregitopes derived from human IgG reproduce immunomodulatory effects of IVIG. Six

collaborating laboratories have evaluated the beneficial effects of Tregitopes in mouse models of MS (EAE), OVA-induced allergic airway disease, cardiac transplant, diabetes (NOD), and AAV-mediated gene transfer. Tregitopes cause Tregs to produce IL-10, and to expand, and iTreg are induced. In OVA-induced allergic airway disease, we observed expansion of Tregs in conjunction with decreased airway reactivity comparable to, if not greater than, IVIG. Additional evidence supports antigen specificity of tolerance when Tregitopes are administered in conjunction with target antigens. Formulation, dose ranging, and safety/toxicity studies for Tregitope therapy are currently underway. Supported by NIH SBIR Phase II R44 DK081261-03A1.

A109

LIPIDOMIC AND TRANSCRIPTOMIC CHARACTERIZATION OF RESOLVIN D1 AND D2 IN A ZYMOSAN-INDUCED PERITONITIS

*V. Baillif, G. Chene, C. Guigne, M. Dubourdeau**

Ambiotis SAS, Toulouse, France. contact@ambiotis.com

Now resolution is a very promising field of investigation in terms of new anti-inflammatory strategies. During resolution, bioactive lipids mediators derived from polyunsaturated fatty acids (PUFA) are produced. Among them, docosahexaenoic acid derivatives termed resolvins of type D (RvD) shows anti-inflammatory and protective properties and are considered to actively participate to orchestrate resolution. In this study, we have evaluated and compared the effects of RvD1 and RvD2 to classical NSAID. The model used was a zymosan-induced peritonitis and studies were done by using lipidomic and transcriptomic technologies. We have shown that RvD1 and RvD2 were able to decrease the recruitment of inflammatory cells in the peritoneal cavity, modulating genes such as LOX, COX, IFN-dependant cytokines pathways. They also increase the secretion of pro-resolving molecules from PUFA metabolism such as mono, di and tri-hydroxy. Injection of resolvins of type D lead to modification in the time of course of inflammation. Activation of specific molecular pathways by RvD could accelerate switching from pro- to anti-inflammatory circuits to promote resolution and development of new surrogate inflammation markers.

A110

BONE MARROW DERIVED EOSINOPHILS: A TOOL FOR STUDYING THE ROLE OF EOSINOPHILS IN INNATE IMMUNITY

Kimberly D. Dyer, Eva M. Sturm and Helene F. Rosenberg*

From the Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, 20892, USA

Among the difficulties encountered by eosinophil biologists is the fact that eosinophil-specific events represent only a fraction of the ongoing hematopoietic activity in bone marrow at any given time even under profound Th2 stimulation. Historically, cytokine-stimulated protocols have been relatively ineffective at generating large quantities of eosinophils from mouse bone marrow progenitors. We have devised an ex vivo culture system which generates large numbers of phenotypically and functionally mature eosinophils at high purity from unselected mouse bone marrow progenitors. Bone marrow derived eosinophils (bmEos) are similar to those isolated from the spleen and peripheral blood of interleukin-5 transgenic mice and can be generated from gene-ablated mice on both BALB/c and C57BL/6 backgrounds. Several research groups have used this protocol to examine the importance of various molecules in eosinophil development and function with an emphasis on the role of eosinophils in allergic inflammation. We have used this protocol to demonstrate that Pneumovirus of Mice (PVM) infects eosinophils and that this infection elicits the release of proinflammatory cytokines such as IL-6, CCL2, CCL3 and IP-10 in a MyD88-dependent manner. We have also demonstrated that platelet activating factor (PAF) stimulates the release of EPO from mouse bmEos and eosinophils isolated from the spleen of IL5Tg mice as well as human eosinophils independent of the known PAF GPCR receptor—PAFR. Lastly, we found that while PAF and lyso-PAF stimulated the release of EPO, these phospholipid mediators did not stimulate the release of cytokines from eosinophils. IL-6 however could and did stimulate the release of stored cytokines indicating that degranulation occurs in a regulated manner. Currently we are working on a method to transplant bmEos into wild-type and eosinophil deficient Delta dblGATA mice in order to study the effects of eosinophils in vivo. Our work and that of others demonstrate that these cells are useful for examining the role of eosinophils in innate immunity.

A111

IMPACT OF CONTRASTING EXTREME INFLAMMATORY GENOTYPES/PHENOTYPES IN ALVEOLAR BONE REPAIR IN MICE

*Vieira AE, Repeke CEP, Francisconi CF, Biguetti CC, Araujo AC, Assis GF, Campanelli AP, Trombone APF, DeFranco M, Garlet GP**

OSTEOimmunology Lab, FOB-USP, University of Sao Paulo, Sao Paulo, Brazil

Since cytokine-inflammation axis role in bone repair remains unknown, our objective was to characterize the impact of extreme inflammatory genotypes/phenotypes in alveolar bone repair after tooth extraction in mice selected to maximal (AIR_{max}) or minimal (AIR_{min}) inflammatory reaction. Following the right upper incisor extraction samples were collected for histomorphometric and real time PCR analysis. AIR_{max} strain presented a higher repair rate, associated with an initially increased but transitory leukocyte influx, higher expression of TNF-a, IL-1b and IL-6, and increased levels of osteogenesis factors COL-I, CBFA-1, ALP, OCN and PHEX. The delayed repair in AIR_{min} strain was associated to a sustained presence of leukocytes in remaining granulation tissue, and overall lower levels of inflammatory cytokines and osteogenic factors. Pharmacological attenuation of inflammatory reaction in AIRmax strain significantly dampens the repair process. The results presented here demonstrate that the overall inflammatory genotype/phenotype interferes in alveolar bone repair through mechanisms that involve the modulation of inflammatory cell migration and osteogenic markers expression during the course of alveolar bone repair. Supported by FAPESP.

A112

CROSS-REACTIVITY ANALYSIS OF THE CHINESE HAMSTER OVARY GENOME

Andres H. Gutiérrez[1], Chris Bailey-Kellogg[2], Kristen DaSilva[1], Frances Terry[3], Matthew Ardito[3], Lenny Moise[1,3], William Martin[3], Anne S. De Groot[1,3]*

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Chinese hamster ovary or CHO cells are the most commonly used cell line to produce therapeutic proteins. Concern has been raised about host cell proteins (HCP) that may be present in the final product; the impact of these proteins on human immune response is unknown. Now that the CHO genome has been sequenced, we've initiated a full-genome analysis of CHO proteins using an established tool to predict T cell epitopes and immunogenicity (EpiMatrix/ISPRI) and a novel tool (JanusMatrix) that compares TCR-facing residues of epitopes to a target genome [CHO vs. Human (self)]. Our initial study included 39 putative CHO secreted proteins, of which 10 proteins were predicted to be immunogenic based on T cell epitope content using our validated immunogenicity screening tools. Cross-conserved epitopes that bind the same MHC allele(s) and presented a similar interface (TCR-facing residues) to the T cell receptor were identified. The impact of cross-conserved epitopes on human immune responses, which may include (1) induction of effector T cells; (2) induction of Tregs; (3) anergy, is described. Our goal is to develop an easily accessible web-based interface enabling rapid screens of CHO and other HCP for immunogenicity and cross-reactive immune response.

A113

LACTOCOCCUS LACTIS EXPRESSING IL-27: A POTENTIAL THERAPEUTIC FOR INFLAMMATORY BOWEL DISEASE

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We aimed to develop a localized delivery of the immunosuppressive cytokine IL-27 that is actively synthesized in situ by the bacterium, *Lactococcus lactis* (LL-IL-27) to treat chronic IBD. The therapeutic effect of LL-IL-27 was tested in a T cell transfer model of IBD. Administration of LL-IL-27 to diseased mice rescued all individuals, whereas the control groups all died. LL-IL-27 mice had normal colon histology, while the control mice had extensive inflammation, crypt abscesses, goblet cell loss, and intraepithelial neoplasia. LL-IL-27 mice had no blood in stool, stool consistency was nearly normal, while weight loss was partially relieved. To elucidate the protective mechanism of LL-IL-27, we analyzed IL-10 expression in the small intestine and colon using ELISA, RT-PCR, and reporter

mice. We determined that T cell-derived IL-10 was necessary for LL-IL-27's therapeutic effect. In addition, we observed significant reductions of inflammatory cytokine levels in colons of LL-IL-27-treated mice relative to control mice. Furthermore, LL-IL-27 was more effective in reducing disease activity and colon pathology than systemic administration of recombinant mouse IL-27. These results suggest that therapeutic application of LL-IL-27 offers promise as a more effective and safer management of IBD in humans.

A114

EFFECT OF *N*-(2-HYDROXY PHENYL) ACETAMIDE, ON NEURONAL HYPERACTIVITY GENE: *C-FOS* IN RESPONSE TO CHRONIC PAIN MODEL OF AIA

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The present study was carried out to study the possible disease-modifying effects of *N*-(2-hydroxy phenyl) acetamide (NA-2) for symptomatic relief of pain and inflammation associated with rheumatoid arthritis (RA) in the adjuvant-induced arthritis (AIA) model of human RA in rats. Gait analysis was used to examine the role of NA-2 (5 mg/kg) in the development of pain. Body weights and paw volumes were also measured to monitor the progression of disease and the systemic anti-arthritic effects of the test compound used in this study. Cellular immediate-early genes (*c-fos*) which reflects pattern of neuronal activity and can directly regulate the expression of the pro-inflammatory cytokines that are responsible for arthritic joint destruction, was also monitored in the present study as an index of arthritic pain. Our results showed that NA-2 inhibits not only the macroscopic inflammatory changes but also significantly reverses gait deficits in AIA. Furthermore, expression of *c-fos* gene and c-Fos protein were also found to be markedly decreased by NA-2 treatment with the parallel significant reduction in pro-inflammatory cytokines IL-1 beta and TNF-alpha and oxidative stress markers, i.e., nitric oxide and peroxide. These observations suggest that NA-2 (5 mg/kg) treatment is effective in controlling the pain related neuronal hyperactivity and may help in reducing the inflammation and have promising immuno-modulatory activity and anti-arthritic properties.

A115

DIFFERENTIAL EFFECTS OF CYCLOSPORINE A AND CRAC INHIBITOR ON CA^{2+} SIGNALING PATHWAYS IN HUMAN CD4+ T CELLS AND REGULATORY T CELLS

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Ca^{2+} signaling is an essential secondary messenger in CD4+ T cells, which drives proliferation, cytokine secretion and activation. It has been suggested that Ca^{2+}/calcineurin/NFAT pathway is also crucial for the development and function of regulatory T cells (Tregs). The *C*alcium-*R*elease-*A*ctivated-*C*alcium (CRAC) channel is believed to be the main mechanism in lymphocytes to increase intracellular Ca^{2+} concentrations, which leads to the activation of downstream signaling proteins. In this study, we used a commercially available selective CRAC inhibitor (CRACi) and calcineurin inhibitor Cyclosporine A (CsA) to interrogate Ca^{2+} signaling pathways in human CD4+ T cells and Tregs. CD4+ CD25+ Foxp3+ natural human Tregs were obtained through in vitro expansion and shown to inhibit T effector cell proliferation. We showed that Ca^{2+} influx; calcineurin activity, Ca^{2+} dependent gene expression, and CD3/CD28/IL2-driven proliferation were differentially affected by CsA and CRACi in CD4+ T cells compared to Tregs. CsA significantly inhibited Foxp3 gene expression and calcineurin activity in Tregs. In contrast, CRACi had little effects on either event, indicating that Tregs might utilize an alternative source of Ca^{2+} or signaling mechanism.

A116

CHONDROPROTECTIVE, ANTI-INFLAMMATORY AND CARTILAGE REGENERATIVE ACTIVITIES OF *CISSUS QUADRANGULARIS* AND LAKSHADI GUGGUL

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Lakshadi guggul is a combination of several ayurvedic herbal products including asthi shrinkhala, *Cissus*

quadrangularis. This herbal formula has been recommended and used clinically for many decades as a complementary and alternative medicine (CAM) for healing bone fractures, osteoporosis and calcium deposition. A clinical study with ten osteoarthritic patients reported that Lakshadi guggul was able to alleviate pain in patients and reduce symptoms associated with osteoarthritis. There is however, very little known at a cellular level, the mechanisms by which these herbals ameliorate anti-inflammatory and cartilage regenerative activities. Therefore, the anti-inflammatory and cartilage regenerative ability of three herbal extracts asthi shrinkhala (AS), lakshadi guggul (LG) and lakshadi guggul (isolate) (LG-isolate) were examined in human osteoarthritic chondrocyte culture pre-exposed to interleukin 1β (IL-1β) for 36 h. Osteotomy was induced in Swiss albino rats in the radial mid diaphyses of the bone and rats were treated with the herbal extracts for 4 weeks. Radiographical images were taken of the operated limb. Alkaline phosphatase (ALP) levels were evaluated in serum and histological sections were examined of the callus. AS, LG and LG-isolate, enhanced the chondrocyte proliferation and viability. AS and LG showed a superior ameliorative activity by reducing chondrocyte apoptosis and production of NO and PGE_2. These herbal extracts significantly inhibited the production of key catabolic mediators like the matrix metalloproteinases (MMPs) and enhanced gene expression of aggrecan and type II collagen. In rats, AS significantly induce serum levels of ALP and histological staining showed AS and LG enhanced formation of cartilaginous tissue and woven bone formation. These findings confirm the anti-inflammatory and cartilage regenerative properties of AS and LG and discovers that their mode of action is through the inhibition of MMPs and nitric oxide. Therefore, these herbs possess significant benefits as a CAM for arthritic and inflammatory diseases as they impose no toxic effects and play a part in hindering disease progression.

A117

UNRESOLVED INFLAMMATION: 'IMMUNE TSUNAMI' AND CANCER CACHEXIA INDUCED BY "TARGETED" THERAPIES

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While the role of inflammation in the induction of acute and chronic diseases or cancer has been recognized for over a century, lack of understanding of the mechanisms involved in acute and chronic inflammation has slowed down progress in preventing or treating inflammatory diseases or cancer. Current approaches in 'targeted' or 'personalized' medicine for site-specific cancers and using potent apoptotic factors often cause life-threatening side-effects such as cachexia, sarcopenia, venous thromboembolism and/or drug-resistance and cancer relapse and multiple organ failure (MOF), features that are noted in potent pathogens-induced sepsis or meningitis. In 2008, acute inflammation was defined to possess two biologically opposing arms, 'Yin' (apoptosis or tumoricidal) and 'Yang' (wound healing or tumorigenic) responses, capable of precise communications between immune, non-immune and neuroendocrine systems that protects the body against external and internal foreign elements including killing of cancer cells (Khatami 2008, 2012). Unresolved inflammation was proposed as the loss of balance between 'Yin' and 'Yang' that causes expression of exaggerated and mismatched mediators that would create immunological chaos or 'immune tsunami' that erode architectural integrity and function of target tissues that are naturally immune-responsive or immune-privileged (Khatami 2009, 2011, 2012a, b). The results of our discoveries in 1980s on experimental models of acute and chronic ocular inflammatory diseases using fluorescein-conjugated ovalbumin (FLOA) in the presence or absence of tumor promoting agents (TPA), adjuvant or infective agents (for up to 30 months) are suggestive of a first evidence for a direct link between inflammation and tumorigenesis. Analyses of a series of clinical and immunopathological findings led to the first report on inflammation-induced identifiable and progressive immune dysfunction in conjunctival associated lymphoid tissues (CALTs) including: (a) acute phase; tissue edema and mast cell (MC) degranulation; (b) intermediate phase; down-regulation phenomenon, minimal edema, partially granulated MC, heavy infiltration of eosinophils into epithelium and goblet cells and neovascularization; (c) chronic phase; induction of massive hyperplastic tissue; partially granulated MC ('leaky' MC), macrophages activation, epithelial thickening and/or thinning in the same section, and angiogenesis. Using a mixture of FLOA and TPA shifted the time course kinetics for the development of tumorigenesis from 12 to 30 to 6 months (Khatami et al. 1984, 1985, 1989; Khatami 2005, 2008). This presentation focuses on assessment of current 'targeted' therapies in the induction of life-threatening diseases such as cachexia and cancer relapse. Mechanisms of variable (reduced or increased) risks for certain cancers in asthmatic, neurodegenerative or diabetic patients are proposed to be due to unresolved inflammation-induced exaggerated expression and release of inflammatory factors

into circulation that are growth-arresting or growth promoting at site-specific tissues (e.g., prostate, liver, lung, pancreas) (Khatami 2011, 2012). A framework for design of cohort clinical studies will be presented based on a concept that inflammation is a common denominator in the genesis of these chronic diseases. Targeting the maintenance of immune surveillance is the correct target for the designs of cost-effective approaches for diagnosis, prevention and therapy of age-associated diseases or cancer.

A118

ACTIVATION OF EPIDERMAL TOLL-LIKE-RECEPTOR 2 ENHANCES TIGHT JUNCTION FUNCTION: IMPLICATIONS FOR ATOPIC DERMATITIS AND SKIN BARRIER REPAIR

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Atopic dermatitis (AD) is a Th2-skewed, allergic skin disease characterized by epidermal tight junction (TJ) defects and a propensity for *Staphylococcus aureus* (*S. aureus*) skin infections. *S. aureus* is sensed by many pattern recognition receptors including toll-like receptor (TLR) 2. We hypothesized that an effective innate immune response will include skin barrier repair and that this response might be impaired in AD subjects. *S. aureus*-derived peptidoglycan (PGN) and synthetic TLR2 agonists enhanced TJ function and increased expression of TJ proteins in primary human keratinocytes. A TLR2 agonist enhanced TJ barrier recovery in human epidermis wounded by tape-stripping which is a model of scratching-induced barrier disruption commonly observed in AD subjects. $Tlr2^{-/-}$ mice had a delayed and incomplete barrier recovery following tape-stripping. AD subjects had reduced epidermal TLR2 expression as compared to nonatopic (NA) subjects, which inversely correlated ($r = -0.654$, $P = 0.0004$) with measures of skin barrier integrity. Lastly, Th2 cytokines (IL4 + IL13) attenuated TLR2-enhanced TJ barrier recovery. These findings indicate that TLR2 activation enhances epidermal TJ function and is an important part of a barrier repair response. The Th2 environment observed in AD subjects may compromise this novel defense function. Antagonizing Th2 cytokines may help restore epidermal integrity in AD.

A119

THERAPEUTIC EFFECTS OF EC0746 ON EXPERIMENTAL AUTOIMMUNE UVEITIS, EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS AND NATURALLY OCCURRING CANINE EROSIVE POLYARTHRITIS

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EC0746 is a rationally designed aminopterin hydrazide-folate conjugate that exhibits folate receptor (FR)-specific anti-inflammatory activity. In this report, we show that EC0746 effectively treats experimental autoimmune uveitis (EAU) and encephalomyelitis (EAE) as well as a dog with naturally occurring erosive polyarthritis. EAU and EAE were induced in rats by active immunization against bovine retinal soluble antigen (PDSAg) and guinea pig myelin basic protein, respectively. In both models, FR-expressing macrophages were found in abundance in the peritoneal cavities of rats with advanced diseases. FR-expressing macrophages were also detected in the retinal tissues of EAU rats. In rats with active EAE, increased uptake of ^{99m}Tc-EC20 (etarfolatide, a FR-specific radiodiagnostic agent), was observed in the brain stem/cerebellum and spinal cord. EC0746 treatment (starting from disease on-set) was found to rapidly suppress the severity of both diseases. Accordingly, aqueous humor protein levels in EC0746-treated EAU rats decreased dramatically ($\sim$4-fold) in comparison to that of the untreated control animals. Immunohistochemical analysis after EC0746 therapy showed no ED1 macrophage reactivity in the brain and lumbar spinal cord of EAE rats. Interestingly, EC0746-treated EAE rats also showed a moderate relapse of the disease $\sim$1 week after the EC0746 dosing had ended. In all clinical/histological parameters assessed, the activities of EC0746 were completely blocked by a folate competitor, suggesting that the therapeutic outcome were FR-mediated. The therapeutic potential of EC0746 was further tested in an 11-year-old, hind-limb lame female-spayed Shetland sheepdog with naturally occurring erosive polyarthritis. Her condition was diagnosed based on the results of clinical presentation, orthopedic examination, complete blood count, serum chemistry, urinalysis joint fluid analysis, radiographic changes and scintigraphy using

^{99m}Tc-EC20. The dog was treated subcutaneously with EC0746 until remission and was confirmed approximately 9 months later. Importantly, the dog has remained in remission for almost a year now. Considering its potent activities across multiple animal models of inflammation, EC0746 may emerge as an effective anti-macrophage agent for the treatment of a variety of "FR-positive" autoimmune and inflammatory disease indications.

A120

GM-0111, A MODIFIED GLYCOSAMINOGLYCAN, PROTECTS MICE FROM DEVELOPING CYSTITIS INDUCED WITH LL-37

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Interstitial cystitis or painful bladder syndrome (IC/PBS) is a chronic disease with unknown cause and affects 3–8 million adult women in the US alone. We investigated the anti-inflammatory effects of GM-0111, a chemically modified glycosaminoglycan, in our murine IC/PBS model. Young female adult mice were first intravesically instilled with various concentrations of GM-0111 followed by intravesical instillation of cystitis inducing peptide LL-37. We sacrificed the animals 24 h after instillation of LL-37, harvested the whole blood and urinary bladders, and analyzed histological and biochemical changes in the blood and tissues. Mice pretreated with GM-0111 showed significantly reduced signs of cystitis: improved body weight gains and decreased inflammatory changes in the tissues that correlate with changes in various biochemical markers that we tested. These data suggest that GM-0111 has therapeutic utility for prevention and potential treatment of IC/PBS.

A121

LACK OF IL-17RA SIGNALING PREVENTS COLLAGEN-INDUCED ARTHRITIS AND RESULTS IN A Th2-LIKE PHENOTYPE

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Here we show that IL-17RA deficient (IL-17RA−/−) mice did not develop collagen-induced arthritis (CIA) even after a third CII/CFA injection. Interestingly and in contrast to the IL-23p19 knockout mice, the IL-17RA deficient showed a Th2-like phenotype in splenic $CD4^+$ T cells at day 69. Moreover, the CII-specific IgG2a levels in the sera of IL-17RA−/− was significantly lower compared to the control group at day 20. At day 69, CII-specific IgG1 levels in the sera of IL-17RA−/− was increased although not statistically significant compared to the control. In conclusion, these data show a Th2-like phenotype in IL-17RA−/− mice immunized with CII, suggesting that IL-17 receptor signaling is involved in the suppression of Th2 cytokines in autoimmune collagen-induced arthritis.

A122

THE MECHANISM OF IMMUNE-MODULATORY ACTIVITY OF INTRAVENOUS IMMUNOGLOBULIN (IVIG) IN A MURINE MODEL OF ALLERGIC ASTHMA

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Anti-inflammatory activity of IVIG has been suggested to be dependent on a minor population of sialylated IgG (SA-IVIG). We have demonstrated that IVIg inhibits airway hyperresponsiveness (AHR) in a murine model of allergic airways disease. We addressed whether the mechanism by which IVIg exerts its immune-regulatory effects is via the sialylated fraction. C57 BL/6 mice were sensitized and challenged with OVA. Prior to challenge, mice were treated with IVIG, enriched SA-IVIG, and nonSA-IVIG fractions. Whole IVIG and enriched SA-IVIG, but not nonSA-IVIG, induced $Foxp3^+$Treg cells, in lung, comparably, as monitored by flow cytometry, and abrogated AHR in OVA-challenged mice, as measured by the FlexiVent small animal ventilator. Adoptive transfer of dendritic cells (DCs) from IVIG and SA-IVIG treated mice to OVA-challenged syngeneic animal induced $Foxp3^+$Treg cells and inhibited AHR similarly. Binding studies with fluorescent-labeled IVIg suggested that a C-type lectin receptor may be the responsible for trapping SA-IVIG on dendritic cells. Binding to this receptor facilitates the internalization

of IgG into the cytoplasm of carrying cells. This study indicates that the anti-inflammatory effect of IVIG is dependent on the sialylated fraction of IVIG. This mechanism might be mediated in part by the immunomodulation of DCs and subsequent induction of Treg. C-type lectin receptors might also be implicated in this pathway.

A123

HTS FOR NOVEL PARP-1 INHIBITORS USING A 5.5 MILLION COMPOUND COLLECTION DERIVED FROM ECLiPS TECHNOLOGY

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*P*oly(*A*DP-*r*ibose) *p*olymerase-1 (PARP-1) plays an important role in the regulation of inflammation in the context of ischemia–reperfusion injury. Numerous PARP-1 inhibitors are being investigated in a variety of in vivo models and in clinical trials for solid tumors, leukemia, lymphoma, and ischemia–reperfusion injury. Our proprietary collection of 5.5 million compounds derived from *E*ncoded *C*ombinatorial *L*ibraries on *P*olymeric *S*upport (ECLiPS) technology was designed for diversity and drug-likeness (Lipinski's rules). We ran an HTS using the ECLiPS collection on purified recombinant PARP-1 in a 1,536-well, 6 μL assay format. The HTS ran with an overall Z'-factor = 0.68, Z-factor = 0.65, and identified hits in 10 of the 129 libraries within ECLiPS. "Synthon Frequency Plots" gave initial indications of SAR within the collection. Promising hits will be re-synthesized to confirm activity and determine potency in a variety of secondary assays.

A124

NEUTROPHIL AND MONOCYTE MIGRATION IS DIFFERENTIALLY REGULATED BY PI3K ISOFORMS

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BioSeek, LLC., South San Francisco, CA, USA Signaling through the phosphoinositide 3-kinase (PI3K) family has been implicated in the development and progression of various disease states including inflammation, autoimmunity, and cancer. Critical to their pathogenic role is the regulation of migration of phagocytic leukocytes (e.g. monocytes and neutrophils), however the role of each PI3K isoform in this process remains to be elucidated. We have developed assays using primary human monocytes and neutrophils to determine the differential impact of selective PI3K inhibitors on their migration. While pretreatment of neutrophils and monocytes with the pan-PI3K inhibitor, wortmannin, blocked migration of both cell types in response to C5a, the PI3K-delta specific inhibitor, IC-87114, blocked neutrophil but not monocyte transmigration through transwell plates. Interestingly, when IC-87114 was added only in the lower chamber with C5a, enhanced transmigration of monocytes but not neutrophils was observed (363 ± 49 RLU vs. to 232 ± 45 RLU for C5a alone). These data suggest that PI3K-delta differentially regulates the migration of neutrophils and monocytes.

A125

DIFFERENTIAL REGULATION OF IL-17A AND IL-17F PRODUCTION IN A HUMAN CO-CULTURE MODEL OF T CELL-DEPENDENT B CELL ACTIVATION

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BioSeek, LLC., South San Francisco, CA, USA BioSeek has pioneered the development of in vitro human primary cell-based co-culture systems (BioMAP®). Improper regulation of lymphocyte activation and the production of IL-17 family cytokines are implicated in many autoimmune diseases. In this study we tested a broad panel of pharmacologic inhibitors in a model of T cell-dependent B cell activation in which B cells are co-cultured with PBMC and stimulated with anti-IgM/superantigens (BT BioMAP system). IL-17A and IL-17F were produced in significant quantities in this system, 261 ± 73 and 1,549 ± 206 pg/ml, respectively. Compounds that induce mitochondrial dysfunction, and inhibitors of p38 MAPK, calcineurin, and COX enzymes, were found to preferentially block production of IL-17A, but not IL-17F. GR agonists and mTOR inhibitors reduced the production of both IL-17A and IL-17F. Interestingly, the only compound class to inhibit IL-17F, but not IL-17A, was EP agonists. Our findings underscore the utility of complex co-cultures for the discovery and validation of targets and compounds specifically involved in the regulation of IL-17 family cytokines.

A126

DIFFERENTIAL INDUCTION OF CHEMOKINES MIP-2 AND KC REGULATES NEUTROPHIL TRAFFICKING AND PROTECTS MICE FROM BACTERIAL SEPSIS

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Previous studies have shown that the CD14-dependent induction of systemic chemokines in response to a local bacterial infection with *E. coli* results in a delay in neutrophil recruitment to the site of infection (peritoneal cavity) and leads to bacterial proliferation and increased mortality. In contrast, in CD14-deficient mice, ip injection of *E. coli* leads to a local induction of chemokines via a CD14-independent pathway that enables rapid neutrophil recruitment, enhanced bacterial clearance and improved survival. This study was initiated to determine whether TLR4 plays a role in the CD14-independent chemokine production. Mice deficient in TLR4 or TLR4 signaling molecules (MyD88−/−TRIF−/−) injected with a lethal dose of *E. coli* exhibited enhanced survival, bacterial clearance, early neutrophil recruitment and chemokine induction that was low in blood and higher in the site of infection. In contrast, LPS alone did not induce neutrophil recruitment. These studies describe a third pathway for chemokine induction in the peritoneal cavity that is both CD14- and TLR4-independent that represents a response to a non-LPS component of *E. coli*. These studies illustrate the existence of multiple pathways for inducing neutrophil-attracting chemokines and suggest that differential induction of these pathways can regulate neutrophil trafficking. GRANT SUPPORT: NIH-NIAID#AI23859 NIH-RCMI #G12RR03060 at City Univ. of New York.

A127

MODULATION OF DENDRITIC CELLS AND COLITIS BY PROBIOTIC BACTERIA

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The interplay between the gut-associated immune system and the intestinal microflora plays an important role in the sensitivity for inflammatory diseases. Accordingly, we demonstrated recently that probiotic bacteria have favorable effects by suppressing inflammation in the recurrent TNBS colitis model and substantiated these effects by genome wide transcriptional analysis of the colons (Mariman et al., Inflamm Bowel Dis, 2011). Probiotic treatment particularly affected local chemokine expression with a major impact on the influx of T cells, macrophages and mast cells. We next studied bone marrow derived dendritic cells (BM-DC) from BALB/c and C57BL/6 mice in response to pure TLR ligands and/or probiotics. Transcriptome analysis with emphasis on TLR-pathways showed unique and overlapping patterns induced by LPS or probiotic bacteria. A set of LPS-induced genes, including *CXCL9* and *CXCL10*, was suppressed by probiotic treatment. Remarkably, IL-12p70 and IL-23 were synergistically induced by LPS and probiotics in C57BL/6 but not in BALB/c BM-DC. It is concluded that immune modulating effects of probiotic bacteria are diverse and may dependent on the genetic background of the host.

A128

LX1606, A PERIPHERAL INHIBITOR OF SEROTONIN SYNTHESIS, ALLEVIATES DEVELOPMENT OF INFLAMMATORY BOWEL DISEASE IN A PRECLINICAL MODEL

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Dysfunctional signaling by the immunoendocrine mediator serotonin (5-HT) may play an important role in the pathophysiology of inflammatory bowel disease (IBD). The first step of 5-HT synthesis in the gut is catalyzed by tryptophan hydroxylase (TPH) 1. We reported the discovery of the TPH inhibitor LX1606 (aka LX1032), which reduced peripheral 5-HT production in preclinical and human studies. We evaluated now the efficacy of LX1606 in the 2,4,6-trinitrobenzenesulfonic acid-induced model of IBD in mice. LX1606 treatment significantly protected mice from weight loss and inflammation, which was associated with lower histopathology scores, decreased expression of pro-inflammatory cytokines, and with reduced peripheral blood neutrophil counts. LX1606 decreased 5-HT content in blood and jejunum, confirming the compound's mechanism of action. These results demonstrate that inhibition of TPH activity by LX1606 may provide a new approach for the treatment of IBD and its serotonin-mediated symptoms.

A129

SERUM LIPIDS, PROTEINS, AND PRO-INFLAMMATORY CYTOKINES LEVELS IN ARTHRITIC RATS FOLLOWING TREATMENT WITH *N*-(2-HYDROXYPHENYL)-ACETAMIDE

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Rheumatoid arthritis is an inflammatory joint disease characterized by synovial proliferation and infiltration of inflammatory cells that leads to the formation of pannus and joints cartilage/bone destruction. TNF-α, and IL-1β are the key mediators in the disease process and shift the whole-body protein metabolism towards net catabolism to increase joint pain and stiffness. In present study, the anti-arthritic/anti-inflammatory activity of *N*-(2-hydroxy phenyl)-acetamide (NA-2) in adjuvant-induced arthritis model of rats was observed by measuring body weight; paw oedema, and latency time. At the end of the experiment, serum was collected and processed for determination of triglyceride, total cholesterol, high density lipoprotein, total protein, and IL-1 β and TNF-β. Our results demonstrated a significant reduction in body weight and increase in paw oedema in arthritic control rats. However, arthritic rats receiving 5 mg/kg dose of NA-2 exhibited a gradual increase in their body weight and reduction in the paw oedema. Moreover, the NA-2 treatment was also found to significantly reduce the levels of the aforementioned serum parameters. Thus based on our results, we suggest that the promising anti-arthritic property of NA-2 makes it a possible therapeutic agent for arthritic patients.

A130

INHIBITION OF PIM KINASE PROTECTS NZBWF1 MICE FROM GLOMERULONEPHRITIS AND PROLONGS SURVIVAL

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AR-472317 and AR-476430 are selective and potent small molecule inhibitors of PIM1 and 3 kinases, with PIM1 IC50 enzyme potencies of 120 and 220 pM and PIM3 IC_{50} potencies of 400 and 700 pM, respectively. Both compounds are also active in a PIM1 cellular assay with respective EC_{50}s of 21 and 31 nM. Herein, we show the activity of these PIM1/3 inhibitors in two in vivo models of inflammation. First, we investigated the ability of AR-476430 to inhibit antigen mediated antibody production in C57Bl6 mice. Mice were immunized and challenged with hen egg lysozyme (HEL) on days 0 and 7. Mice were bled weekly for determination of HEL specific antibody isotypes by ELISA. AR-476430 dosed orally at 30 mg/kg BID for 4 weeks significantly reduced the AUC for total IgG (53 %), IgG2a (32 %), and IgG3 (45 %), activity similar to the positive control, cyclosporine. AR-472317 was evaluated in the NZBWF1 spontaneous murine model of lupus. AR-472317 was administered orally at 3, 10 or 30 mg/kg BID beginning at 29 weeks of age and continuing for 22 weeks. AR-472317 significantly prolonged survival (vehicle survival at 44 weeks = 50 %, 30 mg/kg survival at 50 weeks = 87 %) and inhibited proteinuria at 10 and 30 mg/kg. Histopathological analysis of kidneys showed significant reductions in glomerulonephritis, interstitial nephritis, vessel inflammation and protein cast formation. These data support the hypothesis that inhibition of PIM1/3 kinases is therapeutic in preclinical models of antigen-specific antibody generation and autoimmune mediated glomerulonephritis.

A131

ANTI-INFLAMMATORY AND ANALGESIC EFFECT OF HDAC INHIBITORS; SAHA AND MS-275 IN INFLAMMATORY PAIN MODELS

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Epigenetics is the study of heritable changes in gene expression; without altering deoxy-ribonucleic acid sequence. Drugs modulating epigenetic changes have been

in the focus for treatment of various diseases. Literature evidence suggests a role for epigenetics in the induction and maintenance of neuropathic pain and drugs modulating epigenetic changes are being pursued as promising targets. Histone acetylation/deacetylation plays an important role in inflammation. Histone deacetylases (HDACs) not only cause the modulation of gene transcription, but are also known to directly associate with many signaling proteins. Restoration of epigenetically suppressed GAD65 functions using HDAC inhibitors, leading to analgesic effect in chronic neuropathic pain has been reported. The present work describes the anti-inflammatory and analgesic activity of HDAC inhibitors, suberoylanilide hydroxamic acid (SAHA) and MS-275, in various acute and sub acute inflammatory pain models like carrageenan/Freund's complete adjuvant (FCA) induced paw inflammation and formalin induced pain model. Treatment with SAHA at 10 mg/kg i.p. and MS-275 at 5 mg/kg i.p. significantly inhibited hyperalgesia which is evident from increase in paw withdrawal threshold in carrageenan and FCA induced paw inflammation models and decrease in number of paw licking response in formalin model. SAHA at dose of 10 mg/kg i.p. and MS-275 at dose of 5 mg/kg i.p. were also tested in partial sciatic nerve ligation model. Treatment with SAHA showed improvement in the paw withdrawal threshold while MS-275 failed to show any response. Furthermore, SAHA and MS-275 also significantly inhibited lipopolysaccharide induced TNF-α, IL-6 and nitric oxide in RAW 246.7 murine macrophages. Taken together, present experimental findings demonstrate promising anti-inflammatory and analgesic activity of SAHA and MS-275. It also suggests that inhibition of HDACs may be potential targets for attenuating persistent inflammatory pain.

Keywords: Histone deacetylase inhibitor, Pain, SAHA, MS-275

A132

IRON SATURATED BOVINE LACTOFERRIN, A NOVEL AND SAFE NANOTHERAPEUTIC FOR OSTEOARTHRITIS

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Osteoarthritis (OA) is a degenerative joint disease associated with the progressive destruction of cartilage and joint inflammation. Current treatments have serious side effects and with no known cure, a safe treatment which would protect and/or repair cartilage degradation is urgently needed. In this study, we evaluate the treatment potentials of novel nanocarriers, loaded with anti-inflammatory, non-toxic protein lactoferrin (Lf), using preclinical assays mimicking human joint inflammation. Exposure of chondrocytes to the pro-inflammatory cytokine IL-1β, significantly induced activation of inflammatory mediators such as collagenases, prostaglandins and nitric oxide (NO). Administration of Lf loaded nanocarriers on IL-1β pre-exposed cells dose dependently prevented these catabolic effects and reversed apoptotic cell death stimulated by these mediators. Nanocarriers enhanced chondrocyte proliferation, were non-toxic and ameliorated cartilage matrix synthesis. Further, in a chondrocyte-synoviocyte co-culture mimicking inflammation, nanocarriers prevented production of detrimental NO. Collectively, our findings indicate the anti-inflammatory and cartilage regenerative properties of the nanoformulated Lf and its prospective role in the future development of an oral nanomedicine that can target cartilage and ensure bioavailability of Lf in arthritic joints.

A133

DEVELOPMENT OF A ROBUST RAT MODEL OF GLUCOCORTICOID INDUCED OSTEOPOROSIS AS ASSESSED BY µCT, S.E.M., AND CONFOCAL LM

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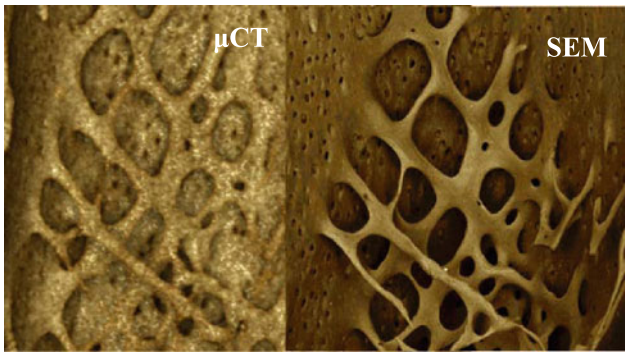
The action of glucocorticoids on bone remains controversial, and a robust animal model is needed. Femurs from prednisolone (Pred) treated rats (p.o. 3–100 mg/kg, 35 days) were imaged by µCT with BoneJ plugin (http://bonej.org) analysis, SEM and confocal LM. Pred reduced (ED_{50} mg/kg) femoral endosteal (12.4) and periosteal (65.2) tetracycline/calcein dual label increment and µCT cortical thickness (1.2), but not trabecular thickness nor cortical mineral density. Epiphyseal trabecular responses were site specific. Active bone modelling from metaphysis to shaft determines the site-specific actions of Pred. Matching of µCT to SEM shows all fine trabeculae (<10 µm) are lost to µCT visualisation and thus analysis. We characterise rat bone responses for comparative glucocorticoid pharmacology.



A134

PRE-CONDITIONED MAPK ACTIVATED PROTEIN KINASE-2 (MK-2) RESPONSES OF MACROPHAGES TO p38 INHIBITION IN THE ABSENCE OF INTRACELLULAR INHIBITOR, SB203580

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Previous work suggests that selective p38 MAPK inhibitors, SB203580 and ML3403, have inhibitory effects on adherent accessory cells such as macrophages lasting up to 120 h after drug washoff, but not lymphocytes. This could be due to drug sequestration and release from within macrophages after drug washout, or due to a conditioning action. PMA (80 nM) differentiated U937 cells were transiently (2 h) exposed to SB203580 (3 µM) and cytosolic and membrane drug content assayed by GCMS and correlated with Western blot analysis of LPS (100 ng/mL, 30 min) stimulated phosphorylation of downstream MK2 and ATF2. LPS stimulated TNF-α production by PMA-U937 cells, which was completely inhibited by SB203580. LPS stimulated a concentration-dependent increase in the phosphorylation of MK-2 which was inhibited by SB203580. SB203580 was undetectable in both cytosol and membrane fractions from 1 h after washout. Washing the cells after drug incubation restored the ability to stimulate phospho-MK-2 in the macrophages shortly after washout (1 min and 2 h), however the inhibition of MK-2, but not AkT, phosphorylation by p38 MAPK was reasserted 4–24 h after drug withdrawal. The inhibition of p38 prior to cell activation selectively preconditions macrophage MK2 and behaviour responsiveness

A135

TOPICAL KAPPA-OPIOID RECEPTOR AGONISTS EXHIBIT ANTI-INFLAMMATORY AND ANTI-PRURITIC ACTIVITY IN A CONTACT DERMATITIS MODEL IN MICE

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The efficacy of topical Nalfurafine or of a proprietary, potent and selective KOR agonist was assessed in a mouse model of contact dermatitis regarding amelioration of itching and inflammation. Mice were sensitized with oxazolone on day 0, challenged on days 7, 9, and 11 and treated daily from day 11 until day 18 with the KOR agonists (1 and 0.2 % in 50 µl DMSO) or betamethasone dipropionate (0.05 % in 50 µl DMSO) as positive control. On day 11 all treatments did reduce scratching not only in the early phase after the challenge with oxazolone but over a period of 22 h. Similar results were obtained on day 18. Ear thickness increased from day 11 to days 14 and 15, resp., in contrast to the positive control. However, ear swelling decreased in mice treated with KOR agonists significantly faster as compared to vehicle control. Histological analysis (H&E) of treated ears showed dose dependent effects on ear thickness, epidermal thickness and dermal infiltrate. Topical KOR agonists warrant further evaluation as treatment for inflammatory and pruritic skin diseases.

A136

THE ROLE OF PHAGOCYTES IN PULMONARY *FRANCISELLA TULARENSIS* INFECTION

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Francisella tularensis is the causative agent of tularemia. Pulmonary infection with *F. tularensis* is highly lethal due to the profound inflammation induced in the airways. Though *F. tularensis* is believed to be an intracellular pathogen that requires replication within macrophages

during respiratory infection, we have found that depletion of alveolar macrophages by intranasal treatment with liposomal clodronate reduced mean time to death and total survival rates of infected mice. Furthermore, mice with a macrophage-specific insensitivity to interferon-γ (MIIG mice) similarly show heightened sensitivity to pulmonary Francisella infection. Such mice exhibited reduced lung-tissue expression of tumor necrosis factor-α, a potent neutrophil chemoattractant, and systemic neutrophil depletion reduced mean time to death and total survival rates. In mice infected with a lethal dose of bacteria, exogenous interleukin-12 promoted survival and bacterial clearance in mice, but this effect was lost upon neutrophil depletion and in IFN-$\gamma^{-/-}$ mice. The results suggest that rather than being a required reservoir for bacterial replication, alveolar macrophages are necessary for defense against *F. tularensis* infection primarily by recruiting neutrophils, which are essential for clearing the bacterium. (Supported by NIH grant PO1 AI 056320).

A137

BTK INHIBITOR RO5465486 IS EFFECTIVE AGAINST EARLY AND LATE PHASE RESPONSES IN A MOUSE ASTHMA MODEL

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Bruton's tyrosine kinase (BTK) is involved in mediating the signaling downstream of both B cell receptor and FCεR1 activation, affecting antibody production and mast cell activation, respectively. RO5465486 is a potent and selective inhibitor of BTK (Kd = 0.3 nM). RO5465486 inhibited IgE-mediated mast cell degranulation and B cell antibody production with IC_{50} values of 6–17 nM in using human and mouse cells. RO5465486 (30 mg/kg, p.o., q.d.) significantly inhibited allergic airway inflammation by 61 ± 11 % ($P < 0.05$) in the mouse ovalbumin model, which is equivalent to the efficacy achieved with the glucocorticoid, budesonide (3 mg/kg, p.o., q.d.). RO5465486 also significantly inhibits allergen induced acute bronchoconstriction (76 ± 23 %; $P < 0.05$), which is equivalent to that of the β-agonist salbutamol (1 mg/kg, i.n., q.d.). These data suggest that a BTK inhibitor can block key features of the allergic response associated with asthma.

A138

TARGETING ALK-5, BUT NOT WISP-1 or LOXL2, PROTECTS AGAINST BLEOMYCIN-INDUCED LUNG FIBROSIS

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Our aim was to characterize and assess candidate mechanisms using the bleomycin (BLM) induced lung fibrosis model. Intratracheal administration of bleomycin (BLM) (2 U/kg) to male, C57BL6/J mice led to expression changes in the elements of the Wnt and TGF-β pathways as well as matrix remodeling enzymes. These changes paralleled increases in lung fibrosis, which showed a significant correlation to alterations in pulmonary mechanics (e.g. work of inflation) over time ($R = 0.74, p < 0.05$). Subsequently, the efficacy of an ALK-5 inhibitor, Pirfenidone, anti-LOXL2, and anti-WISP1a were assessed. The ALK-5 inhibitor effectively prevented BLM-induced fibrosis from developing, whereas none of the other molecules assessed had any impact on changes in this model. These data indicate that the clinically relevant pathophysiological changes mimicked in the BLM model are dependent on TGF-β signaling.

A139

POLY IC INDUCED GENE EXPRESSION CHANGES IN NON-HUMAN PRIMATES

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Polyinosinic:polycytidylic acid (poly I:C) is a synthetic form of double-stranded (ds)RNA commonly used to mimic viral infections. Nasal scrapes from naïve and *Ascaris suum* (*A. suum*) sensitized NHPs ($n = 12$ per group) were obtained before (0 h) and after (6 h) nasal challenge with 100 μg/ml HMW poly:IC or saline.

Histological sections indicated that respiratory epithelium and submucosal tissues were present within the scrapes. Treatment with poly I:C induced 161 differentially expressed genes (DEGs) and largely up-regulated genes involved in innate immunity and inflammatory responses, including toll-like receptor signaling pathways ($p = 6.31e{-}05$), RIG-1 signaling($p = 0.03$), IL-6 signaling ($p = 1.7e{-}04$). Interaction analyses did not show significant differences in response between naïve and *A. suum* sensitized NHPs. These data indicate that the poly I:C nasal challenge model mimics acute responses similar to those associated with a viral infection.

A140

RESULTS OF A PHASE I CLINICAL STUDY WITH ORALLY ADMINISTERED OCID 2987: A NOVEL PDE4 INHIBITOR

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OCID 2987 is a novel PDE4 inhibitor being developed for treatment of patients with COPD/asthma. A double-blind, randomized, placebo-controlled, sequential group, single- and multiple-ascending dose study; Part 1 (SAD 2, 5, 10, 15, 20 and 30 mg) consists of six groups of eight healthy subjects each receiving a single oral dose of OCID 2987 or placebo (six verum and two placebo); Part 2 (MAD 10, 20 and 30 mg) consists of three groups of eight healthy subjects each receiving an oral dose of OCID 2987 or placebo (six verum and two placebo) once daily for 14 days. Safety, tolerability, pharmacokinetics and pharmacodynamics were evaluated. The most common adverse event observed in SAD was postural dizziness, and in MAD were headache and backache. Oral administration of single and multiple ascending doses, OCID 2987 was rapidly absorbed with a mean T_{max} and $T_{1/2}$ of 3 and 8 h, respectively. $AUC_{(0-\infty)}$ and C_{max} were found to be dose proportional. In MAD, OCID 2987 steady state plasma concentration and $AUC_{(0-\infty)}$ increased proportionately with increasing dose on day 14 compared to day 1 with a marginal decrease in clearance, consequent to increase in $T_{1/2}$. Further, OCID 2987 showed a dose dependent ex vivo inhibition of inflammatory marker TNF-α >60 %. Based on these results and the preclinical data, OCID 2987 is expected to be beneficial to asthma/COPD patients with a good safety margin.

A141

OCID 2987: A POTENTIAL DRUG FOR THE TREATMENT OF ATOPIC DERMATITIS AND UVEITIS

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PDE4 inhibitors have been extensively investigated as a treatment for several inflammatory diseases such as asthma, chronic obstructive pulmonary disease (COPD), rheumatoid arthritis and psoriasis. OCID 2987, a potent PDE4B2 enzyme inhibitor showed prominent inhibition of TNF-α, IFN-γ, IL-1β, IL-2, IL-5 and IL-17 in lipopolysaccharide (LPS)/phytohaemagglutinin (PHA) induced peripheral blood mononuclear cell (PBMC), it also showed strong inhibition of TNF-α in human whole blood and THP-1 cell line. OCID 2987 showed significant inhibition of nitric oxide in LPS activated macrophage cell line (RAW 246.7 cells) and leukotriene B4 (LTB4) inhibition in human neutrophils. OCID 2987 was studied for its potential in treatment of atopic dermatitis and uveitis. OCID 2987 showed profound improvement in mouse phorbol ester (TPA) and oxazolone induced ear edema, ear thickness and ear weight which were further confirmed by the histological examination. OCID 2987 topical application as an ocular suspension in endotoxin induced uveitis (EIU) rat model, showed dose dependent decrease in cell count in aqueous humor sample. These results indicate that OCID 2987 has a great therapeutic potential in the treatment of inflammatory skin and eye diseases. Phase 1 clinical study of OCID 2987 conducted in the healthy volunteers indicated that drug is well-tolerated with a very favorable safety profile and good PK/PD relationship on oral dosing.

A142

IMAGING ARTHRITIC INFLAMMATION & THERAPEUTIC RESPONSE BY ^{19}F MRI

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Non-invasive imaging of inflammation to measure the progression of autoimmune diseases, such as rheumatoid

arthritis (RA), and to monitor responses to therapy is critically needed. V-Sense, a perfluorocarbon contrast agent that preferentially labels inflammatory cells, which are then recruited out of systemic circulation to sites of inflammation, enables detection by fluorine-19 magnetic resonance imaging (^{19}F MRI). Arthritic but not naïve rats had significant accumulation of the contrast agent in the hind limbs. Quantification of ^{19}F signal measured by MRI in affected limbs was linearly correlated with disease severity assessed by caliper measurements. In serial imaging studies, increasing ^{19}F signal reflected animals with progressive disease, while no increase was observed in animals receiving prednisolone treatment which resulted in clinical and microscopic resolution of disease. These results indicate that inflammation in arthritis may be imaged by ^{19}F MRI, and this approach can be used to quantitatively evaluate responses to a therapeutic regimen longitudinally in a non-invasive manner.

A143

DEVELOPMENT OF A POTENT AND SELECTIVE LPA R1 ANTAGONIST FOR THE TREATMENT OF FIBROTIC DISEASES

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Lysophosphatidic acid regulates several cellular processes including motility, proliferation, survival, and differentiation via specific G-protein-coupled receptors (LPAR1, LPAR2, LPAR3, and LPAR4). Recently, a number of studies, using both knockout mice and pharmacological tools, have implicated LPAR1 as a key mediator promoting wound healing and fibrosis. Here, we report the in vitro and in vivo pharmacological profile of a potent, orally available and LPAR1-selective antagonist. In vitro, AR476479 inhibits LPA-stimulated GTPgammaS binding (61.5 nM) and intracellular calcium release (91 nM) from a Chem1 cell line (Millipore) stably expressing human LPAR1. After oral dosing (30 mg/kg) in rats, AR476479 plasma concentrations peaked at 3 h with a C_{max} of 7.3 μg/ml and an oral bioavailability of 57 %, demonstrating a good pharmacokinetic profile. Moreover, in a LPA-induced in vivo histamine release model AR476479 treatment results in dose-related inhibition of histamine release ($ED_{50} = 1.45$ mg/kg) in the mouse confirming in vivo pharmacodynamic activity of AR476479. In a rat renal fibrosis model, induced by unilateral ureteral obstruction (UUO) for 21 days, AR476479 attenuated the expression of collagen 1a, α-smooth muscle actin (α-SMA), and fibronectin. Furthermore, treatment with the LPAR1 antagonist reduced the expression of the profibrotic cytokine connective tissue growth factor (CTGF). These data show that AR476479 is a small molecule that potently and selectively antagonized LPAR1, which represents a novel and exciting target for fibrotic diseases.

A144

VALIDATION OF A REPRODUCIBLE ANIMAL MODEL FOR CROHN'S DISEASE

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Background: Inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease (CD) affecting up to 1.4 million Americans (CDC data) are chronic inflammatory disorders of the small and large intestine leading to pain and debilitation, sometimes with life-threatening complications. Recent studies suggest that disruption of the intestinal mucosal immune system is involved in the pathogenesis of IBD. To evaluate the therapeutic effects of new drugs to treat these autoinflammatory disorders, a validated animal model that mimics the human condition and offers a test system with translational potential is needed. The hapten-induced model of colitis induced by intrarectal administration of trinitrobenzene sulfonic acid (TNBS) is widely used, but as yet, not standardized. The pathophysiology of TNBS-induced colitis is a $CD4^+$ T cell mediated autoimmune disease that results in a Th1-polarized immune response and inflammatory bowel disease that affects the colon and resembles CD. Although the experimental procedures are in principle easy to perform, individual differences in the intestinal microflora among animals, the genetic heterogeneity of inbred susceptible mouse strains, as well as pathogen status of animal facilities require optimization of the dose levels and formulation of TNBS.

Method: Female BALB/C mice were intrarectally administered either TNBS (1–3 mg/animal) formulated in ethanol, vehicle control, or untreated control. Animals were observed for an 8 days period and evaluated for body weight, rectal bleeding, and terminal evaluation of the colon weights, length as well as macroscopic and microscopic colonic changes.

Results: TNBS induction resulted in a rapid onset of colonic inflammation associated with up to 22 % decrease in body weight and severe rectal bleeding throughout the 8 days of study, as compared to constant body weight and transient mild rectal bleeding in the ethanol control group or untreated control animals. In addition, colon weight (not length), macroscopic scoring and microscopic histological evaluation of colon samples resulted in severe damage to the colonic mucosa when animals were treated with TNBS, but no tissue damage was noticed with vehicle alone.

Conclusion: These data demonstrate our proficiency in generating a reproducible auto-inflammatory model for IBD. We have optimized and establish a TNBS induction method that provides a repeatable, reliable, and robust IBD model for testing novel therapeutic agents.

A145

CCL9 MEDIATES TGF-β REGULATION OF TUMOR CELL SURVIVAL AND LUNG METASTASIS

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Cancer metastasis is an ominous feature of tumor progression and accounts for over 90 % of cancer-associated deaths. It is a process in which tumor cells spread from the tumor of origin and colonize in the distant organs. In order to achieve a successful metastasis, tumor cells must first survive and then proliferate in the distant organ. Therefore, tumor cell survival is the rate-limiting step in cancer metastasis. Our lab has previously observed a significant infiltration of Gr-1+CD11b+ cells or myeloid derived suppressor cells (MDSCs) in lungs of mice bearing mammary adenocarcinomas before the arrival of tumor cells. These cells contribute to an inflammatory, proliferative, and immune suppressive pre-metastatic lung environment. To further understand the function of MDSCs, we co-cultured or co-injected MDSCs with tumor cells. MDSCs promote tumor cell survival in vitro/ex vivo as well as metastasis in vivo. Using a cytokine protein array screening, we found that CCL9 (MIP1-Y, macrophage inflammatory protein-1 gamma) is highly secreted in the supernatant as well as the metastatic lung. We then identified that MDSCs is the main cell type express CCL9. Interestingly, deletion of transforming growth factor-β receptor II (TβRII) in myeloid cells, including these MDSCs (Tgfbr2^{MyeKO}), results in significantly decreased lung metastasis and CCL9 expression. Importantly, CCR1, the only receptor of CCL9, is expressed by the tumor cells. We are currently aiming at addressing the function of CCL9/CCR1 chemokine/receptor axis in promoting tumor cell survival and the consequential metastasis.

A146

A MOUSE MODEL OF ACUTE EXACERBATIONS OF COPD LUNG INFLAMMATION WITH BOTH STEROID-SENSITIVE AND STEROID-INSENSITIVE FEATURES

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Exposure to tobacco smoke (TS) induces a robust steroid-resistant inflammation in mouse lung. Mice previously exposed on a daily basis to TS were challenged with the viral mimetic polyinosinic-polycytidylic acid (pIC). This caused an enhanced inflammation above that of TS or pIC alone. This exaggerated response was inhibited by oral dexamethasone (DEX). The time-course of inflammatory mediator increases and inflammatory cell influx in the bronchoalveolar lavage fluid (BALF) in response to pIC/TS-exposure was followed for 5 days post-challenge. Neutrophils were the main infiltrating cell type, peaking 24 h after final TS-exposure, followed by increases in macrophages and lymphocytes. The enhanced inflammation did not fully resolve over the 5 days. DEX (0.3 mg/kg) inhibited the TS/pIC-exaggerated cellular response. BALF levels of KC, MCP-1, IL-6, GM-CSF, IL-1β, TNFα and VEGF were significantly increased by TS/pIC at 24 h. This model has excellent translational potential with both steroid-insensitive baseline inflammation and steroid-sensitive exacerbated inflammation, features reflecting key aspects of the treatment of human COPD.

A147

CHARACTERIZATION OF PULMONARY FIBROSIS IN MICE FOLLOWING SYSTEMIC ADMINISTRATION OF BLEOMYCIN USING HISTOPATHOLOGICAL, FUNCTIONAL AND IMAGING ENDPOINTS: RELEVANCE TO HUMAN IPF

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Human idiopathic pulmonary fibrosis (hIPF) is a progressive disease marked by the development of a unique pattern of subpleural fibrotic tissue and decreases in lung function. Since the pattern of fibrosis may reflect mechanisms associated with the induction and progression of this disease it is important to recreate various aspects of this fibrotic lesion in mice for disease modeling and for screening potential therapeutic agents. In the current study, fibrotic lesions were induced in the lungs of mice by the systemic release of bleomycin (BM) from subcutaneous minipumps (MP) over a 7-day period. Mice treated with BM/MP exhibited a distinct subpleural fibrosis after 28 days that was distributed throughout numerous lobes of the lung. The parenchymal border of the fibrotic regions showed alveolar destruction (emphysema) marked by a characteristic honeycomb appearance that is observed in hIPF. Active inflammatory processes involving macrophages were also occurring simultaneously with fibrotic processes. Analysis of lung function revealed marked alterations in numerous endpoints including increased peripheral (not large) airway resistance, decreased compliance and total lung capacity. MicroCT image analysis of fixed lungs supported the histopathological findings and functional measurements and provided insight into compensatory structural changes occurring in the lung during the development of the fibrotic lesions. These findings were in contrast to the characteristics of the fibrotic lesions induced by BM administered intratracheally. The results of the current study indicate that BM/MP treatment of mice induces a pulmonary fibrosis that has many histopathological and functional characteristics similar to those that occur in hIPF. Further characterization of the BM/MP model of pulmonary fibrosis may increase our knowledge of the etiology of hIPF and reveal new targets for therapeutic intervention.

Van Arman Award Competition Abstracts

Van Arman Award Competition Abstracts

The Inflammation Research Association sponsors a competition for the encouragement of young scientists to perform exploratory and applied research in the general area of inflammation. Contestants must be candidates for advanced degrees: M.S., Ph.D., M.D., D.O., D.D.S., D.V.M., etc., or first-year post-doctoral fellows. Those who have won first place in a previous year are ineligible to compete again.

These awards are in recognition of the late C. Gordon Van Arman, who had a long and distinguished career as an industrial scientist, during which he published over 100 scientific papers. The development of the drugs diphenoxylate, disopyramide, sulindac, and diflunisal can be directly attributed to his work. In 1970, Dr. Van Arman with Edward Takesue, Marvin Rosenthale, and Mary Lee Graeme founded the Inflammation Research Association as an informal forum for bench scientists to exchange research ideas in inflammatory diseases. Through this award, the IRA wishes to develop a commitment to high quality inflammation research in young scientists.

Prior to the conference, the scholarship committee selects the five finalists based on submitted mini-papers. Finalists will attend the conference and participate in poster and oral presentations to the committee. Based on these presentations and the mini-papers, awards will be presented to the finalists.

VA01

ABSENCE OF THE γ_c CHAIN, A CRITICAL COMPONENT OF THE TYPE I IL-4 RECEPTOR, INCREASES THE SEVERITY OF ALLERGIC INFLAMMATION

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The T_H2 cytokines, IL-4 and IL-13, play critical roles in inducing allergic lung inflammation and also drive alternative activation of macrophages (AAM). Aside from controlling T_H2 differentiation, the contribution of IL-4 signaling via the Type I receptor in modulating effector responses in airway inflammation remains unclear. Here, we adoptively transferred wild-type OVA primed $CD4^+$ T cells into $Rag2^{-/-}$ and gamma c $(\gamma_c)^{-/-}$ mice. $\gamma_c^{-/-}$ mice developed increased peribronchial and perivascular inflammation and eosinophilia upon OVA challenge, compared to $Rag2^{-/-}$ mice. Characteristic AAM proteins FIZZ1 and YM1 were expressed by lung epithelial cells in both mouse strains. Absence of γ_c in macrophages however, caused reduced YM1 expression. There was dysregulated T cell and dendritic cell (DC) activation in the γ_c-deficient environment; greater percentages of $CD4^+$ T cells expressed T_H2 cytokines and IFNγ, while DCs expressed less CD11b and more OX40L. This suggests that the Type I R partially regulates AAM protein expression. In absence of the Type I IL-4R, the Type II R can mediate allergic responses.

VA02

ABNORMAL LIPOPROTEIN PARTICLES AND CHOLESTEROL EFFLUX CAPACITY IN PATIENTS WITH PSORIASIS, AN INFLAMMATORY SKIN DISEASE

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Psoriasis is an inflammatory skin disease associated with increased risk of cardiovascular disease (CVD). Systemic inflammation may modulate lipoprotein particle number and impair HDL function. We sought to study how chronic in vivo inflammation modulates these parameters in psoriasis. We enrolled consecutive samples of psoriasis patients ($n = 122$) and asymptomatic age- and gender-matched controls ($n = 134$). Fasting lipids were measured and HDL-efflux capacity quantified with a validated ex vivo assay; lipoprotein concentration and size were measured with NMR. Though patients with psoriasis had lower traditional lipid levels, they demonstrated significantly reduced normalized HDL efflux capacity (0.83 vs. 0.98, $p = 0.008$) and a more atherogenic lipoprotein profile (increased LDL particle number and decreased LDL particle size). These associations persisted after adjusting for traditional lipid levels and BMI. The abnormal particle composition and HDL function in psoriasis may provide a link between this inflammatory condition and CVD.

VA03

NEONATAL THYMECTOMY PROLONGS THE PERMEABILITY OF ENTERIC ANTIGENS AND PROMOTES THE STRONG ACTIVATION OF PERIPHERAL CD4 T CELLS

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Thymectomy (Tx) during the neonatal period (d2–4) leads to the development of a spectrum of organ-specific autoimmune diseases in several strains of adult mice. The etiology of this intriguing phenomenon is yet to be fully elucidated, although it is widely believed to be a consequence of two controversial mechanisms: (1) production of pathogenic $CD4^+$ T cells from neonatal-specific defect in central tolerance, and (2) abolition in the production of regulatory T cells (Tregs), which are believed to develop later in ontogeny than typical T cells. We hereby suggest an additional mechanism that may contribute to the onset of autoimmunity, perhaps by instigating the chronic activation of autoreactive T cells. We found that neonatal mice display a defect in their ability to induce efficient tolerance to enteric antigens during the first several days of their life, and neonatal thymectomy significantly prolongs the period of this defect. Hence, while enteric antigens induce strong activation of peripheral $CD4^+$ T cells only during the first several days in euthymic neonatal mice, such responses continue for several weeks in d3 thymectomized mice. The unregulated responses to enteric antigens in return may lead to inadvertent activation of autoreactive T cells.

VA04

INFECTION-DEPENDENT VLA-3 INTEGRIN UPREGULATION REVEALS TWO NEUTROPHIL SUBPOPULATIONS IN SEPSIS FOR MICE AND HUMANS

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To study the expression kinetics of various beta1 integrin heterodimers on neutrophils during inflammation, we employed two animal models of septic peritonitis, as well as evaluated human ICU samples. We report that only integrin alpha3 beta1 (VLA-3) levels became significantly enhanced. Interestingly, the surface expression of VLA-3 on neutrophils from patients with severe sepsis, but not non-infectious SIRS, was significantly elevated. This suggests that VLA-3 could be used as a novel neutrophil marker to discriminate sepsis from SIRS. In fact, VLA-3 expression was elevated within 24–36 h of diagnosis, and the levels returned to normal upon recovery. Moreover, two populations of neutrophils could be discerned, based on their relative VLA-3 expression, in human septic patients and sepsis mouse models (cecal ligation and puncture surgery, and endotoxemia). Compared with the VLA-3^{low} neutrophils, VLA-3^{high} cells from septic animals displayed a hyper-inflammatory phenotype, including relatively greater production of pro-inflammatory cytokines and increased myeloperoxidase (MPO) activity. VLA-3^{low} cells, on the other hand, were the major producers of IL-10.

VA05

DNA DIRECTS NUCLEOSOME REDISTRIBUTION IN THE RESPONSE TO KSHV

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In the eukaryotic nucleus, DNA is organized with histone proteins forming the nucleosome: the fundamental subunit of chromatin. The distribution of nucleosomes is controlled by a combination of chromatin regulatory complexes and features intrinsic to DNA sequence, which can affect genome response. Upon infection, immune activation brings about well-characterized response and chromatin structural changes have been documented at a handful of loci. Kaposi's sarcoma-associated herpesvirus (KSHV), an oncogenic virus, provides an excellent model system for studying this response. We measured nucleosome distribution at the transcription start site (TSS) of 472 immunity-related genes. KSHV reactivation caused changes in nucleosome distribution at the TSS of 233 loci with time-dependent kinetics. DNA sequence directed this nucleosome redistribution in a majority of loci. The results indicate that certain loci, essential to the response of KSHV reactivation, are held in a chromatin state that is unfavorable to activation. We propose that a genetically directed "spring-loaded" repositioning of nucleosomes has evolved on these promoters to facilitate the concerted immune response.

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