



Book of Abstracts

FIFTEENTH INTERNATIONAL CONFERENCE

Sunday, September 21 – Wednesday, September 24, 2008
Westfields Marriott, Chantilly, VA, USA

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sion of scientific and technological advances that can be used to develop new therapeutic agents for the wide diversity of serious diseases with inflammatory processes.

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Contents

Inflammation Research

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IX Welcome

X Officers and Board Members

XI Conference Organizers

XII Keynote Speaker

Symposia Abstracts			
SA01	Intestinal immunomodulation by the emerging B7-like butyrophilin family	S77	SA14 The protein C system resides at the crossroads of hemostasis and inflammation S84
SA02	The role of antigen presenting cell subsets in regulating the balance between immunity and tolerance in the intestine	S77	SA15 Biological therapeutics, T effector and T regulatory cells in autoimmune disease S84
SA03	The Th17 lineage: development, function and regulation	S78	SA16 Characterization and optimization of monoclonal antibodies for the treatment of inflammatory diseases - case studies S85
SA04	Experimental breakthrough therapies in IBD	S78	SA17 Biological therapeutic approach to atherosclerosis: challenges and opportunities, two case studies in experimental disease S85
SA05	Combinatorial genomics leads to rapid discovery of integrated pathways for targeted therapies in IBD	S78	SA18 Dissecting the IL-17 receptor: Signaling and Function S86
SA06	What do T regulatory cells (Treg) see and do?	S80	SA19 The identification of a human IL-17F/IL-17A heterodimer and the receptor complex utilized in signaling S86
SA07	Reshaping the immune system to induce tolerance in autoimmunity using gene therapy approaches	S80	SA20 The IL-23/IL-17 axis in arthritis S86
SA08	An antigen-specific tolerance approach for therapy of mouse models of MS and type 1 diabetes	S81	SA21 Stat transcription factor signaling in mouse and human Th17 polarization S87
SA09	Tepizumab, a humanized, Fc Receptor (FcR) non-binding anti-CD3 monoclonal antibody in recent-onset type 1 diabetes (T1DM)	S81	SA22 Inhibition of IL-4R α : a promising therapeutic strategy for asthma S88
SA10	The surprising complexity of pain testing in the laboratory mouse	S82	SA23 Potential for bronchial thermoplasty to reverse airway remodeling associated with asthma S88
SA11	Role of ASICs in muscle and joint hyperalgesia associated with inflammation	S82	SA24 BIO-11006: targeting the MARCKS protein in COPD S89
SA12	Pronociceptive actions of CCL2 and CXCL12 after peripheral nerve injury	S83	SA25 Anti-IL-12/IL-23 treatment for Crohn's disease and psoriasis S89
SA13	Both EP4 antagonists and mPGES-1 inhibitors relieve pain in rodent models of arthritis	S83	SA26 Baminercept: targeting LT- β and LIGHT pathway in RA S89

Mini-symposia and Poster Session Abstracts					
			A116	Chronic inflammation in asthma airway remodeling	S98
A100	Nociceptin antagonists for the treatment of inflammatory bowel disease	S93	A117	Effects of recombinant human growth hormone on <i>Staphylococcus aureus</i> sepsis in mouse	S98
A101	Design, synthesis and biological evaluation of azole derivatives of diphenyl acetic acid as anti-inflammatory agents	S93	A118	Suppression of C - reactive protein and lipoprotein levels in arthritic rats by novel glucosamine-analog GN1	S98
A102	Inflammatory changes in schizophrenia with antipsychotic treatment	S93	A119	Inhibitory effect of EGCG on acute pancreatitis	S99
A103	Pharmacological characterization of a new model of cynomolgus skin inflammation measured with novel methodologies	S93	A120	Inhibitory effect of inflammatory mediator by <i>Orostachys japonicus</i> in mouse peritoneal macrophages	S99
A104	Biacore analysis reveals distinct receptor ($\alpha 1$ and $\alpha 2$) binding inhibition characteristics of anti IL-13 antibodies	S94	A121	4-Aryl-5-heteroaryl-2-thio-substituted imidazoles: Approach to p38 MAP kinase inhibitor prodrugs	S99
A105	Micro-computed tomography (μ CT) is a powerful imaging technique for evaluating bone pathology in arthritis models	S94	A122	IL-1 drives pathogenic Th17 cells during spontaneous arthritis in IL-1Ra-deficient mice	S100
A106	Soft, topical immunosuppressants derived from cyclosporin A and FK-506 for the treatment of atopic dermatitis	S94	A123	IL-17 synergy with TNF causes striking cartilage erosion <i>in vivo</i>	S100
A107	SD0006; a potent, selective, and orally-available p38 kinase inhibitor	S95	A124	Formylpeptide receptor-like-1 mediates amyloid β -induced inflammatory response in Alzheimer's disease	S100
A108	A role for cathepsin K inhibitors in rheumatoid arthritis: VEL-0230 Phase I clinical trial results	S95	A125	Optimization of a quinolone class of dissociated glucocorticoid mimetics	S101
A109	Genomic-based high throughput screening identifies small molecules that differentially inhibit the antiviral and immunomodulatory effects of IFN- α	S95	A126	Inflammatory signaling processes and cardiovascular complication	S101
A110	The induction of nuclear factor κ B in laryngeal cancer cells by human papillomavirus-16 oncoprotein E7 is associated with increased inflammatory cytokines	S96	A127	The golgi-associated protein p115 mediates the secretion of macrophage migration inhibitory factor (MIF)	S101
A111	Comparative effects of an anti-inflammatory blend on reducing skin irritation caused by UVB or a chemical irritant to 1% hydrocortisone	S96	A128	GABA(A)-mediated alteration of autoimmune-mediated inflammation using the natural plant product, honokiol	S101
A112	Imaging an inflammatory response using ^{19}F MRI	S96	A129	Human apolipoprotein C1 transgenic mice: a unique novel model of atopic dermatitis	S102
A113	A novel mouse model of <i>Mycobacterium tuberculosis</i> -induced granuloma necrosis: Implications for adjuvant immunotherapy targeting TH2 cytokines in tuberculosis	S97	A130	Modulation of inflammation in two humanized mouse models of psoriasis	S102
A114	p38 MAPK inhibitor for the treatment of rheumatoid arthritis	S97	A131	Efficacy of probiotics in TNBS-induced colitis	S102
A115	Oral lactoferrin and glycine display <i>in vivo</i> synergistic anti-inflammatory activity	S97	A132	Predicting efficacy and side effects of the p38MAP kinase inhibitor class using BioMAP [®] primary human cell-based systems	S102
			A133	Histamine is not released in acute thermal injury in human skin <i>in vivo</i> : A microdialysis study	S103

A134	Effects of a selective, potent p38 inhibitor in <i>in vivo</i> models of rheumatoid arthritis	S103	A150	Evaluation of novel topical drug delivery systems of colchicine in mono sodium urate (MSU) model of gout in rats	S108
A135	A global gene expression profiling analysis to study the role of endothelial CD40 during inflammation	S103	A151	Gadolinium decreases inflammation related to myocardial ischemia and reperfusion injury	S109
A136	Dipeptidyl peptidase I mediates cigarette smoke-induced pulmonary inflammation and alveolar destruction	S104	A152	The discovery of a series of novel small molecule macrocyclic TNF- α antagonists	S109
A137	The involvement of nitric oxide in the peripheral antinociceptive effects of opioids during chronic inflammatory pain	S104	A153	Identification of functional roles for both IL-17RB and IL-17RA in mediating IL-25 induced activities	S109
A138	<i>Campylobacter jejuni</i> -induced activation of murine dendritic cells involves cooperative signaling through MyD88 and TRIF	S104	A154	The anti-obesity effect of Gyeongshinhae Gihwan T2 is associated with a decreased nitric oxide synthesis	S110
A139	AN2898: a novel anti-inflammatory compound that inhibits phosphodiesterase 4 and 7 enzyme activity and IL-12 and IL-23 release	S105	A155	Effective mechanism of herb complex prescription 'Anssichegamsan' in obesity	S110
A140	Discovery of 3-{3-(2-piperidinylethoxy)phenyl}-5-(1 <i>H</i> -1,2,4-triazol-3-yl)-1 <i>H</i> -indazole (CC-401), a potent JNK inhibitor	S105	A156	Exploration of the MAP3K TAK1 as a target for modulating inflammatory arthritis	S110
A141	Resolvin E1 (RvE1) inhibits inflammation in acute and chronic murine models of colitis	S105	A157	Treatment with anti-CD30 ligand prevents disease progression in murine systemic lupus erythematosus	S111
A142	The poly-anionic sugar sucrose octa-sulphate is an oral anti-rheumatic and anti-erosive metabolite of sucralfate	S105	A158	Elimination of Tolmetin Ulcers by Anisodamine (ANSA)	S111
A143	Preconditioning lymphocytes with p38 MAPK inhibitors, and not accessory cells, prevents Con-A-induced lymphocyte responses	S106	A159	Small molecule CXCR2 antagonist prevents hyperoxia-induced neutrophil accumulation in the lungs of newborn rats	S111
A144	Preventive role of ZH-67-2-1 in adjuvant-induced arthritis in rats	S106	A160	Synergistic induction of IL-10 by a TLR agonist and a phospho-ceramide analog is mediated by cAMP	S111
A145	Design of small molecule inhibitors for inflammatory bowel disease mechanism: inflammatory/immune mechanisms	S106	Van Arman Award Competition Abstracts		
A146	L-17 signaling induces sequential phosphorylation of C/EBP β	S107	VA01	Differential modulatory effects of TLR2 and TLR4 on T cell balance in experimental arthritis: possibilities for new therapeutic strategies	S114
A147	Modulatory role of 2-acetamidophenol on the expression of CD44 cell surface markers in the brain of Adjuvant Induced Arthritic model (AIA) of rats	S107	VA02	The role of CC chemokine receptor 7 during invasive aspergillosis	S114
A148	Protective effect of non-selective and selective COX-2-inhibitors in hypoxia stress-induced behavioral and biochemical alterations	S108	VA03	Pro-inflammatory cytokines inhibit chondrogenesis of human mesenchymal stem cells through NF- κ B dependent pathways	S114
A149	Rosiglitazone prevents hyperhomocysteinemia-induced myocardial remodeling through mast cell stabilization in rats	S108	VA04	Bz-423 improves GVHD-associated tissue damage and mortality by specifically targeting effector T cells	S115
			VA05	Mapping the binding of macrophage migration inhibitory factor (MIF) to the chemokine receptor CXCR4	S115
			Author Index		S117

WELCOME

September 21, 2008

Dear Colleague,

On behalf of the Inflammation Research Association Officers and the Board of Directors, as well as the Organizing Committee, we welcome you to the 15th International Conference of the Inflammation Research Association.

In addition to excellent meeting content, we have implemented several format changes, including choosing a new location conveniently located just 7 miles from Dulles International Airport for easy access and providing attendees a greater choice of topics through concurrent morning symposia. Of course, we will still stress the hallmark of all IRA Conferences, the ability for close scientific and social interactions in a relaxed, fun atmosphere.

The meeting kicks off with a symposium entitled **“Inflammatory Bowel Disease: From Basic Mechanisms to the Clinic”** scheduled from **3-6 PM on Sunday, September 21st**. The scientific program continues that evening with a Keynote Lecture from **Dr. Diane Mathis**, Professor of Medicine at Harvard Medical School, speaking about **“Regulation of Auto-Inflammatory Responses.”** Dr. Mathis is a preeminent authority on autoimmunity and T-cell biology as it relates to inflammatory diseases such as diabetes and rheumatoid arthritis.

Our Main Symposia on Monday the 22nd and Tuesday the 23rd feature invited speakers in the following areas:

- Tolerance Induction as a Therapeutic Option for Autoimmunity
- Inflammation and Pain
- Biologics as a Platform for Inflammation Therapies
- The IL-17/IL-23 Axis in Autoimmunity

Our final symposium on Wednesday the 24th in the morning features exciting clinical data on **“Novel Therapeutics.”**

In addition to the Main Symposia, we will hold four Mini-Symposia as well as two Poster Sessions as part of the program. All poster presenters are automatically entered into a competition for the poster with the ‘greatest therapeutic potential’ that is supported by a grant from GE Healthcare®. For scientists earlier in their careers we have: 1) the Van Arman Scholarship Competition, which allows five scientists to compete for cash awards while attending the meeting with all expenses paid, and 2) networking and breakfast events for investigators new to the inflammation arena, which will foster interaction with established IRA members and allow attendees to gain valuable advice on career development.

On behalf of the IRA Officers and Board of Directors, I look forward to welcoming you to the 15th International Conference at the elegant Westfields Marriott in Chantilly, Virginia, and to participating in an event that fosters both great science and social interactions.

William Selig, PhD
President
Inflammation Research Association

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Keynote Speaker



Diane Mathis, Ph.D.

Diane Mathis obtained a B.Sc. from Wake Forest University and a Ph.D. from the University of Rochester. She performed postdoctoral studies at the *Laboratoire de Génétique Moléculaire des Eucaryotes* in Strasbourg, France and at Stanford University Medical Center. She returned to France at the end of 1983, establishing a laboratory at the *Institut de Génétique et de Biologie Moléculaire et Cellulaire* in Strasbourg, in conjunction

with Dr. Christophe Benoist. The lab moved to the Joslin Diabetes Center at the end of 1999. Dr. Mathis is currently a Professor of Medicine at Brigham and Women's Hospital and Harvard Medical School, and is an Associate Research Director and Head of the Section on Immunology and Immunogenetics at Joslin, where she holds the William T. Young Chair in Diabetes Research. She is Director of the JDRF Center on Immunological Tolerance in Type-1 Diabetes at HMS, a Principle Faculty Member at the Harvard Stem Cell Institute and an Associate Faculty Member of The Broad Institute. Dr. Mathis was elected to the U.S. Academy of Sciences in 2003 and to the German Academy in 2007.

The lab works in the fields of T cell differentiation and autoimmunity, with a special emphasis on exploiting the most advanced transgenic and gene-targeting technology to engineer new mouse models. Studies on autoimmunity explore the immunological mechanisms of diabetes, rheumatoid arthritis and APECED, a polyglandular autoimmune disease. Major questions tackled are what initiates these diseases, how is their progression regulated, and what are the final effector mechanisms. In addition, modern genetic and genomic approaches are used to identify disease-modifying genes in both human patients and mouse models, and the application of computational and bioinformatic strategies to these and other issues is explored. Whole-animal imaging of inflammation and its tissular effects in diabetes and arthritis models is pursued as part of a long-standing collaborative program.

The Mathis/Benoist laboratory has produced over 250 publications and trained over 100 students and postdoctoral fellows.

Symposia Abstracts

IBD from Basic Mechanisms to the Clinic

Co-chairpersons: **Jo Viney** (Amgen)
Stephan Targan (Cedars Sinai/UCLA)

In this session the audience will begin by learning about the newest family of costimulatory immune regulators recently demonstrated to play a role in intestinal homeostasis, as well as the emerging knowledge surrounding the function of innate immune receptors at mucosal surfaces. The speakers will also cover the latest information regarding the elicitation of effector cells and regulatory cells in the intestine, with particular emphasis on the uniqueness of intestinal antigen presenting cells and the major role that IL-17 plays in the gut. An overview of the

recent experimental therapeutic approaches that have been tried in the clinic will provide context for highlighting the latest breakthrough and potentially promising therapies for treating both Crohn's disease and ulcerative colitis. Finally, an update on the latest ground-breaking genetic studies in IBD will provide the foundation for how genetic information can be transformed into understanding more about the complex mechanistic basis of disease when integrated pathways are studied together, with an emphasis on how this type of approach can reveal novel therapeutic approaches for intervention in the disease process.

SA01

INTESTINAL IMMUNOMODULATION BY THE EMERGING B7-LIKE BUTYROPHILIN FAMILY

*R.M. Swanson, H.A. Arnett and J.L. Viney**

*Inflammation Research, Amgen,
Seattle, WA and Thousand Oaks, CA*

Activation of T cells is known to be modulated by positive or negative co-signaling molecules. The B7-family of costimulatory molecules has received the greatest attention so far, and intervention in B7-family signaling pathways has proven to be an efficacious strategy for treating autoimmune diseases in the clinic. Recently, a new family of molecules has garnered interest – the butyrophilins – and early data is suggesting that these butyrophilin and butyrophilin-like molecules have the potential for influencing the nature of immune and inflammatory responses. In vitro studies have revealed that butyrophilins, such as butyrophilin-like 2 (BTNL2), can negatively regulate T cell proliferation and cytokine production. Alterations in intestinal BTNL2 expression have been reported in mouse models of IBD as well. And, genetic studies have described polymorphisms in BTNL2 which are reported to be associated with disorders such as sarcoidosis, myositis and ulcerative colitis. The potential for interdicting in the butyrophilin pathways highlights possible opportunities for developing new therapeutic strategies for treating autoimmune and inflammatory disorders. This presentation will focus on the emerging information of this new class of regulatory molecules.

SA02

THE ROLE OF ANTIGEN PRESENTING CELL SUBSETS IN REGULATING THE BALANCE BETWEEN IMMUNITY AND TOLERANCE IN THE INTESTINE

Bali Pulendran, Rosa Maria Salazar, Rajesh Nair,
Timothy L. Denning*

*Emory Vaccine Center, Emory University,
954 Gatewood Road, Atlanta, GA 30329, USA*

The intestinal immune system must elicit robust immunity against pathogens, while restraining reactivity to commensals and dietary antigens. The mechanisms that mediate this dichotomy are poorly understood. Our recent work has demonstrated a population of CD11b⁺F4/80⁺CD11c⁻ macrophages in the intestinal lamina propria, which express several anti-inflammatory molecules including IL-10, but little or no pro-inflammatory cytokines, even upon stimulation with TLR ligands. Furthermore, intestinal macrophages induce FoxP3⁺ regulatory T cells, via a mechanism dependent on IL-10, and retinoic acid and exogenous TGF- β . In contrast, adjacent CD11b⁺ dendritic cells stimulate Th17 responses, which are suppressed by intestinal macrophages. Thus, lamina propria macrophages and DCs differentially induce T regulatory and Th17 cells, and the dynamic interplay between these subsets, likely plays a critical role in balancing immune responsiveness versus tolerance. Our subsequent analysis is beginning to reveal a rich diversity of macrophage and dendritic cell subsets, which seem to be functionally specialized to perform

distinct function. Importantly, the representation of the various subsets of cells appear to be strikingly different in the large versus small intestine, and the endogenous microflora appear to play a major role in influencing this representation and function of the antigen presenting cells.

SA03

THE TH17 LINEAGE: DEVELOPMENT, FUNCTION AND REGULATION

Casey T. Weaver*

Department of Pathology, University of Alabama, BBRB 870, 845 19th Street South, Birmingham, AL 35294-2170

Naïve T cells differentiate into effector T cells with enhanced functional potential for orchestrating pathogen clearance largely under the guidance of cytokines produced by cells of the innate immune system that have been activated by recognition of those pathogens. This “secondary” education of post-thymic T cells provides a mechanism for appropriately matching adaptive immunity to cues of the innate immune system. Recently, factors that specify the differentiation of a new effector T cell subset – Th17 – have now been identified, providing a new arm of adaptive immunity and presenting a unifying model that can explain many heretofore confusing aspects of immune regulation, immune pathogenesis and host defense. An update on Th17 development and function, and consideration of the close links of Th17 development to regulatory cell development of this pathway will be presented.

SA04

EXPERIMENTAL BREAKTHROUGH THERAPIES IN IBD

Maria T. Abreu*

University of Miami, Miller School of Medicine Miami, Florida 33101

Understanding the pathogenesis of inflammatory bowel disease (IBD) has formed the basis of therapy for these complex disorders. Immunologically IBD is characterized by a defective innate immune response and an inappropriate adaptive immune response to luminal bacterial antigens. Data also suggest a component of defective barrier function. Therapies such as trophic growth factors directed at barrier function have thus far not been effective. At present, monoclonal antibodies are used to target cytokines important in perpetuation of chronic inflammation. Anti-TNF Abs are effective in the treatment of both ulcerative colitis and Crohn’s disease. Two studies have shown efficacy of an anti-p40 Ab that antagonizes both IL-12 and IL-23. Given the emerging role of IL-17 in IBD pathogenesis, this approach is likely to be effective. Lymphocytes are recruited to the intestine through the action of chemokines. Small molecule

antagonists of CCR9, the receptor for CCL25 (TECK), have some benefit in CD. Antibodies against alpha-4 integrin are effective in UC and CD. Concern with all of these approaches relates to the systemic effects of therapy, in particular, infectious complications. Finally, mesenchymal stem cells are in clinical trials for CD and may offer an alternative approach. The biggest advances will come when we can personalize therapy based on immunologic/genetic profiling.

SA05

COMBINATORIAL GENOMICS LEADS TO RAPID DISCOVERY OF INTEGRATED PATHWAYS FOR TARGETED THERAPIES IN IBD

Stephan Targan*

Cedars-Sinai Division of Gastroenterology, Inflammatory Bowel Disease Center, and Immunobiology Institute, UCLA School of Medicine, Los Angeles CA

In recent years, the scientific community has benefited from an explosion of technological advances in available methods to study the genetic variations that exist in common diseases. For the last two years there have been multiple genome wide association studies (GWAS) performed in many common diseases. These have been able to pinpoint specific biologic processes in specific genes and genetic variants that may well play a role in these diseases. A need remains for researchers to conduct these studies on extremely large collections of patients in a given disease entity. Studies of Crohn’s disease, one of the two major disease entities which comprise inflammatory bowel disease (IBD) have led the way in this new research approach. Very recently, a meta analysis of several genome wide association studies have yielded 31 confirmed genes associated with Crohn’s disease. It is well established, as evidenced by the many genetically manipulated animal models of intestinal inflammation, that multiple types of inflammation can be generated by this means and suggests functional variants in many different genes. Most of these animals generate a dysregulation at the mucosal surface leading to spontaneous and/or enhanced mucosal inflammation upon perturbation of inflammation. It is clear that the abnormal interactions between commensal bacterial products and antigens and the genetically susceptible host is what leads to different forms of IBD. The major question arising from these findings is which of these genetics alterations in animals are actually relevant to human. An approach for determining human correlates for results generated by animal based research is to separate the disease process into physiologic subtypes. We know that the mucosal dysregulation in human Crohn’s disease can be defined by specific immune serologic responses to particular bacterial antigens and that these responses can be used to sub classify this disease into more homogeneous groups ranging from very benign to very severe disease that leads to complications. Very recently, it was determined that these serologic responses individually and in combination appear to be representative of mucosal genetic variants leading to different mechanisms of mucosal

inflammatory dysregulation. This advance not only allows targeted discovery of pathways involved within a definable patient population but allows further analysis into

pathogenic interactions, thus, leading to coordinated discovery of relevant targets in a given population and for potentially accelerating novel drug development.

Tolerance Induction as a Therapeutic Option for Autoimmunity

Co-chairpersons: **Steve Nadler** (Bristol-Myers Squibb)
John Iacomini (Harvard)

The induction of tolerance to autoantigens is the “holy grail” for the treatment of autoimmune disease. Many therapeutic modalities for tolerance induction have been studied over the years, both preclinically and in the clinic. This session will address the basic science and some of the more recent approaches towards the induction of tolerance including antigen specific tolerization strategies, effector T-cell targeted therapies and utilization of T regulatory cells.

SA06

WHAT DO T REGULATORY CELLS (TREG) SEE AND DO?

*Ethan M. Shevach**

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Treg can be identified by the expression of the transcription factor, Foxp3, and can be generated both in the thymus and in peripheral lymphoid tissues. TGF β in concert with IL-2 is a potent induction factor for the differentiation of Foxp3⁺ Treg from naïve precursors. Polyclonal TGF β -induced Tregs (iTreg) are capable of preventing the autoimmune syndrome that develops in scurfy mice that lack Foxp3⁺ Treg. Antigen-specific iTreg can be used to both prevent and treat autoimmune gastritis that is induced by transfer of naïve or primed autoantigen-specific T cells. The antigen-specific iTreg inhibit the expansion of the effector T cells by disabling the antigen presenting function of dendritic cells in the lymph node draining the target organ. TGF β complexed with latency-associated peptide is expressed on the surface of activated thymus-derived Treg. Coculture of activated Treg with naïve responder T cells results in the de novo generation of fully functional Foxp3⁺ T cells in a contact-dependent, and TGF β -dependent manner. Generation of functional Foxp3⁺ T cells via this pathway may represent a mechanism by which Treg maintain tolerance and expand their repertoire.

SA07

RESHAPING THE IMMUNE SYSTEM TO INDUCE TOLERANCE IN AUTOIMMUNITY USING GENE THERAPY APPROACHES

*John Iacomini**

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The principal determining genetic factor in autoimmune type I diabetes (T1D) is the inheritance of MHC class II genes associated with susceptibility to autoimmune disease. We hypothesized that it may be possible to specifically address the problem of inheritance of at-risk MHC alleles in T1D by providing genetically predisposed individuals with MHC genes associated with protection from disease using a gene therapy based approach. We have shown that reconstitution of diabetes prone non-obese diabetic (NOD) mice with autologous bone marrow transduced with retroviruses carrying genes encoding MHC class II molecules associated with protection from T1D, resulting in a state of molecular chimerism, provided robust protection from the development of diabetes. Induction of molecular chimerism was also able to prevent the recurrence of T1D following islet transplantation in NOD mice with pre-existing diabetes. These data suggest that gene therapy based approaches can be used to overcome the underlying problem of autoimmunity in T1D. These results as well as the development of additional gene therapy based approaches to prevent or cure T1D will be discussed.

SA08**AN ANTIGEN-SPECIFIC TOLERANCE APPROACH FOR THERAPY OF MOUSE MODELS OF MS AND TYPE 1 DIABETES**

S. D. Miller*, D. M. Turley, C. Smith, A. Martin, E. Feeney, S. Prasad, D. McCarthy, A. Kohm and X. Luo

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Chronic progression of relapsing experimental autoimmune encephalomyelitis (R-EAE) in the SJL mouse (a model of MS), and spontaneous diabetes in the NOD mouse (a model of type 1 diabetes) are dependent on the activation of T cells to endogenous tissue epitopes, *i.e.* epitope spreading. Provided the correct autoepitope(s) are targeted, disease progression in both models can be both prevented and treated by induction of antigen-specific tolerance using the intravenous injection of syngeneic autoepitope-pulsed splenic antigen presenting cells (Ag-SP) fixed with the chemical cross-linker, ethylene carbodiimide (ECDI). Our results indicate that the insulin B chain epitope (Ins B₉₋₂₃) is the initiating diabetogenic epitope in NOD mice as tolerance induced with either intact insulin (Ins-SP) or Ins B₉₋₂₃ (InsB₉₋₂₃-SP), but not with GAD65₅₀₉₋₅₂₈, GAD65₅₂₄₋₅₄₃, or IGRP₂₀₆₋₂₁₄ coupled splenocytes at 4-6 weeks of age inhibits diabetes development. In contrast, regulation of new onset diabetes in 20 week old NOD mice is only induced by tolerization with Ins-SP, not InsB₉₋₂₃-SP, suggesting spreading to a distinct insulin epitope. Ag-SP tolerance is due primarily to re-presentation of apoptotic Ag-SP by host splenic plasmacytoid dendritic cells which induce both PD-1-dependent clonal anergy and the activation antigen-specific Foxp3⁺ regulatory T cells (Tregs).

SA09**TEPLIZUMAB, A HUMANIZED, FC RECEPTOR (FCR) NON-BINDING ANTI-CD3 MONOCLONAL ANTIBODY IN RECENT-ONSET TYPE 1 DIABETES (T1DM)**

Ronald L. Wilder*

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Teplizumab, which is also called hOKT3γ1 (Ala-Ala) and MGA031, have the same binding specificity and avidity for CD3 epsilon as murine OKT[®]3, but the Fc component of the antibody does not bind FcR or activate complement. Humanization and the changes in the Fc component of teplizumab have resulted in profoundly different biological activity compared to the parent monoclonal antibody. It is 1000-10,000-fold less mitogenic, is only weakly activating and does not appear to engage costimulatory signals. Data available to date suggest that teplizumab inactivates/anergizes T effector cells and expands T regulatory cells. Its capacity to induce a cytokine-release syndrome is dramatically blunted compared to OKT[®]3. In addition, teplizumab does not produce long-term lymphopenia. It has potential applications in a wide spectrum of autoimmune diseases and transplant indications. Published data have indicated that teplizumab has the capacity to preserve and possibly improve pancreatic islet cell function in subjects with recent-onset T1DM. It is under evaluation in 3 trials for this indication, *i.e.*, two phase 2 trials (the Abate and Delay trials), and a phase 2/3 trial (the Protégé trial). These trials are actively enrolling.

Inflammation and Pain

Co-chairpersons: **Jane Connor** (Medimmune)
Jeff Mogil (McGill U.)

Pain (along with redness, heat and swelling) is one of the four hallmarks of inflammation. However, in spite of well-established animal models of inflammatory pain (such as carrageenan-induced hyperalgesia) providing confidence in rationale and currently marketed clinical therapies (such as NSAIDs and COX-2 inhibitors) in this area, there continues to be a significant unmet medical need for the treatment of pain driven by inflammation. This symposium will focus on more recent approaches to evaluating pain in animals as well as novel targets recently identified that possess the potential to treat those patients whose pain goes untreated in spite of current therapies.

SA10

THE SURPRISING COMPLEXITY OF PAIN TESTING IN THE LABORATORY MOUSE

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Researchers studying pain in animal models face a conundrum. Any number of assays are available with high face validity, but the mechanisms underlying pain processing in these assays are more and more understood to be of little relation to mechanisms underlying clinical pain. However, the production of more clinically relevant pain states in laboratory animals yields few scorable dependent measures. As a result, most pain researchers rely on the measurement of reflexive, evoked hyper-sensitivity responses after neuropathic or inflammatory injury, whereas clinical pain in humans features much spontaneous pain and an important cognitive and emotional overlay. Making matters worse, it has become very clear that pain can be modulated both quantitatively and qualitatively by factors such as genetic background, sex, arousal, social factors and laboratory environment. Such complexities have served to discourage measurement of pain in inflammatory models; this is unfortunate because the rather poor correlation between inflammation and pain in these states suggests that pain really does need to be studied separately. The challenges and promise

of pain testing in the laboratory mouse will be discussed, with a focus on inflammatory pain.

SA11

ROLE OF ASICs IN MUSCLE AND JOINT HYPERALGESIA ASSOCIATED WITH INFLAMMATION

K.A. Sluka, R.Y. Walder, L.A. Burnes, S.J. Kolker, and M. Ikeuchi*

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Acid Sensing Ion Channels (ASICs) are important for sensing decreases in pH and are found on nociceptive afferents. Using ASIC1 and ASIC3 knockout mice, we mechanical sensitivity of the muscle or joint (primary hyperalgesia) and of the paw (secondary hyperalgesia) before and after induction of inflammation with carrageenan. In ASIC3 knockout mice, withdrawal thresholds of the muscle and joint decreases similarly to wild-type mice after induction of inflammation. However in ASIC1 knockout mice, withdrawal threshold of the muscle did not decrease after induction of inflammation. In contrast, ASIC3 knockout mice did not show decreases in withdrawal thresholds of the paw; ASIC1 knockout mice still showed increased mechanical sensitivity similar to wild-type controls. A non-selective ASIC antagonist, A-317567, reverses both the primary and secondary hyperalgesia in wild-type mice. In ASIC3 knockout mice the primary hyperalgesia is also reversed by A-317567 and in ASIC1 knockout mice the secondary hyperalgesia is reversed by A-317567. Further there is an upregulation of ASIC3 in primary afferent fibers innervating joint, and in DRG retrogradely labeled from joint. This upregulation in DRG occurs in non-peptidergic neurons. Thus ASICs are upregulated by inflammation; ASIC3 appears to be important for development of secondary hyperalgesia; and ASIC1 appears to be important for development of primary hyperalgesia. Supported by National Institutes of Health AR053509.

SA12**PRONOCICEPTIVE ACTIONS OF CCL2 AND CXCL12 AFTER PERIPHERAL NERVE INJURY***Fletcher A. White**

Department of Cell Biology, Neurobiology & Anatomy, Anesthesiology Research Laboratory, Burn and Shock Trauma Institute, Loyola University-Chicago, Maywood, IL 60153

C-C and C-X-C chemokines and their receptors may play crucial roles in the pathophysiology of pain. Evidence of a pronociceptive contribution includes a delayed upregulation of monocyte chemoattractant protein-1 (MCP-1/CCL2) and its respective receptor, CCR2, in both injured and adjacent, non-injured sensory neurons. Activation of neuronal CCR2 by CCL2 elicits membrane depolarization, triggers action potential and sensitizes nociceptors via transactivation of transient receptor potential channels, TRPA1 and TRPV1. Peripheral nerve injury also produces a latent increase in both neuronal expression of the chemokine, stromal-derived factor-1 (SDF1/CXCL12) and functional signaling by its cognate receptor, CXCR4. Treatment of nerve injured rodents with a CCR2 receptor antagonist, ((R)-4-Acetyl-1-(4-chloro-2-fluorophenyl)-5-cyclohexyl-3-hydroxy-1,5-dihydro-2H-pyrrol-2-one, at post-injury day (PID) 14 or 28, but not PID 7, temporarily attenuates hypernociceptive pain behavior. The non-peptide CXCR4 receptor antagonist, AMD3100, produces similar reversal of hypernociception in the rodent at PID 28, but not PID 14. Taken together, chemokine receptor antagonists may be important therapeutic interventions for pain syndromes. Funded by NIH RO1NS049136, RO1NS043095; Falk Medical Research Trust.

SA13**BOTH EP4 ANTAGONISTS AND mPGES-1 INHIBITORS RELIEVE PAIN IN RODENT MODELS OF ARTHRITIS***Daigen Xu*, Bernard Côté, Yongxin Han, Yves Ducharme, Richard W. Friesen, Joseph Mancini, Denis Riendeau, Laurent Audoly, and Alex Therien.*

Merck Frosst Centre for Therapeutic Research, 16711 Transcanada Hwy, Kirkland, Qc, Canada, H9H 3L1

Traditional non-steroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase-2 (COX-2) inhibitors provide pain relief in arthritis by blocking the synthesis of prostaglandins (PGs). Recent evidence demonstrates that deletion of the EP4 receptor or microsomal PGE synthase 1 (mPGES-1) renders mice resistant to auto-immune arthritis, similarly to the deletion of COX-2, suggesting that PGE₂ is a major pro-inflammatory prostaglandin in arthritis and mPGES-1 or EP4 may be a useful target for the treatment of the disease. In support of the hypothesis, we demonstrated that a selective EP4 antagonist was as effective as a COX-2 inhibitor or an NSAID at mitigating adjuvant-induced arthritis in rats and relieving iodoacetate-induced osteoarthritic pain in guinea pigs. We also noted a similar analgesic efficacy by a selective mPGES-1 inhibitor in guinea pigs. Unlike NSAIDs, both the EP4 antagonist and the mPGES-1 inhibitor were well tolerated by the gastrointestinal tract, causing no mucosal erosions or leakage. Together, these findings suggest that pharmacological inhibition of PGE₂ synthesis or activity is a useful approach for treating inflammatory diseases.

Biologics as a Platform for Inflammation Therapies

Co-chairpersons: **John Beals** (Lilly)
Frank Castellino (Notre Dame)

Biological therapeutics are increasingly being used to treat diseases that are exacerbated by inflammation, e.g., rheumatoid arthritis (anti-TNF α and IL-1 antagonist therapies) and sepsis (activated Protein C). The goal of this session is to explore the hurdles associated with using biologics as inflammation therapies and solutions to address these challenges.

SA14

THE PROTEIN C SYSTEM RESIDES AT THE CROSSROADS OF HEMOSTASIS AND INFLAMMATION

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Abstract not available at time of printing, see Supplement.

SA15

BIOLOGICAL THERAPEUTICS, T EFFECTOR AND T REGULATORY CELLS IN AUTOIMMUNE DISEASE

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Immunogenicity elicited by protein therapeutics can cause serious side effects in humans. Tools designed by expert immunoinformaticians have enabled the prediction of immunogenicity and the prospective identification of subjects who may be at increased risk of developing adverse immune responses. These same techniques can also be used to elucidate the dynamic balance between T effector and T regulatory cells in the development and treatment of autoimmune diseases. We have used HLA typing in conjunction with EpiMatrix, an *in-silico* epitope-

mapping tool, to predetermine the immunogenicity of biological therapeutics. In a recent published case, we identified promiscuous epitopes ("EpiBars") in the C-terminal region of a recombinant fusion protein (FPX)(1). On administration of FPX in 76 healthy human subjects, 37% developed antibodies after a single injection; immune responses were limited to individuals who had the HLA that could present the immunogenic regions of the peptide. A memory T-cell response against the carboxy-terminus of the peptide was both predicted and observed, and also as predicted, HLA-haplotype DRB1*0701/1501 was associated with the highest T-cell and antibody response. For monoclonal antibodies, a close correlation between *silico* immunogenicity assessment and the confirmed immunogenicity of monoclonal antibodies in the clinic has also been documented(2). *In silico* tools such as Optimatrix can be used in combination with *in vitro/in vivo* approaches to diminish the T cell epitope content of monoclonal antibodies and protein therapeutics. In addition, exploitation of T regulatory cell suppression of immune response may be another means of suppressing T dependent antibodies to protein therapeutics. The latter approach, which has been the main focus of our work for the last 18 months, also holds some promise for the treatment of autoimmune diseases. This presentation will illustrate the use of these readily available tools to pre-determine immunogenicity. Techniques for salvaging immunogenic therapeutics will also be addressed.

1. Koren E, De Groot AS, et al. Clinical validation of the "in silico" prediction of immunogenicity of a human recombinant therapeutic protein. *Clin Immunol.* 2007 May 7; epub. doi:10.1016/j.clim.2007.03.544.
2. Hai S, McMurry JA, Knopf P, Martin W, De Groot AS. Immunogenicity screening using *in silico* methods: Correlation between T-cell epitope content and clinical immunogenicity of monoclonal antibodies. In *Therapeutic Antibodies: from Theory to Practice*. Zhiqiang An, Editor. John Wiley and Sons. (Scheduled for publication Fall 2008).

SA16

CHARACTERIZATION AND OPTIMIZATION OF MONOCLONAL ANTIBODIES FOR THE TREATMENT OF INFLAMMATORY DISEASES - CASE STUDIES

Jirong Lu*

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LY634923 is an interleukin-1 β (IL-1 β) antagonist antibody. Deamidation of Asn55 (-PGN β GNI-) in the CDR2 of the heavy chain was identified. This site is readily deamidated at neutral pH and presents challenges for the development of this antibody as a therapeutic agent. To better understand the impact of deamidation on antigen binding, site-directed mutagenesis was used to replace Asn55 and Gly56 to eliminate or slow-down deamidation. Ten analogs, together with the wild-type molecule, were produced and evaluated. Substitution of Asn55 eliminated chemical instability but resulted in a 3 to 200-fold decrease in binding affinity to IL-1 β that translated into lower neutralization activity *in vitro*. Both charge and size of the side chain at position 55 appeared to impact binding of the antibody to IL-1 β . The analog Asn55Asp, which mimics fully deamidated form, displayed a 20-fold reduction in activity. Mutation of Asn55 to Ser, Ala or Thr has the lowest impact on binding (~3-fold). In contrast to limited site-directed mutagenesis, when directed evolution *in vitro* was used to evaluate the impact of all possible amino acid substitutions at every position throughout the CDR, a number of antibody variants with improved stability and enhanced affinity were generated.

SA17

BIOLOGICAL THERAPEUTIC APPROACH TO ATHEROSCLEROSIS: CHALLENGES AND OPPORTUNITIES, TWO CASE STUDIES IN EXPERIMENTAL DISEASE

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Atherosclerosis is a chronic inflammatory state of various sites in large and medium size arteries. It is the pathology

that underlies much of the cardiovascular disease and morbidity found in a major portion of the first world populations. I will briefly review the pathogenesis of atherosclerosis as studied in mouse models of the disease. It is clear that it results from increases in plasma lipids and other risk factors and from local inflammatory reactions in the vessel wall itself. Much of the current preventative approach relies on agents that lower plasma cholesterol and LDL in particular coupled with attempts to increase HDL. Our research is directed at two major questions: how does the adaptive immune system influence atherosclerosis and how does HDL composition influence the anti-inflammatory role of this lipoprotein. In studying the immune system modulation of atherosclerosis we have noted in a number of cases that various vascular sites are differentially influenced by immune regulators. This poses a significant challenge in using immune modulation as a window to an approach to the prevention and treatment of the vascular disease.

We have recently found a close connection between the immune system and lipoprotein homeostasis. This is mediated by the co-stimulatory set of molecules (second signals in antigen stimulation) of the TNF superfamily and LIGHT and lymphotoxin in particular, expressed on the surface of T cells. These two membranes bound cytokines signal via the lymphotoxin beta receptor expressed on among other cells, the hepatocytes. Interdiction of this pathway with soluble synthetic receptor lowers plasma lipids. This is mediated by effects on the enzyme, hepatic lipase which may function either as an enzyme or as a clearance receptor ligand. We are studying the particular role of NKT cells in this regulatory network. The latter cells are enriched among the lymphocytes of the liver. This pathway and the potential of the soluble receptor as a therapeutic will be reviewed.

HDL is thought to be therapeutic. The major protein of HDL is apolipoprotein A-1 and overexpression of the latter results in atheroprotection. There has been great interest recently in the use of synthetic apolipoprotein A-1 mimetics to treat atherosclerosis. These agents have many potential actions in mediating cholesterol removal from the vessel wall macrophages and in their potential as antioxidants and anti-inflammatories.

I will review our studies on several variants of the mimetics as anti-oxidative, anti-inflammatory and anti-atherogenic agents in animals with the usual HDL and those lacking HDL. (This work is supported by grants from the National Heart, Lung and Blood Institutes of the NIH).

The IL-17/IL-23 Axis in Autoimmunity

Co-chairpersons: **Joel Tocker** (Amgen)
Sarah Gaffen (U. Pittsburgh)

The regulation of IL-17 production from a distinct and pathogenic population of T helper cells and the contribution of these cells and IL-17 in animal models of autoimmune disease and inflammation is a hot topic. Further the complexities in the regulation of IL-17 production, the IL-17R signaling complex, and the nature of the IL-17 ligand itself offer numerous approaches and opportunities to modulate IL-17 biologic activity.

SA18

DISSECTING THE IL-17 RECEPTOR: SIGNALING AND FUNCTION

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IL-17 is the hallmark cytokine of a newly-discovered inflammatory T cell subset termed "Th17." IL-17 has been shown to mediate disease pathology in a variety of autoimmune conditions including rheumatoid arthritis (RA). Not surprisingly, blockade of IL-17 or its receptor is effective in limiting disease in several animal models of RA, and trials in humans are now ongoing. The IL-17 receptor is the founding member of a new superfamily of cytokine receptors that bears little homology to other families of cytokine receptors. Therefore, few predictions regarding signaling mechanisms can be made simply on primary sequence. Our laboratory has established systems to define structure-function relationships in the IL-17 receptor complex in terms of receptor subunit assembly and downstream signal transduction. Here, I will discuss recent progress in defining the important structural and functional elements that dictate how this unique receptor system operates.

SA19

THE IDENTIFICATION OF A HUMAN IL-17F/IL-17A HETERODIMER AND THE RECEPTOR COMPLEX UTILIZED IN SIGNALING

*Jill Wright**

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IL-17A and IL-17F are members of the IL-17 pro-inflammatory cytokine family and have been linked to a variety of inflammatory and autoimmune conditions. IL-17A and IL-17F signal through a receptor complex that consists of IL-17RA and IL-17RC. The crystal structure of IL-17F shows that IL-17F forms a disulfide-linked dimer that contains a cysteine knot motif. Given the high degree of amino acid homology between IL-17A and IL-17F and the conservation of the four cysteines that form the knot, it is likely that IL-17A and IL-17F adopt a similar structure. We hypothesized that IL-17F and IL-17A could form a heterodimer due to their sequence homology and overlapping pattern of expression. In this symposium, I will present data that shows that activated human CD4+ T cells not only produce the IL-17A and IL-17F homodimers but also produce the IL-17F/IL-17A heterodimer that utilizes the same receptor complex as IL-17A and IL-17F homodimers.

SA20

THE IL-23/IL-17 AXIS IN ARTHRITIS

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Interleukin-17A (IL-17A) contributes to the pathogenesis of arthritis. Data from experimental arthritis indicate IL-17 receptor signaling as a critical pathway in turning an acute synovitis into a chronic destructive arthritis. The identification of six IL-17 family members (IL-17A-F) may extend the role of this novel cytokine family in the pathogenesis of chronic destructive joint inflammation. Whether the successful anti-IL-17A cytokine therapy in murine arthritis can be effectively translated to human

arthritis need to be tested in clinical trials in humans. Interestingly, IL-17A and IL-17F are secreted by the novel T helper subset named Th17. This novel pathogenic T cell population induces autoimmune inflammation in mice and is far more efficient at inducing Th1-mediated autoimmune inflammation in mice than classical Th1 cells (IFN- γ). IL-23 plays a critical role in Th17 survival and activity in experimental autoimmune models. Studies will be discussed to show the role of IL-23 in IL-17/IFN-gamma subgroup polarization in the autoimmune collagen-induced arthritis and its role in chronic non-autoimmune arthritis. In addition, modulation of Th17 polarization in experimental mouse models as well as in early rheumatoid arthritis patients will be presented.

SA21

STAT TRANSCRIPTION FACTOR SIGNALING IN MOUSE AND HUMAN TH17 POLARIZATION

*Arian Laurence**

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Studies in mice have shown that T helper (Th) cell lineages are critical in defending against classes of pathogens: Interferon gamma (IFN γ) producing Th1 cells for defense against viruses and interleukin-4 (IL-4) producing Th2 cells for defense against nematode worms. The novel IL-17 producing Th17 cells are critical for defense against bacterial infections, and mice that lack the ability to make IL-17 have difficulty in forming abscesses and overcoming bacterial pneumonia. Studies in Stat3 knockout mice have shown that this transcription factor is necessary for the formation of Th17 cells. Several groups have documented the key cytokines required for the induction of Th17 cells in both mice and humans yet there remains disagreement as to the correct cocktail in the latter. Despite the controversy, a common feature of all the human studies reported is that a Stat3 activating cytokine is required. Patients with Job syndrome, an inability to defend against bacterial infections, have mutations in their Stat3 gene and are a unique opportunity to study the role of Stat3 in human Th cell polarization. Job syndrome patients are unable to make Th17 cells. Further studies are required to determine how this contributes to the patho-physiology of the syndrome.

Novel Therapeutics

Co-chairpersons: **Bruce Tomczuk** (Chemnomics LLC)
Rey Panettieri (U. Pennsylvania)

This symposium will present novel therapies for Asthma, COPD, Crohn's Disease/Psoriasis, and Rheumatoid Arthritis. The Asthma presentations will focus on a pharmacological (IL-4 receptor antagonist, AMG-317, in Phase II) and a non-pharmacological (Bronchial Thermo-plasty) approach toward this complex disease. Due to the high unmet medical need, the presentation on COPD will provide an early stage opportunity for the symptomatic relief of mucus production. A late-stage clinical candidate, ABT-874, for Crohn's Disease (Phase II) and Psoriasis (Phase III) targets the inflammatory cytokines, IL-12 and IL-23 at their shared IL-12 receptor beta 1. A therapeutic for rheumatoid arthritis (Baminercept in Phase IIb) targets the lymphotoxin- β /LIGHT pathway for inhibition of lymphoid structures in inflamed joints. An additional talk will cover late-breaking clinical results.

SA22

INHIBITION OF IL-4R α : A PROMISING THERAPEUTIC STRATEGY FOR ASTHMA

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The cytokine interleukin-4 (IL-4) plays a key role in the development of type 2 helper T lymphocyte (Th2) immune responses. Th2 mediated immunity underlies allergic inflammation and protective immunity to extracellular organisms. Other cytokines associated with Th2 immune response include IL-13, which also signals through the IL-4 receptor alpha (IL-4R α) complex. Evidence from murine models implicates IL-4 and IL-13 in the induction and maintenance of asthma. The efficacy of IL-4R antagonism was demonstrated with a surrogate antibody in mouse models of allergic inflammation. In a cockroach allergen re-challenge model in mice, treatment with Mu317RaXMu reduced lung inflammation, airway hyperresponsiveness, total serum IgE, and accumulation of whole lung hydroxyproline. In cynomolgus monkeys, weekly administration of Cy317HuXCy was well tolerated in 4-, 13-, and 26-week repeated dose safety studies. AMG 317 is a fully human monoclonal antibody being

investigated in asthma based on its ability to inhibit IL-4 and IL-13 signaling through IL-4R α . In Phase 1 studies, no clinically relevant safety signal was evident following administration of single doses of AMG 317 up to 1000 mg. Proof of concept for IL-4R α blockade has been demonstrated in inhaled allergen challenge studies of Pitrakinra, a molecule that blocks IL-4 and IL-13 through IL-4R α . Results showed a reduction in the decrease in FEV₁ during the late phase response, and decreased FeNO post-treatment. AMG 317 is currently being evaluated in a Phase 2 dose ranging study in subjects with moderate to severe atopic asthma.

SA23

POTENTIAL FOR BRONCHIAL THERMOPLASTY TO REVERSE AIRWAY REMODELING ASSOCIATED WITH ASTHMA

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Chronic asthma is associated with remodeling of the bronchial wall and especially with increased mass and activity of smooth muscle that is found in airways. Medical therapies can address many of the key features of asthma, such as bronchoconstriction and airway inflammation, but none of the currently available therapies can reverse remodeling. We have explored a physical approach, using direct thermal treatment of the bronchi, to reduce the mass of airway smooth muscle and thus reduce the potential for bronchoconstriction. The Alair[®] system comprises a radiofrequency generator that delivers energy through a specially designed catheter that has at the distal end an expandable basket of 4 electrodes. When positioned at the time of bronchoscopy, the electrodes are expanded to contact the airway wall and a controlled bolus of energy is supplied over 10 seconds that achieves a target temperature in the airway wall. In a series of studies in canine airways, we found a "dose-response" effect of temperature on subsequent loss of airway muscle mass, and on reduced bronchoconstriction in response to directly administered methacholine. Furthermore, the reduction in methacholine-induced bronchoconstriction correlated with the degree to which

smooth muscle was diminished. These effects persisted out to three years.

In 3 clinical trials in patients with a range of asthma severity from mild to severe/steroid-dependent, we found that bronchial thermoplasty led to improvements in subjective and objective measures of asthma control including improvements in peak flow readings and reduced need for symptom-relieving medications. These benefits could be related to reduced activity of airway smooth muscle. The benefits of bronchial thermoplasty are most evident in those with the most severe asthma. While bronchial thermoplasty is associated with frequent short term increase in airway symptom, no long term adverse effects have been found.

SA24

BIO-11006: TARGETING THE MARCKS PROTEIN IN COPD

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Work from this laboratory and from others has shown that MARCKS protein (myristoylated alanine-rich C kinase substrate) has an integral role in two components of chronic bronchitis: inflammation and mucus secretion in the airways. A peptide related to the N-terminus of MARCKS (the MANS peptide) had dramatic ameliorating effects in both *in vitro* and *in vivo* models of mucin secretion and inflammation. Here, we report that a soluble analog of MANS, BIO-11006, produced by BioMarck, RTP, North Carolina, has similar effects in a variety of model systems that relate to asthma and chronic bronchitis. BIO-11006 attenuated, in a concentration-dependent manner, both mucin secretion and airway obstruction in mice sensitized to OVA and treated with MCH. While all doses ranging from 1-20 mM were found effective, 10 mM solution aerosolized for 30 min was found to be most effective. The effect of BIO-11006 was maximal immediately after the aerosol was administered and declined thereafter with t_{1/2} of 2-3 hrs. Statistically and biologically significant effects were seen as late as 4 hrs after pre-treatment. BIO-11006 also showed significant effects in two different mouse models of chronic bronchitis: it markedly inhibited ozone-induced neutrophil migration into the airways and also inhibited expression of cytokines such as IL-6, TNF α and KC (the murine equivalent of IL-8). In addition, it had significant inhibitory effects on mucin secretion in mice treated with human neutrophil elastase. Collectively, the results indicate that BIO-11006, a peptide related to

MARCKS protein, possesses potent anti-mucus and anti-inflammatory properties. BIO-11006 currently is in Phase 2 clinical trials in COPD patients.

SA25

ANTI-IL-12/IL-23 TREATMENT FOR CROHN'S DISEASE AND PSORIASIS

*Trudi Veldman**

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The anti-IL-12/23p40 antibody ABT-874 is a fully human recombinant antibody isolated using phage display technology. Abbott has completed several clinical studies with ABT-874 in Crohn's disease, multiple sclerosis and psoriasis. The clinical data from the Phase 2a Crohn's disease study and the 12-week Phase 2 psoriasis study will be presented. ABT-874 was significantly more efficacious than placebo in the treatment of moderate to severe plaque psoriasis, and appears to have a favorable safety profile. A potential mechanism that may explain the extended pharmacodynamic effect of drug treatment will be discussed.

SA26

BAMINERCEPT: TARGETING LT- β AND LIGHT PATHWAY IN RA

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Lymphotoxins (LT- α and LT- β), LIGHT [homologous to LT, inducible expression, competes with herpes simplex virus (HSV) glycoprotein D for HSV entry mediator (HVEM), a receptor expressed on T lymphocytes], and their specific receptors LT β R and HVEM, are members of the immediate family of the larger TNF superfamily. These cytokines establish a critical communication system required for the development of secondary lymphoid tissues. Such reactions are hypothesized to play an important role in autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus. This presentation will discuss the pre-clinical and emerging clinical evidence suggesting that targeting LT β R will modulate disease-related biology and provide clinical benefit in rheumatoid arthritis.

Mini-symposia and Poster Session Abstracts

A100**NOCICEPTIN ANTAGONISTS FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASE**

Carsten Alt, Ken Shew, Than Thuy Tran, Willma Polgar, Lawrence Toll, and Annalisa D'Andrea*

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Inflammatory bowel disease (IBD) is represented by a complex of heterogeneous gastrointestinal diseases that include two major entities, ulcerative colitis and Crohn's disease. Nociceptin is a neuropeptide that has been hypothesized to be a stimulator of inflammation, and the Nociceptin receptor is expressed at high levels in the gut. In this study we evaluated the prophylactic and therapeutic potential of Nociceptin antagonists in the mouse model of DSS-induced colitis. To this end we induced colitis by feeding C57BL/6 mice with 3.5% DSS solution in the drinking water for 5 subsequent days. Nociceptin antagonists were administered intraperitoneally starting at day 0 until sacrifice at day 12. Our results show that treatment with Nociceptin antagonists dramatically ameliorated the development of colitis as observed by changes in body weight, fecal consistency, and colon length. Additional studies are in progress to evaluate the mechanism of action of nociceptin antagonists during IBD.

Our results show that treatment of the animals with Nociceptin antagonists significantly ameliorates the DSS-induced colitis, suggesting that inhibition of Nociceptin might be an effective therapy for IBD.

A101**DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF AZOLE DERIVATIVES OF DIPHENYL ACETIC ACID AS ANTI-INFLAMMATORY AGENTS**

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Long-term use of arylalkanoic acids such as ibuprofen, diclofenac and flurbiprofen has been associated with gastrointestinal ulceration, bleeding and nephrotoxicity. To reduce their gastric toxicity a number of derivatives have been prepared and in some of these carboxylic group has been replaced by five membered heterocyclic moieties such as 1,3,4-oxadiazole/thiadiazole and 1,2,4-triazole. In our attempt to discover new and useful agents for treating inflammatory diseases, we have replaced the carboxylic acid group of diphenyl acetic acid with these heterocyclic moieties. The cyclized derivatives were screened by carrageenan induced rat paw edema method and showed 49.03% to 83.02% inhibition, where as standard drug ibuprofen showed 74.71% inhibition at the same oral dose. Compounds showing significant anti-

inflammatory activity were selected to study their analgesic, ulcerogenic and lipid peroxidation activities. The tested compounds showed reduction in ulcerogenic activity compared to ibuprofen. The compounds that showed less ulcerogenic effect also produced less malondialdehyde (MDA) content in gastric mucosa, which is one of the end products of lipid peroxidation.

A102**INFLAMMATORY CHANGES IN SCHIZOPHRENIA WITH ANTIPSYCHOTIC TREATMENT**

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Patients with schizophrenia (SCH) are at increased risk of developing metabolic disease. Furthermore, some atypical antipsychotics are associated with increased risk of weight gain and metabolic changes, increasing the risk for metabolic disease. Previous research indicated that both schizophrenia and atypical antipsychotic treatment modulated phospholipid concentrations and apparent production of arachidonic acid, a critical precursor for the inflammatory lipids. We used a targeted metabolomic approach to evaluate the inflammatory lipid and cytokine changes occurring in schizophrenia and with antipsychotic treatment. Plasma concentrations of specific prostaglandins (PG) and other inflammatory lipids were decreased in patients with schizophrenia compared to control subjects. Plasma concentrations of PGD₂ were decreased after treatment with antipsychotics. Adipokine concentrations, including adiponectin and PAI1 were different in controls vs. schizophrenia subjects and were further changed by antipsychotic treatment. Inflammation might be an important common process relating schizophrenia and antipsychotic treatment to the development of weight gain and metabolic disease.

A103**PHARMACOLOGICAL CHARACTERIZATION OF A NEW MODEL OF CYNOMOLGUS SKIN INFLAMMATION MEASURED WITH NOVEL METHODOLOGIES**

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The development of a novel delayed-type hypersensitivity (DTH) in Cynomolgus monkeys was used to assess the role of T-cells in skin inflammation. The inflammation was scored by traditional DTH methods, including immunohistochemistry. Further analysis of skin biopsies by gene expression provided a more thorough and efficient characterization of the response that correlated with the DTH response. The skin inflammation was blocked by Dexamethasone but not FK506, revealing a surprising

lack of effect of T cell blockade in the model. This data demonstrates a minimally invasive and rapid method to fully characterize skin inflammation in non-human primates that has the potential for translation to humans.

A104

BIACORE ANALYSIS REVEALS DISTINCT RECEPTOR ($\alpha 1$ AND $\alpha 2$) BINDING INHIBITION CHARACTERISTICS OF ANTI IL-13 ANTIBODIES

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Human IL13 is a glycoprotein, which plays an important role in inflammatory cytokine production. It functions by binding to its cell surface receptors IL13R $\alpha 1$ and IL13R $\alpha 2$. It has been shown that IL13 blockade by the soluble muIL13 R $\alpha 2$ inhibited the signs and symptoms of asthma in the mouse OVA-induced asthma model. Therefore, inhibition of IL13 signaling has been deemed a potential therapy for asthma. Several *in vitro* assays have been set-up to select rat anti mouse antibodies to block interaction of IL13 with both receptors. An Elisa-based assay has been useful to select rat antibodies inhibiting binding of muIL-13 to both receptors. However, due to technical difficulties ELISA could only be used in selecting antibodies inhibiting binding of muIL13R $\alpha 1$. Here, we describe a Biacore competition method to select rat antibody that inhibit binding to IL13R $\alpha 1$ and R $\alpha 2$. Use of this method has been valuable in selection and characterization of rat anti mouse antibodies for in vivo studies.

A105

MICRO-COMPUTED TOMOGRAPHY (μ CT) IS A POWERFUL IMAGING TECHNIQUE FOR EVALUATING BONE PATHOLOGY IN ARTHRITIS MODELS

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Adjuvant Arthritis (AA) and Collagen-induced arthritis (CIA) are well-established rodent models for rheumatoid arthritis (RA). The disease in these models is initiated either by an immunization of an antigen of mycobacterial origin (AA) or xenogenic type II collagen (CIA). These antigens induce a robust pro-inflammatory response, consequently animals develop progressive arthritis involving the front and hind limbs with maximal joint swelling most often observed in the affected metatarsal/metacarpal region. One of the hallmarks of AA and CIA models is the bone pathology due to an invasive inflammatory pannus tissue as well as osteoclast mediated

bone resorption. Monitoring these changes in bone are critical for assessing disease modifying potential of novel anti-rheumatic agents.

Micro-computed tomography (μ CT) offers a powerful imaging technique for evaluating the changes in bone in these preclinical models. The information captured by μ CT can be used to quantify bone volume loss, which is a measure of bone destruction. In addition, more subtle changes in the bone can be measured by an alternative μ CT generated parameter, bone roughness.

We have used the μ CT to characterize the changes in bone volume and roughness in the arthritic tarsals. Our data for adjuvant or mouse collagen-induced arthritis models showed a 40-50% reduction in bone volume with a two hundred percent increase in roughness index compared to normal age matched controls on days 19 and 21 post-immunization for adjuvant arthritis or day 42 post-immunization for mouse CIA. Also, a significant increase in bone roughness was observed in the rat CIA model twenty-one days following immunization with bovine collagen. Finally, we have used anti-TNF as a comparator to assess the impact of a disease modifying agent on bone erosion in our models.

A106

SOFT, TOPICAL IMMUNOSUPPRESSANTS DERIVED FROM CYCLOSPORIN A AND FK-506 FOR THE TREATMENT OF ATOPIC DERMATITIS

Laurence E. Burgess, Kevin W. Hunt, Stephen T. Schlachter, David A. Mareska, Robert D. Groneberg, David Chantry, Jed Pheneger, Suzy A. Brown, Ken Brameld, Patrice Lee and Kevin Koch*

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Topical immunosuppressants (pimecrolimus, tacrolimus) are efficacious therapeutics for the treatment of atopic dermatitis. When delivered topically, these calcineurin inhibitors offer advantages over topical steroids; however, these marketed drugs have received a black box warning because of a potential cancer risk that may be due to systemic exposure. Accordingly, we have designed and discovered a series of "soft" calcineurin inhibitors as potentially safer drugs. Soft drugs are engineered, via medicinal chemistry, to be effective upon local delivery but, upon systemic exposure, are rapidly metabolized to inactive species resulting in a significant enhancement in therapeutic index. Relying on olefin metathesis technology, CsA and FK-506 derivatives were prepared and tested in calcineurin enzyme and IL-2 dependent cell assays. Based on the resulting SAR and further profiling in hepatocyte and plasma stability studies, ARRY-003 was identified as a potent, soft derivative of FK-506. In a swine model of delayed-type hypersensitivity, ARRY-003 displayed excellent efficacy in controlling allergic inflammation, as measured by erythema and induration, when delivered topically.

A107**SD0006; A POTENT, SELECTIVE, AND ORALLY-AVAILABLE P38 KINASE INHIBITOR**

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Interest has been high in the pharmaceutical industry in p38 α kinase (p38 α) as a target for therapeutic drug intervention for diseases such as rheumatoid arthritis because of its role in the expression of and the signaling by pro-inflammatory proteins. Here, SD0006 (1-(4-(3-(4-chlorophenyl)-4-(pyrimidin-4-yl)-1H-pyrazol-5-yl) piperidin-1-yl)-2-hydroxyethanone) was prepared as an inhibitor of p38 α and evaluated both *in vitro* and *in vivo*. *In vitro*, SD0006 was selective for p38 α kinase over 50 other kinases screened, including the γ and δ p38 isoforms and with modest selectivity over the β isoform. Crystal structures with p38 α show binding at the ATP site with additional residue interactions outside the ATP pocket unique to p38 α that can confer advantages over other ATP competitive inhibitors. SD0006 suppressed lipopolysaccharide (LPS) stimulated expression of pro-inflammatory cytokines TNF α , IL-1 β , and IL-6 with potencies (IC₅₀) <200 nM, and with direct correlation to inhibition of p38 α activity both in cellular models and *in vivo*, including a clinical trial. SD0006 demonstrated good oral anti-inflammatory efficacy and gave dose-dependent inhibition or arthritis incidence in the mouse CIA model equivalent to anti-TNF α treatment.

A108**A ROLE FOR CATHEPSIN K INHIBITORS IN RHEUMATOID ARTHRITIS: VEL-0230 PHASE I CLINICAL TRIAL RESULTS**

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VEL-0230* is a potent small molecule inhibitor of cathepsin K with potential for disease-modifying effects in RA. A Phase Ia rising-dose safety and tolerance trial indicated both a rapid uptake of the compound (T_{max} = 45 min) and a rapid reduction of serum collagen type 1 cross-linked C-telopeptide (CTx), a marker of cathepsin K-mediated bone resorption. CTx reduction was equivalent following all doses (150-450 mg), suggesting delivery of levels of VEL-0230 that achieved or exceeded inhibition-saturating exposures at all doses. CTx was significantly reduced below that of placebo-treated subjects as early as 15 min post-dose and remained significantly below placebo control levels through 12 hrs post-dose. An ~80% reduction from pre-dose levels of CTx occurred 6 hr post-dose. These results demonstrate sustained inhibition of bone collagen turnover following single oral doses of VEL-0230. Plasma concentrations of VEL-0230 were dose-proportional and rapidly cleared, with a T_{1/2} of ~1 hr.

A single AE of grade 1 dizziness was recorded for one VEL-0230-dosed patient. Taken together with the recently reported modulation of autoimmune inflammation by this compound**, these results support further assessment of VEL-0230 for clinical efficacy in RA and other diseases involving accelerated bone turnover.

*NC-2300; (2S,3S)-3-[[[(1S)-3-methyl-1-[[2-methoxypropoxy]methyl]butyl]amino]carbonyl]oxorane-2-carboxylate, Na⁺ salt; NC-2300 **Asagiri et al (2008) Science 319, 624.

A109**GENOMIC-BASED HIGH THROUGHPUT SCREENING IDENTIFIES SMALL MOLECULES THAT DIFFERENTIALLY INHIBIT THE ANTIVIRAL AND IMMUNOMODULATORY EFFECTS OF IFN- α**

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Multiple lines of evidence suggest that inhibition of Type I Interferons, including IFN- α , may provide a therapeutic benefit for autoimmune diseases. Using a chemical genomics approach integrated with cellular and *in vivo* assays, we screened a small compound library to identify modulators of IFN- α biological effects. A genomic fingerprint was developed from both *ex vivo* patient genomic information and *in vitro* gene modulation from IFN- α cell-based stimulation. A high throughput genomic-based screen was then applied to prioritize 268 small molecule inhibitors targeting 41 different intracellular signaling pathways. Active compounds were further profiled for their ability to inhibit the activation and differentiation of human monocytes using disease-related stimuli. Inhibitors targeting NF- κ B or JAK/STAT signaling emerged as "dissociated inhibitors" since they did not modulate IFN- α anti-viral effects against HSV-1 but potently inhibited other immune-related functions. This work describes a novel strategy to identify small molecule inhibitors for the treatment of autoimmune disorders.

A110**THE INDUCTION OF NUCLEAR FACTOR κ B IN LARYNGEAL CANCER CELLS BY HUMAN PAPILOMAVIRUS-16 ONCOPROTEIN E7 IS ASSOCIATED WITH INCREASED INFLAMMATORY CYTOKINES**

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Laryngeal cancers are frequently infected by high risk HPV-16 and HPV-18. E7 is an oncoprotein of HPV16 and HPV18. Human epithelial cells that express E7 exhibit a variety of growth control defects. The relationship between E7 and Nuclear Factor-kappaB (NF- κ B) in laryngeal cancer is unclear. In this study we have employed 2 human laryngeal cancer cell lines (UMSCC 12 and UMSCC11) to explore how E7 interacts with NF-kappaB. We have shown that E7 induced increased levels of NF- κ B subunit p65 in the nucleus of laryngeal cancer cells. Since NF- κ B is a well known survival factor, this finding indicates that activation of NF- κ B may contribute to cell growth and proliferation of HPV-16-infected laryngeal cancer cells. Using immunohistochemical staining method, we also showed that p65 was increased in human laryngeal cancer tissues, compared with non-cancer tissues. We have also observed a significant infiltration of lymphocytes in the cancer tissues infected with HPV-16, suggesting the occurrence of inflammation. Measurement of inflammatory cytokines indicated that IL-1beta and IL-8 were obviously increased in laryngeal cancer cells infected by E7. Accompanying the increase of IL-1beta and IL-8, the level of p65 was significantly increased and the level of p65 was positively associated with IL-1beta and IL-8. These changes led to the elevation of the proliferation of tumor cells. Such changes could be prevented when p65 was blocked. In conclusion, The level of NF- κ B can be induced by E7, which leads to the over-production of inflammatory cytokines IL-1beta and IL-8 and subsequently the high proliferation of the tumor cells.

A111**COMPARATIVE EFFECTS OF AN ANTI-INFLAMMATORY BLEND ON REDUCING SKIN IRRITATION CAUSED BY UVB OR A CHEMICAL IRRITANT TO 1% HYDROCORTISONE**

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It is of considerable interest to develop a topical agent, containing no hydrocortisone (HC), that can both reduce the onset of chemically or environmentally induced skin

irritation and ameliorate this irritation once it occurs. A blend of ingredients with anti-inflammatory activities has been developed that outperforms topical 1% HC with regard to UVB induced or Balsam of Peru (BOP) induced skin irritation. This blend contains an inhibitor of histamine release, inhibitors of the PLA2, 5-LO, COX-2, collagenase, elastase and PDE IV enzymes, neutrophil chemotaxis and adhesion blockers, a histamine receptor blocker and an inhibitor of NF κ B activation. A cosmetically acceptable oil/water emulsion containing the anti-inflammatory ingredients was prepared and applied to human subjects either 20 minutes before exposure to the irritant (UVB or BOP) or after irritant exposure once erythema was achieved. When applied before the irritant, this blend was able to reduce BOP induced erythema by 82% and UVB induced erythema by > 90%. When applied after the irritant, the blend was able to reduce existing UVB irritation by 22% and existing BOP induced erythema by 28%.

A112**IMAGING AN INFLAMMATORY RESPONSE USING ¹⁹F MRI**

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Accumulation of mononuclear phagocytes is the hallmark of chronic inflammation. We present a powerful method for noninvasively detecting and quantifying inflammatory cells using ¹⁹F MRI. V-Sense[®], a ¹⁹F-based MRI tracer agent is a perfluorocarbon nanoemulsion that is internalized by macrophages following intravenous injection. An inflammatory response was induced in mice using PVA sponges that were soaked in either complete Freund's adjuvant (CFA) or PBS (control) and implanted subcutaneously in the dorsal surface of Balb/C mice (day 0). V-Sense (0.2 ml) was injected intravenously at day 4. Mice were imaged on days 5 and 6 at 7T using MRI with a dual ¹⁹F/¹H coil. A ¹H anatomical scan was acquired with slices covering the sponge, liver, spleen and kidneys followed by a ¹⁹F scan over the same slices. Significant ¹⁹F signal was observed surrounding the CFA-soaked sponges, consistent with macrophage accumulations but no ¹⁹F signals were detectable in the control PBS-soaked sponge animals. Necropsies showed that the animals with CFA sponges contained 10–20 fold more inflammatory cells than control animals, consistent with the ¹⁹F MRI findings indicating that V-Sense is a specific in vivo biomarker for inflammation that can potentially be used to quantify macrophage activity.

A113**A NOVEL MOUSE MODEL OF MYCOBACTERIUM TUBERCULOSIS-INDUCED GRANULOMA NECROSIS: IMPLICATIONS FOR ADJUVANT IMMUNOTHERAPY TARGETING TH2 CYTOKINES IN TUBERCULOSIS**

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TH2 reactions have been implicated in reactivation of and cavity formation in human tuberculosis (TB). In a large SNP association study with Ghanaian TB patients, we found a structural variant of the IL-4R-alpha to be significantly associated with increased lesion and cavity size. To experimentally address the mechanism of action, IL-13-overexpressing (IL-13tg) mice were aerogenically infected with *Mycobacterium tuberculosis* (*Mtb*). A profound induction of alternative macrophage activation in IL-13tg mice led to increased bacterial loads only during the chronic stage of infection. Importantly, *Mtb* infection in IL-13tg mice, but not in wild type mice, resulted in increased tissue pathology similar to granuloma caseation in human TB. IL-4Ralpha-dependent mechanisms represent a novel target for adjuvant immunotherapy aimed at reducing pathology during chronic and reactivation TB.

A114**P38 MAPK INHIBITOR FOR THE TREATMENT OF RHEUMATOID ARTHRITIS**

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Abbott Bioresearch Center

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic synovial inflammation and progressive destruction of cartilage and bone. p38 α is a serine/threonine mitogen-activated protein kinase (MAPK) that is part of a signaling cascade activated by pro-inflammatory stimuli. Inhibition of p38 α is expected to block the production of pro-inflammatory cytokines (e.g. TNF- α , IL-1 β , IL-6, IL-8) and downstream mediators of inflammation (e.g. COX-2, MMP's) that are important in the pathogenesis of RA. Small molecule p38 α inhibitors, from several different chemotypes, have been shown to block the production of TNF- α and other cytokines in rodent models. Additionally, these compounds have been shown to prevent inflammation

of the joint and bone erosion in various preclinical models of arthritis. Using a collagen induced arthritis model in rats, we assessed the impact on inflammation, and bone/cartilage degradation with oral administration of small molecule inhibitors of p38 α . A serum biomarker of arthritis, cartilage oligomeric matrix protein (COMP): a measure of cartilage breakdown that is produced during inflammation was measured at study termination. In addition, we used micro-computed tomographic (μ CT) imaging to assess bone destruction in the joints of arthritic rats. We demonstrate that these different parameters of measuring bone/cartilage destruction correlate well with each other and with the traditional paw volume measurements. These results suggest that p38 α inhibition may be an effective disease modifying therapy for the treatment of RA.

A115**ORAL LACTOFERRIN AND GLYCINE DISPLAY IN VIVO SYNERGISTIC ANTI-INFLAMMATORY ACTIVITY**

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There is a growing awareness of the interaction of food constituents with the immune system. The present studies aim to evaluate immunomodulatory effects of two of these nutritional components, i.e. glycine and lactoferrin. Mice orally supplemented with glycine, lactoferrin or a combination, were injected intradermally in the ear, with zymosan. Ear swelling, as a measure for inflammation, as well as IL-1, TNF- α and IL-6 levels in the ear and the number of TNF- α producing spleen cells were analysed. In the collagen induced arthritis (CIA) model mice were orally supplemented with a combination of glycine and lactoferrin starting after the second collagen booster. Arthritis development was scored and the pro-inflammatory cytokine levels in the serum were detected.

Glycine and lactoferrin were able to decrease the zymosan induced inflammatory response locally (decreased ear swelling and pro-inflammatory cytokine levels) as well as systemically (reduced number of TNF- α producing spleen cells). Glycine effects (20, 50 and 100 mg/mouse/day) were concentration dependent whereas for lactoferrin only the lowest doses (0.1 and 1 mg/mouse/day) inhibited the inflammatory response significantly. Surprisingly higher doses of lactoferrin (5 and 25 mg/mouse/day) failed to influence the inflammatory reaction. A combination of both nutrients (lactoferrin 0.1mg/mouse/day in combination with glycine 20 or 50 mg/mouse/day) inhibited the zymosan induced ear swelling synergistically. Additionally, an enhancing effect of both components was seen on the number of TNF- α producing spleen cells. In the CIA model the combination of glycine and lactoferrin (lactoferrin 0.1mg/mouse/day with glycine 20 mg/mouse/day) was able to inhibit arthritis develop-

ment and to decrease the IL-6 and TNF- α level in the serum significantly.

The present data show anti-inflammatory activity of glycine and lactoferrin using the zymosan induced inflammation model. Moreover a combination of both components demonstrated a synergistic effect on inflammation of the skin and a strong anti-inflammatory effect as detected in mouse serum (CIA model).

A116

CHRONIC INFLAMMATION IN ASTHMA AIRWAY REMODELING

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Chronic inflammation has become a dominant factor in our understanding of chronic asthma. While for many years the important role that inflammation played in asthma was not recognized and pharmacology recommendations were to avoid steroid treatment if at all possible. Today, clinical authorities recommend inhaled steroids for all patients with persistent asthma. However, although many studies of chronic asthma patients treated with long-term steroids show suppression of inflammation in the lung, patients continue to have exacerbations of their disease and relentless decline of FEV1. This progression has been attributed to airway remodeling – structural changes in the airway that obstruct airflow. While experts state that remodeling is triggered by chronic inflammation, the links between inflammation and remodeling and decline in FEV1 are still speculative. We have developed an initial computational model to explore the physiology of airway remodeling. This systems-engineering approach uses physiological modeling and simulation to quantitatively evaluate hypotheses in the progression of the disease. We will present the model with details of how it was developed and how it is being applied to drug development decisions.

A117

EFFECTS OF RECOMBINANT HUMAN GROWTH HORMONE ON STAPHYLOCOCCUS AUREUS SEPSIS IN MOUSE

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Objective: To investigate the effects of recombinant human growth hormone (rhGH) on Staphylococcus aureus sepsis and explore its possible mechanism.

Methods: Sepsis was mimicked by intraperitoneal (i. p.) injection of Staphylococcus aureus. Male Kun Ming mice were randomly divided into three groups as follows: control group, injected with physiologic saline (i.p.); sepsis group, received a bolus injection of Staphylococcus aureus E311122 (1.75×10^{12} cfu/L, 40ml/kg, i.p.) followed by intramuscular physiologic saline injection; and rhGH treatment group, intramuscularly injected with a dose of 1.0U/kg rhGH after Staphylococcus aureus challenge. Sepsis group and rhGH treatment group were further divided into 6-, 12- and 24-hr subgroups, respectively. 24h cumulative survival rate, bacteria positive rate of blood smear, bacteria count in bacteria plate culture, tumor necrosis factor- α (TNF- α) and interleukin-10 (IL-10) concentrations in plasma, lung injury score, expression of lung intercellular adhesion molecule-1 (ICAM-1) at levels of protein and message-RNA (mRNA), nuclear positive rate of nuclear factor-kappa B (NF- κ B) and its mRNA in the lung were determined.

Results: After Staphylococcus aureus challenge, bacteria positive rate of blood smear, bacteria count in bacteria plate culture, plasma TNF- α concentrations, lung injury score, and NF- κ B and ICAM-1 expression at the levels of protein and mRNA in the lung were significantly elevated, whereas 24h cumulative survival rate and plasma IL-10 levels showed obviously decrease ($P < 0.05$ for all vs. control group). Receiving rhGH treatment, 24h cumulative survival rate and plasma IL-10 levels were significantly increased, whereas bacteria positive rate of blood smear, bacteria count in bacteria plate culture, lung injury score, ICAM-1 and NF- κ B protein expression in the lung were markedly decreased, and the up-regulation of plasma TNF- α was also suppressed. Furthermore, rhGH administration could significantly inhibit the gene expression of ICAM-1 and NF- κ B in the lung ($P < 0.05$ for all vs. sepsis group).

Conclusions: Treatment with rhGH had beneficial effects on Staphylococcus aureus sepsis in mouse, which may be attributed to maintaining a balance of inflammatory cytokines network, reducing the bacterial translocation, inhibiting activation of NF- κ B and decreasing ICAM-1 expression in lung tissue.

A118

SUPPRESSION OF C - REACTIVE PROTEIN AND LIPOPROTEIN LEVELS IN ARTHRITIC RATS BY NOVEL GLUCOSAMINE-ANALOG GN1

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The anti-arthritic effects of GN1 (6-hydroxymethyl-3-(1-methylene-allylamino)-tetrahydro-pyran-2, 4, 5-triol) an analog of glucosamine was investigated on collagen induced arthritis (CIA) in SD rats. Arthritis was induced in rats by multiple intradermal injections of emulsion containing bovine type II collagen in IFA and challenged

again with the same antigen preparation 7 days later. Increased hind paw swelling was significantly suppressed with no further noticeable retardation of body weight in the groups treated with glucosamine ($P < 0.05$) and its analog GN1 ($P < 0.02$) as compared to control arthritic rats. In contrast, the animals in the arthritic control group showed a gradual decrease in their body weight. The histopathological evaluation of isolated knee joints by grading system, classification of the stages in arthritic lesion development, revealed suppression of the inflammatory changes in the GN1 treated animals. In addition, both the proinflammatory markers C-reactive protein (CRP) and low-density lipoproteins (LDL) levels were also found to be significantly decreased in animals treated with GN1 ($P < 0.03$ for CRP and $P < 0.05$ for LDL). The arthritic animals receiving no other treatment or vehicle only showed a significantly elevated CRP and lipoproteins levels. These results suggest that GN1 may have anti-arthritic properties and also act as an anti-inflammatory agent.

A119

INHIBITORY EFFECT OF EGCG ON ACUTE PANCREATITIS

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EGCG has been frequently used as a remedy for inflammatory diseases. The aim of this study was to investigate the effect of EGCG on cholecystokinin(CCK)-octapeptide-induced acute pancreatitis in rats. EGCG at 10 mg/kg was orally administered, followed by 75 µg/kg CCK octapeptide injected subcutaneously three times after 1, 3 and 5 h. This whole procedure was repeated for 5 d. We determined the pancreatic weight/body weight ratio, the levels of pancreatic HSP60 and HSP72, and the secretion of pro-inflammatory cytokines. Repeated CCK-octapeptide treatment resulted in typical laboratory and morphological changes of experimentally-induced pancreatitis. EGCG significantly decreased the pancreatic weight/body weight ratio in CCK octapeptide-induced acute pancreatitis. EGCG also increased the pancreatic levels of HSP60 and HSP72. Additionally, the secretion of IL-6 and TNF- α decreased in the animals treated with EGCG. EGCG may have a protective effect against CCK octapeptide-induced acute pancreatitis

A120

INHIBITORY EFFECT OF INFLAMMATORY MEDIATOR BY OROSTACHYS JAPONICUS IN MOUSE PERITONEAL MACROPHAGES

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Orostachys japonicus (OJ) is an herb widely used herb medicine for the treatment of a variety of pathologies. In this study, the effect of OJ on interferon- γ (IFN- γ) and lipopolysaccharide (LPS)-induced production of nitric oxide (NO), tumor necrosis factor- α (TNF- α), interleukin (IL)-12, and IL-6 were examined using mouse peritoneal macrophages. OJ inhibits IFN- γ /LPS-induced NO in a dose-dependent manner. The decrease in NO synthesis was reflected as a decreased amount of inducible NO synthase protein. We also found that OJ inhibits pro-inflammatory cytokine, IL-12, and TNF- α production. However, OJ doesn't affect the IL-6 production. In addition, OJ inhibited nuclear factor- κ B activation and I κ B- α degradation. Our study suggests that an important molecular mechanism by OJ reduce inflammation, which might explain its beneficial effect in the regulation of inflammatory reactions.

A121

4-ARYL-5-HETEROARYL-2-THIO-SUBSTITUTED IMIDAZOLES: APPROACH TO P38 MAP KINASE INHIBITOR PRODRUGS

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The p38 α mitogen-activated protein kinase (MAPK) is a key component of the cascade leading to pro-inflammatory cytokines like TNF- α and IL-1 β . Inhibition of p38 MAPK is therefore a promising therapeutic strategy for the treatment of many inflammatory disorders such as IBD or RA. To date, however, p38 MAPK inhibitors failed in late stage clinical development, presumably due to not tolerable side effects. In order to reduce toxicity, which is mediated by systemic p38 MAPK inhibition, a prodrug concept was established. To this end, new highly

potent pyridinyl imidazoles were synthesized and linked to non-antibiotic macrolides. The drug carrier is orally available and concentrated in macrophages and neutrophils. Using this prodrug system, selected compounds were investigated for the release of various inflammatory parameters, like interleukins (IL-2, IL-6), IFN- γ , NO production and cell proliferation in mouse spleen and peritoneal exudates cells. Most promising candidates and conjugates underwent *in vivo* studies, e.g. in an acute DSS induced IBD model and CIA model in mice.

A122

IL-1 DRIVES PATHOGENIC TH17 CELLS DURING SPONTANEOUS ARTHRITIS IN IL-1RA-DEFICIENT MICE

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IL-1Ra-deficient mice spontaneously develop an inflammatory and destructive arthritis due to unopposed excess IL-1 signaling. In this study, the role of Th17 cells and the effect of neutralizing IL-17, IL-1, and TNF were investigated. T cells were isolated from IL-1Ra^{-/-} and WT mice, stained for IL-17 and IFN γ , and analyzed by FACS. These FACS data showed that an increase of Th17 cells already precedes the onset of arthritis in IL-1Ra^{-/-} mice while % Th1 remains unaffected, and that the percentage of IL-17-positive T cells clearly correlates to the severity of arthritis. IL-1, IL-17, and TNF were highly expressed in the serum of IL-1Ra^{-/-} mice. Blocking IL-1 or TNF demonstrated that this IL-1-driven model cannot be suppressed by anti-TNF treatment. Interestingly, this anti-IL-1 treatment also significantly reduced the % Th17 cells in these arthritic mice. Our blocking study also demonstrated that IL-17 contributes to the inflammation and bone erosion in this model, and suggests that IL-1 is the driving force behind the IL-17+ Th17 cells.

A123

IL-17 SYNERGY WITH TNF CAUSES STRIKING CARTILAGE EROSION *IN VIVO*

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To study the combined effects of TNF and IL-17 on joint inflammation and destruction *in vivo*, mice were intrarticularly injected into the knee joint with adenoviruses encoding for TNF and IL-17.

Local overexpression of either TNF or IL-17 alone causes synovial inflammation and reversible cartilage proteoglycan depletion. This modest joint pathology was clearly exaggerated by combining TNF and IL-17. Interestingly, only in the combination group severe chondrocyte death and cartilage surface erosions were found, indicating synergy between TNF and IL-17 on irreversible cartilage destruction. Overexpression of TNF plus IL-17 significantly aggravated cartilage VDIPEN expression, a marker for MMP-driven cleavage of aggrecan. QPCR analysis revealed synergistically elevated mRNA levels of MMP3 and MMP13 in the cartilage of the TNF+IL-17 group. In conclusion, the synergy between TNF and IL-17 *in vivo* results in limited increase in bone erosion, but striking exaggeration of cartilage erosion, in line with synergistic upregulation of the erosive enzymes MMP3 and MMP13.

A124

FORMYLPEPTIDE RECEPTOR-LIKE-1 MEDIATES AMYLOID β -INDUCED INFLAMMATORY RESPONSE IN ALZHEIMER'S DISEASE

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Amyloid beta (A β) is a major contributor to the pathogenesis of Alzheimer's disease (AD). Accumulating evidence indicates that A β causes neuronal damage by activating glial cells that accumulate in and around senile plaques. Our previous studies revealed that formylpeptide receptor-like-1 (FPRL1) mediated the chemotactic effect of A β_{42} on mononuclear phagocytes (monocytes and microglia), and elevated FPRL1 gene expression was detected in CD11b-positive mononuclear phagocytes that infiltrate the plaques in brain tissues of the AD patients. In this study, we examined the involvement of FPR2, the mouse homologue of human FPRL1, in A β_{42} -induced inflammatory response both *in vitro* and *in vivo*. We found that lowering the expression of FPR2 using lentiviral vector expressing siRNA targeting FPR2 abolished A β_{42} -induced IL-1 β , IL-6 and MCP-1 expression at mRNA level and protein levels in mouse primary microglia and astrocytes, as well as iNOS and TNF- α mRNA expression in mouse primary astrocytes. Furthermore, lentivirus-mediated FPR2 RNA interference in hippocampus of mice inhibited A β_{42} -induced microglia and astrocyte activation as well as TNF- α and IL-6 upregulation. Thus, FPRL1 may mediate inflammation seen in AD and is a potential target for developing therapeutic agents.

A125**OPTIMIZATION OF A QUINOLONE CLASS OF DISSOCIATED GLUCOCORTICOID MIMETICS**

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Glucocorticoids (GCs) and their derivatives have found wide use in the treatment of many inflammatory diseases. Research efforts are now focused on “dissociated” GC derivatives or mimetics that exhibit reduced side effect profiles versus traditional GCs while maintaining the potent anti-inflammatory activities. Recently, we disclosed a quinolone class of dissociated GC mimetics with potent anti-inflammatory activities in cellular and *in vivo* assays. These earlier compounds with poor PK were not suitable for testing in a chronic disease relevant model (CIA). Further optimization led to BI-306 (3-chloro-1-[4-[5-(2,6-dimethyl-pyridin-4-yl)-2,3-dihydro-benzofuran-7-yl]-2-hydroxy-4-methyl-2-trifluoromethyl-pentyl]-1*H*-quinolin-4-one) with the following improved profile: (a) potent and selective over other nuclear receptors, (b) dissociated in cellular systems, (c) improved plasma level after oral dosing, (d) potent inhibition of LPS-induced TNF α production, and (e) efficacious in an Ab CIA model.

A126**INFLAMMATORY SIGNALING PROCESSES AND CARDIOVASCULAR COMPLICATION**

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Inflammatory signaling processes are critically involved in the pathogenesis and resolution of various inflammatory diseases such as atherosclerosis and hypertension. The interleukin-1 receptor associated kinase 1 (IRAK-1) is a critical modulator regulating the inflammatory signaling processes. We have demonstrated that IRAK-1 is a kinase responsible for the phosphorylation and inactivation of the Nuclear Factor of Activated T-cell (NFAT). Expression of IRAK-1 suppressed NFAT reporter activity. Correspondingly, the levels of both nuclear NFATc1 and NFATc4 were constitutively elevated in IRAK-1^{-/-} cells. Furthermore, the phosphorylation of NFATc4 at the S₁₆₈PS₁₇₀P site was significantly diminished in IRAK-1^{-/-} cells. Mechanistically, we observed that IRAK-1 interacted with NFATc4 via the C-terminus of IRAK-1 and the N-terminal NHR region of NFATc4. IRAK-1 mutants that ablated either its kinase activity or its interaction with NFATc4 failed to suppress NFAT reporter activity. The expression level of COX-2, which is under the control of NFAT, was elevated in IRAK-1^{-/-} cells. Functionally, ApoE^{-/-}/IRAK-1^{-/-} mice were protected from high-fat-diet induced hypertension

and atherosclerosis. Taken together, our findings reveal that IRAK-1 serves as a novel target for treating cardiovascular diseases.

A127**THE GOLGI-ASSOCIATED PROTEIN P115 MEDIATES THE SECRETION OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF)**

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The leaderless pro-inflammatory cytokine MIF is secreted from cells by an unconventional export pathway. The release of MIF plays an important role in diverse inflammatory diseases. We identified the Golgi complex-associated protein, p115, as an intracellular binding partner for MIF. MIF localizes with p115 in the cytoplasm and the stimulated secretion of MIF results in the accumulation of both proteins in supernatants, which is consistent with MIF release from cells in conjunction with p115. The depletion of p115 from monocytes/macrophages decreases the release of MIF but not other cytokines following inflammatory stimulation or intracellular bacterial infection. Notably, the small molecule MIF inhibitor, 4-iodo-6-phenylpyrimidine, inhibits MIF secretion by targeting the interaction between MIF and p115. These data reveal p115 to be a critical intermediary component in the regulated secretion of MIF from monocytes/macrophages.

A128**GABA(A)-MEDIATED ALTERATION OF AUTOIMMUNE-MEDIATED INFLAMMATION USING THE NATURAL PLANT PRODUCT, HONOKIOL**

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Honokiol (HNK) is a natural, purified, product of the leaves and root stems of the *Magnolia* plant that has long been used in traditional Asian medicine without dangerous side effects. To determine if HNK has anti-inflammatory properties, we tested its effects in a mouse model of inflammatory rheumatoid arthritis (RA). Our initial studies show that both *in vivo* inflammation and disease progression and *in vitro* pro-inflammatory cytokine production and cellular activation are markedly inhibited, without increased cell death or *in vivo* toxicity. HNK also inhibits signaling via CD40 in B cells, an immune receptor that has been implicated as playing a role in RA, as well as an EBV-encoded CD40 mimic called LMP1, that has been implicated in exacerbating

autoimmune disease. Furthermore, the anti-inflammatory effects of HNK could be reversed using inhibitors of the neurotransmitter GABA(A), a previously reported target for HNK interaction. These findings are particularly exciting and suggest that the nontoxic anti-inflammatory properties of HNK could prove more effective than treatments targeting a single cytokine or receptor.

A129

HUMAN APOLIPOPROTEIN C1 TRANSGENIC MICE: A UNIQUE NOVEL MODEL OF ATOPIC DERMATITIS

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Mice with a chronic overexpression of human apolipoprotein C1 in liver and skin display increased levels of cholesterol and triglycerides, and spontaneously develop clinical symptoms of atopic dermatitis (AD). These mice show increased trans-epidermal water loss suggestive for an impaired skin-barrier function. Development of AD in these mice is associated with increased pruritus. Histological analysis shows hyperplasia of both the epidermis and dermis and increased numbers of CD4⁺ T cells, eosinophils and IgE⁺ mast cells in the dermis. Serum levels of IgE are increased as well. Development of AD in this model was found to be sensitive to topical treatment with triamcinolone-acetonide, fluticasone-isopropionate or tacrolimus. Moreover, oral treatment with dexamethasone successfully inhibits several aspects of disease in this model. Therefore, this novel animal model may help to gain new insight into the pathogenesis and be of great value to develop novel therapeutic strategies for the management of AD.

A130

MODULATION OF INFLAMMATION IN TWO HUMANIZED MOUSE MODELS OF PSORIASIS

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Several mechanisms involved in the development and progression of psoriasis are considered as potential therapeutic targets for biologicals. To ensure that such reagents properly predict efficacy in the clinic it is of high importance to evaluate those in a humanized model of psoriasis. We here describe two humanized mouse models of psoriasis by grafting either non-lesional or lesional skin from psoriasis vulgaris patients onto immunodeficient mice. In the non-lesional skin graft model a psoriatic

process is induced by intradermal injection of SEB-activated autologous T cells. In both models a psoriatic process is demonstrated by assessment of epidermal hyperplasia, and proliferation (Ki67) and differentiation (CK16) of keratinocytes, in conjunction with the involvement of innate cells, activated T cells and cytokines like TNF- α . Either model was shown to be sensitive to a wide range of therapeutics, including anti-TNF- α and anti-IL-23. Therefore, these two humanized mouse models represent a powerful tool for the identification or validation of potential therapeutics in psoriasis.

A131

EFFICACY OF PROBIOTICS IN TNBS-INDUCED COLITIS

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Defects related to epithelial barrier function, NOD2, altered production of IL-12 and IL-23 by dendritic cells, strong Th17 responses and impaired regulatory T cells have been implicated in Inflammatory Bowel Disease. We evaluated the sensitivity of TNBS-induced colitis in BALB/c mice with respect to immunosuppressive drugs and probiotics. Systemic administration of corticosteroids, CsA, IL-10 or Etanercept were mostly ineffective, or even resulted in increased mortality. Rectally instilled budesonide resulted in up to 50 % inhibition of colitis as measured by effects on colon damage and cellular infiltration. Of note, treatment with *L.plantarum* or VSL#3 had substantial effects, evident from a decrease in infiltrating CD4⁺, CD8⁺ and CD11b⁺ cells in the colon. Interestingly, these observations were associated with decreased serum levels of IL-17, IFN- γ , IL-1 β and MIP-1 α . Such effects were also found in mice treated with IL-10 or Etanercept, indicating – in view of the increased mortality – that immunosuppression may not always be the right strategy of treatment in experimental colitis. Our data are in favor of treatment with probiotics as an approach to restore intestinal immune homeostasis.

A132

PREDICTING EFFICACY AND SIDE EFFECTS OF THE P38MAP KINASE INHIBITOR CLASS USING BIOMAP[®] PRIMARY HUMAN CELL-BASED SYSTEMS

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Inhibitors of p38 are safe and strongly anti-inflammatory in preclinical animal models, but have reported dose-limiting liver and skin toxicity in human clinical trials. BioSeek has pioneered the development of *in vitro*

human cell-based BioMAP® Systems that incorporate the complexity of environmental factors observed in diseased tissues *in vivo*. Analysis of multiple experimental and clinical p38 inhibitors in BioMAP® identified class-specific biomarker and pathway modulation information that is predictive for both efficacy (monocyte/macrophage-driven inflammation) and side effects (liver and skin tissue stress responses) with strong correlation to clinical data. In particular, the liver and skin stress responses seen with p38 inhibitors in BioMAP® are shared with other therapies known to cause such responses (i.e. EGF pathway inhibitors). Inhibitors of downstream p38 targets (e.g. MK-2 or MNK1) have reduced anti-inflammatory activity, but also induce less of a stress response. These findings demonstrate how BioMAP® human disease models can drive the discovery and optimization of safe and efficacious therapeutics.

A133

HISTAMINE IS NOT RELEASED IN ACUTE THERMAL INJURY IN HUMAN SKIN *IN VIVO*: A MICRODIALYSIS STUDY

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Background Animal models have shown histamine to be released from the skin during the acute phase of a thermal injury. The role of histamine during the early phase of thermal injuries in humans remains unclear.

Purpose The objectives of this trial were to study histamine release in human skin during the acute phase of a standardized thermal injury in healthy volunteers.

Methods Histamine concentrations in human skin were measured by skin microdialysis technique. Microdialysis fibers were inserted into the dermis in calf skin in male healthy volunteers. A standardized thermal injury was elicited by a heating thermode. Histamine in dialysate was analyzed for up to 120 min.

Results In separate investigations, histamine was analyzed in 2-min samples over 20 min (n=6) and at 10-min intervals over 120 min (n=8) after the injury. Histamine levels at baseline and in most post-injury samples were at or below the quantification limit of the spectrofluorometric analysis. Confirmatory analysis using a sensitive radioimmunoassay showed no significant histamine release during the first 60 min after a thermal injury using a (baseline histamine 11.6 ± 1.8 nM versus 14.8 ± 1.8 nM post injury).

Conclusions Histamine is not released in human skin during the acute phase of a thermal injury.

A134

EFFECTS OF A SELECTIVE, POTENT P38 INHIBITOR IN *IN VIVO* MODELS OF RHEUMATOID ARTHRITIS

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ARRY-371797 (ARRY-797; (N-substituted-5-(2,4-difluorophenoxy)-1-isobutyl-1H-indazole-6-carboxamide).) is a selective, potent inhibitor of the p38 α enzyme (IC₅₀<5nM). This activity is maintained in human whole blood cytokine assays (IC₅₀<2nM). Here we show the effects of ARRY-797 in two rat models of rheumatoid arthritis (CIA and AIA) when given alone or in combination with methotrexate. In CIA, ARRY-797 (3, 10, or 30 mg/kg, BID,PO) dosed as a single agent (d10-16) inhibited paw diameter increases with an ED₅₀ of ~5 mg/kg and histological lesions with an ED₅₀ of ~3 mg/kg. In AIA, ARRY-797 (3, 10, or 30 mg/kg, PO, BID) was dosed as a single agent (d0-16) or with methotrexate (MTX;0.05 mg/kg QD, PO, d0-16). ARRY-797 alone inhibited paw diameter (~40%) at 30 mg/kg and was very effective in the inhibition of bone resorption (ED₅₀ of <3 mg/kg). ARRY-797 with MTX resulted in additive inhibition of paw swelling. We have shown the potent p38 inhibitor ARRY-797 has excellent anti-inflammatory and bone protective effects in CIA and potent inhibitory effects on bone resorption in AIA. ARRY-797 can be combined with MTX to produce at least additive effects on in-life and histological endpoints. ARRY-797 is in clinical trials to study its safety and efficacy in patients with pain and other inflammatory disease.

A135

A GLOBAL GENE EXPRESSION PROFILING ANALYSIS TO STUDY THE ROLE OF ENDOTHELIAL CD40 DURING INFLAMMATION

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The CD40-CD154 interaction plays a key role in the immune-inflammatory response triggered by endothelial cell (EC)-T cell communication. To study the involvement of CD40 in EC activation we have carried out a global gene expression profiling analysis of ECs interacting with (CD154+) T cells. We have assessed the consequences of blocking CD40 signaling with a specific human anti-CD40 siRNA in a time course experiment and have observed an extensive transcriptional response after CD40-CD154 engagement in ECs. There is an early up-regulation of the major pro-inflammatory NF- κ B and MAPK/SAPK pathways and their associated transcription factors. Moreover, CD40-mediated up-regulation of the viral immune surveillance system, notably TLR3,

IFIH1, RIG-I and RNASEL, establishes a reverse link from adaptive to innate immunity. This suggests that CD40 triggers the antiviral innate immune response in ECs when challenged with activated T cells. In summary, we have identified novel genes and signaling pathways involved in the induction of the inflammatory response through CD40 activation in the endothelium.

A136

DIPEPTIDYL PEPTIDASE I MEDIATES CIGARETTE SMOKE-INDUCED PULMONARY INFLAMMATION AND ALVEOLAR DESTRUCTION

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Based on evidence that a protease-antiprotease imbalance is pivotal in the development of chronic obstructive pulmonary disease (COPD), we hypothesized that dipeptidyl peptidase I (DPPI), the physiological activator

of neutrophil elastase (NE), may contribute to the etiology of COPD. DPPI gene-deficient (DPPI^{-/-}) mice exposed to 4% mainstream cigarette smoke (CS) for 2 weeks exhibited reduced numbers of BAL fluid neutrophils, macrophages, and lymphocytes, as well as decreased levels of KC, MCP-1 and IL-12p40, compared to DPPI^{+/+} and DPPI^{+/-} mice. Following 15 weeks

of CS exposure, DPPI^{-/-} mice exhibited reduced numbers of BAL fluid neutrophils and macrophages, but not lymphocytes, compared to DPPI^{+/+} and DPPI^{+/-} mice. DPPI^{-/-} BAL fluid levels of KC and MCP-1 were also decreased, while IL-12p40 levels were not. Importantly, DPPI^{-/-} mice were completely protected from CS-induced airspace enlargement. In a second model of COPD, DPPI^{+/+} mice exposed to ozone (3 ppm for 3 hours) twice weekly for 6 weeks exhibited significant airspace enlargement, while DPPI^{-/-} mice were not different from sham-exposed mice. These results suggest that DPPI inhibition offers a promising approach to the treatment of COPD.

A137

THE INVOLVEMENT OF NITRIC OXIDE IN THE PERIPHERAL ANTINOCICEPTIVE EFFECTS OF OPIOIDS DURING CHRONIC INFLAMMATORY PAIN

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We investigated using inducible nitric oxide synthase (NOS2) knockout mice the role of nitric oxide in the local

antinociceptive effects of μ and δ -opioid receptor agonists during CFA-induced peripheral chronic inflammatory pain. The presence of paw inflammation, mechanical allodynia and thermal hyperalgesia induced by CFA were assessed by measuring paw diameter and using the von Frey filaments and plantar tests, respectively. NOS2 knockout mice exhibited reduced paw edema and diminished thermal hyperalgesia after CFA as compared to WT mice. The subplantar administration of morphine (μ -agonist) or [D-Pen2,5]-enkephalin (δ -agonist) which completely reversed the thermal hyperalgesia induced by chronic inflammatory pain in WT mice was completely ineffective in NOS2 knockout mice. These results indicate that nitric oxide derived from NOS2 participates in paw edema and thermal hyperalgesia induced by CFA as well as in the antinociceptive effects of μ - and δ -opioid receptors during chronic inflammatory pain. Supported by FIS (05/1604) & Fundació Marató TV3 (07/0810), Spain.

A138

CAMPYLOBACTER JEJUNI-INDUCED ACTIVATION OF MURINE DENDRITIC CELLS INVOLVES COOPERATIVE SIGNALING THROUGH MYD88 AND TRIF

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Campylobacter jejuni (Cj) is a frequent cause of human enteritis that resembles inflammatory bowel disease. We showed that Cj induces activation of bone marrow-derived dendritic cells (BM-DCs). Toll-like receptors (TLRs) of DCs induce immune responses to pathogens through adapters such as MyD88 and TRIF. We hypothesized that Cj-induced inflammatory activation of BM-DCs is mediated by TLR2 and TLR4 and that MyD88 or TRIF signaling is necessary for activation. WT, MyD88^{-/-}, TRIF^{-/-}, TLR4^{-/-} or TLR2^{-/-} BM-DCs were exposed to Cj and immune responses were assessed. Up-regulation of MHC-II after Cj challenge was significantly impaired by MyD88-, TRIF-, TLR4- or TLR2-deficiency. Increase in expression levels of CD80, CD86 and CD40 and secretion of IL-12, IL-6 and TNF- α following Cj challenge was significantly inhibited in MyD88^{-/-}, TRIF^{-/-}, TLR4^{-/-} and TLR2^{-/-} DCs compared to WT DCs. However, the magnitude of inhibition was greater in MyD88^{-/-}, TRIF^{-/-} and TLR4^{-/-} DCs than in TLR2^{-/-} DCs. Our results show for the first time that cooperative signaling through TLR4-MyD88 and TLR4-TRIF axes represents a novel mechanism mediating Cj-induced maturation and inflammatory responses of DCs. (Funded by NIH contract NO1-AI-30058 and grant K26 RR023080-01).

A139**AN2898: A NOVEL ANTI-INFLAMMATORY COMPOUND THAT INHIBITS PHOSPHODIESTERASE 4 AND 7 ENZYME ACTIVITY AND IL-12 AND IL-23 RELEASE**

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AN2898 (5-(3,4-dicyanophenoxy)-1-hydroxy-1,3-dihydro-2,1-benzoxaborole) is a dual-inhibitor of PDE4 and PDE7 enzymes. Additionally, AN2898 inhibits IL-12 and IL-23 release from PBMCs. This new small molecule compound is currently in preclinical development for psoriasis and atopic dermatitis, common skin diseases that are characterized by chronic inflammation. AN2898 IC₅₀ values for inhibition of PDE4 and PDE7 activity is 0.06 and 0.21 μM respectively. AN2898 is a competitive, reversible inhibitor of PDE4 with a K_i of 65 nM. The AN2898-PDE4B2 catalytic domain co-crystal structure shows that AN2898 binds directly to the metal ions and water molecule in the active site. This binding configuration is unique compared to classical PDE4 inhibitors (i.e. rolipram). AN2898 has equal activity against the four PDE4 subtypes and does not significantly inhibit PDE 1, 2, 3, 5, or 6. Unlike classical PDE4 inhibitors, AN2898 has additional activity and inhibits IL-12 (IC₅₀ = 0.016 μM) and IL-23 (IC₅₀ = 1.1 μM). These are important cytokines in the treatment of psoriasis, and are not affected by PDE4 inhibition. Like other PDE4 inhibitors, AN2898 inhibits the release of TNF α, IL-2, IFN γ, IL-5, and IL-10 in the low to mid-nanomolar range, and does not inhibit IL-1 β, IL-6 and IL-8. AN2898 after topical administration demonstrates significant *in vivo* activity. AN2898 has much greater efficacy compared to rolipram in a PMA induced ear edema model. Additionally, AN2898 has anti-inflammatory activity in the oxazolone model (a model of allergic contact dermatitis). In conclusion, AN2898 shows excellent activity against cytokines associated with psoriasis and atopic dermatitis.

A140**DISCOVERY OF 3-{3-(2-PIPERIDINYLETHOXY)PHE-NYL}-5-(1H-1,2,4-TRIAZOL-3-YL)-1H-INDAZOLE (CC-401), A POTENT JNK INHIBITOR**

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Activation of Jun N-terminal kinases (JNK's) in ischemia-reperfusion (IR) injury is well documented. Through the template modification efforts on a screening hit, anthrapyrazolone (SP600125), we identified a potent, indazole-based JNK inhibitor, CC-401, which inhibited JNK's (JNK2; Ki = 24 nM), blocked PMA/PHA induced IL-2 production in Jurkat T-cells (IC₅₀ = 0.8 μM), and prevented deaths in a rat liver IR injury model (84% survival at 10 mg/kg iv CC-401 vs. 0% in control). CC-401 was investigated in a number of normal volunteers and patients in Phase 1 trials to monitor pharmacokinetics and proof of JNK inhibitory activity.

A141**RESOLVIN E1 (RVE1) INHIBITS INFLAMMATION IN ACUTE AND CHRONIC MURINE MODELS OF COLITIS**

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Resolvix Pharmaceuticals Inc, Bedford MA

Resolvins are a recently discovered family of naturally-occurring, small molecule lipid mediators that act to resolve inflammation, protect healthy tissue and restore immune homeostasis. Resolvins show highly potent efficacy in rodent models of rheumatoid arthritis, asthma and other inflammatory diseases. These molecules may represent a novel class of therapeutics for the treatment of a wide range of inflammatory diseases. In these studies, the objective was to evaluate the therapeutic effect of RvE1 in acute and chronic models of inflammatory bowel disease (IBD), including oral consumption of dextran sodium sulfate (DSS) and adoptive transfer of CD45RB^{hi} CD4⁺ cells T cells into immunodeficient recipients. Therapeutic endpoints in these studies included body weight, colon length, colon edema, myeloperoxidase (MPO) activity, and histological scores. The results from these studies suggest that RvE1 effectively modulates disease pathology and could have therapeutic potential in the treatment of IBD.

A142**THE POLY-ANIONIC SUGAR SUCROSE OCTA-SULPHATE IS AN ORAL ANTI-RHEUMATIC AND ANTI-EROSIVE METABOLITE OF SUCRALFATE**

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We have reported that polyanionic diglucopyranosylamines are antirheumatic *in vivo*, and here the disaccharide analogue sucrose octasulphate (SOS). Antigen induced arthritis (AIA) C57bl mice sensitized to methylated BSA challenged *ia*, and joint *dia*. Assessed at 24hrs. Murine (dba-1, MCIA) and rat (Lewis, RCIA) collagen

arthritis was induced, scored and paw volume (plethysmometry) measured. CT images of fixed paws were acquired (Siemens Microcat II instrument), isosurface plots obtained with reference to an untreated control, and scored. 100mg/kg p.o. sucralfate inhibited AIA, and this reversed by lansoprazole. SOS (100, 30, 10mg/kg p.o.) dose relatedly inhibited AIA. RCIA was inhibited when dosed p.o. from day 0-11, and MCIA dosed p.o. prophylactically. An MCIA bone erosion score was developed, and erosion scores reduced from 12.0 ± 3.4 to 6.5 ± 2.7 ($p < 0.07$, $n=9$ & 10). Dexamethasone reduced scores from 10.4 ± 2.9 to 2.4 ± 0.8 ($p < 0.01$, $n=1$ & 12). Orally active polyanionic drugs may provide a novel approach to oral anti-rheumatic anti-erosive therapy.

A143

PRECONDITIONING LYMPHOCYTES WITH P38 MAPK INHIBITORS, AND NOT ACCESSORY CELLS, PREVENTS CON-A-INDUCED LYMPHOCYTE RESPONSES

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We have shown DC-T cell MLR requires active T cell p38 MAPK before stimulation, but not in DCs. We here assess whether this is specific for antigen dependent responses. Balb/c mouse spleen cells were stimulated with $2.5 \mu\text{g/ml}$ Con-A in the presence or absence of $3 \mu\text{M}$ SB203580 (4-(4-Fluorophenyl)-2-(4-ethylsulfinylphenyl)-5-(4-pyridyl) 1H-imidazole, SB) or $3 \mu\text{M}$ ML3403 ((RS)-[4-[5-(4-Fluorophenyl)-2-methylsulfonyl-3H-imidazol-4-yl]pyridine-2-yl]-(1-phenylethyl)amine, ML) and incubated for 72 hours. Accessory cells (AC) were separated from L \emptyset by adherence to plastic and washed 3 times. Separated cells were pre-conditioned with drugs for 2 hours, washed 3 times, resuspended (1×10^6 /mL), mixed and stimulated with Con-A. Con-A induced L \emptyset proliferation was dependent on ACs. SB and ML both inhibited whole spleen cell proliferation. 2hr preconditioning of ACs with SB or ML had no effect, whilst preconditioning of L \emptyset inhibited proliferation. Thus both SB and ML are effective after washout. L \emptyset , not AC, require functional p38 prior to Con-A stimulation in order to proliferate. P38 inhibition conditions L \emptyset responsiveness to AC and DCs.

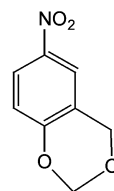
A144

PREVENTIVE ROLE OF ZH-67-2-1 IN ADJUVANT-INDUCED ARTHRITIS IN RATS

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In the present study, the anti-arthritic and anti-oxidative effects of the synthetic compound ZH-67-2-1 (6-nitro-1,3-benzodioxane, Fig. 1), was evaluated in the adjuvant-induced arthritic (AIA) rats. It was observed that the increased hind paw swelling was significantly suppressed ($p < 0.007$) in the ZH-67-2-1 (20 mg/kg , i.p.) treated arthritic rats as compared to arthritic control rats. Body weights were also measured to monitor the progression of disease and systemic anti-arthritic effects of the test compound used in this study. Our results show that ZH-67-2-1 not only inhibited the macroscopic changes such as erythema and swelling of limbs but also exhibited reversal of nociception as measured by traction test. Moreover, the body weight reduction which was observed in arthritic and saline treated arthritic control rats was not observed in the arthritic rats treated with ZH-67-2-1. The anti-oxidative activity of ZH-67-2-1 was measured in the serum of all treated and untreated arthritic and non-arthritic rats. The ZH-67-2-1 treatment was observed to significantly suppress both the nitric oxide (NO) ($p < 0.014$) and peroxide levels ($p < 0.00$) as compared to the arthritic control group. Based on these observations, we suggest that one of the possible mechanism through which ZH-67-2-1 exerts its anti-arthritic effect is through its free radicals scavenging activity.



Exact Mass: 181.04

Mol. Wt.: 181.15

C, 53.04; H, 3.89; N, 7.73; O, 35.33

A145

DESIGN OF SMALL MOLECULE INHIBITORS FOR INFLAMMATORY BOWEL DISEASE MECHANISM: INFLAMMATORY/IMMUNE MECHANISMS

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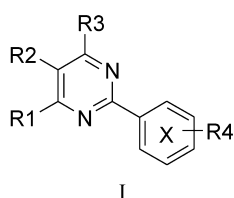
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Inflammatory bowel disease is a chronic inflammatory condition of the gastrointestinal tract that manifests as ulcerative colitis and Crohn disease^{1,2}. We have focused our approach towards the design and synthesis of novel small molecule inhibitors for treating inflammatory bowel disease.

Several analogs of **I** were synthesized and screened for activity using TNF- α as a preliminary screen followed by finding the mechanism of action (lead compound

OCID2287 was found to be a potent thromboxane synthase inhibitor with nanomolar IC_{50}).

DRC of the lead molecule was performed using DSS induced IBD model in mice, TNBS induced IBD in rat model, and Oxazolone induced colitis in mice. Disease Activity Index (DAI) in DSS induced IBD model in mice showed the potency of the lead compound OCID2287 at 0.1mg/kg comparable to that of Sulfasalazine at 100mg/kg.



To summarize, several analogs of **I** were synthesized and screened for thromboxane synthase activity followed by the activity in IBD disease models which facilitated the identification of a lead compound OCID2287.

References:

1. R.S.Blumberg and W. Strober, Journal of American Medical Association, 2001, 285, 643-647
2. M.G. Neumann, Romanian Journal of Gastroenterology, 2004, Vol.13, No.4, 309-316

A146

IL-17 SIGNALING INDUCES SEQUENTIAL PHOSPHORYLATION OF C/EBP β

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IL-17 induces target gene expression via activation of the transcription factors NF- κ B and C/EBP β . C/EBP β is subject to many posttranslational modifications. To investigate phosphorylation of C/EBP β following IL-17 signaling, ST-2 stromal cells were stimulated by IL-17 and C/EBP β was enriched by immunoprecipitation. After in-gel trypsin digestion, ESI nano-spray tandem MS spectrometry was used to evaluate phosphorylation sites. Within 1 hour, IL-17 stimulation induced sequential phosphorylation of two distinct Thr/Ser residues in the regulatory domain of C/EBP β . IL-17-induced C/EBP β phosphorylation was completely blocked by an ERK specific inhibitor. Fibroblasts expressing the IL-17RA receptor V553H mutation, which is defective in activating the MAPK pathway, also failed to induce C/EBP β phosphorylation after IL-17 stimulation. Thus, IL-17 triggers multiple phosphorylation events on C/EBP β that correlate with signaling function.

A147

MODULATORY ROLE OF 2-ACETAMIDOPHENOL ON THE EXPRESSION OF CD44 CELL SURFACE MARKERS IN THE BRAIN OF ADJUVANT INDUCED ARTHRITIC MODEL (AIA) OF RATS

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Rheumatoid Arthritis is a chronic inflammatory autoimmune disease involving damage to the joints and synovial tissue. The inflammatory process is triggered by up regulation of cell surface marker CD44 inflammatory cells especially during the progression of inflammation in rheumatoid arthritis. In addition, the interaction between CD44 and extracellular hyaluronan has been reported to be involved in a variety of pathological processes at the site of inflammation. In the present study, we have investigated the effect of 2-acetamidophenol ($HOC_6H_4NHC(O)CH_3$) on the expression of CD44 markers and its potential role as anti-arthritis agent in AIA rats. To our knowledge, much of the work done on the CD44 up-regulation in case of rheumatoid arthritis were carried out in the synovial fluid aspirate from the inflamed joint. However, we have evaluated the effect of 2-acetamidophenol on the expression of CD44 in the brain of arthritic rats. The CD44 immunohistochemistry was done using monoclonal anti-CD44 antibodies. Our results demonstrate a marked decrease in the CD44 expression in the 2-acetamidophenol treated arthritic rats compared to the arthritic control group and indomethacine treated arthritic rats. The progression of the disease was monitored by measuring body weight and paw volume. The arthritic rats treated with 2-acetamidophenol not only inhibited the macroscopic changes such as erythema and swelling of limbs, but also exhibited significant attenuation of the increase in paw volume produced as a result of arthritis induction. There was also a slight but non-significant reduction in the body weights of 2-acetamidophenol treated arthritic rats however it was recovered in the end. Based on these results, we suggest that 2-acetamidophenol is not only anti-arthritis but also control the underlying mechanism of the inflammatory processes associated with arthritis. In addition, the determination of CD44 in brain samples can also be a strong predictive marker of the inflammatory processes involved in the arthritis.

A148**PROTECTIVE EFFECT OF NON-SELECTIVE AND SELECTIVE COX-2-INHIBITORS IN HYPOXIA STRESS-INDUCED BEHAVIORAL AND BIOCHEMICAL ALTERATIONS**

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¹Chandigarh College of Pharmacy, Landran (Mohali), India

²Pharmacology Division, Department of Pharmaceutical Sciences and Drug Research, Panjabi University, Patiala, India.

Hypoxia stress has been reported to produce several behavioral, neurochemical and biochemical alterations. Cyclooxygenase (COX) enzymes are playing a vital role in pathogenesis of several brain disorders including Alzheimer disease, epilepsy, depression, in addition to pain and inflammation. In the present work, we examined the role of non-selective (naproxen) and selective (rofecoxib, valdecoxib) COX-2 inhibitors against hypoxia stress-induced behavioral alterations and oxidative damage in mice. Mice were subjected to hypoxia stress for a period of 4 h. Naproxen (5, 10 and 20 mg/kg, ip), rofecoxib (5, 10 and 20 mg/kg, ip) or valdecoxib (5, 10 and 20 mg/kg, ip) were administered 30 min before hypoxia stress. Four-hour hypoxia stress significantly caused anxiety-like behavior, memory deficit and impaired motor activity as well as oxidative damage (raised lipid peroxidation, nitrite activity, depletion of reduced glutathione and catalase activity) as compared to naive animals placed on sawdust ($p < 0.05$). Pretreatment with Naproxen (5, 10 and 20 mg/kg, ip), rofecoxib (5, 10 and 20 mg/kg, ip) or valdecoxib (5, 10 and 20 mg/kg, ip) significantly improved locomotor activity, antianxiety effect, memory retention (memory deficit) and attenuated oxidative damage (lowering of raised malondialdehyde, nitrite activity, restoration of reduced glutathione and catalase activity as compared to hypoxia stress group ($p < 0.05$)). Results propose the neuroprotective and antioxidant effect of both non-selective and selective COX-2 inhibitors.

A149**ROSIGLITAZONE PREVENTS HYPERHOMOCYSTEINEMIA-INDUCED MYOCARDIAL REMODELING THROUGH MAST CELL STABILIZATION IN RATS**

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Objectives: The present study has been designed to investigate the effect of rosiglitazone, a peroxisome proliferator receptor agonist- γ (PPAR- γ) in hyperhomocysteinemia-induced myocardial hypertrophy and fibrosis in rats. Methods: Rats were administered L-methionine

(1.7mg/kg/day *p.o.*) for 8 weeks to produce hyperhomocysteinemia. Rosiglitazone (3 and 5mg/kg/day, *p.o.*) treatment was started from the first day of administration of L-methionine and were continued for 8 weeks in rats. The development of myocardial remodeling was assessed in terms of measuring ratio of left ventricular (LV) weight to body weight (LVW/BW), LV wall thickness (LVWT), LV protein content (mg/g of LV) and LV collagen content (mg/g of LV). The haematoxylin-eosin staining, picrosirius red and toluidine blue staining was done to measure cardiomyocyte diameter, collagen deposition and the mast cell density in left ventricular sections. Results: The hyperhomocysteinemia significantly increased LVW/BW, LVWT, LV protein, collagen content. Histological studies revealed increased cardiomyocyte diameter, extensive fibrosis and increased mast cell density. However, rosiglitazone treatments significantly attenuated hyperhomocysteinemia-induced pathological cardiac hypertrophy and fibrosis without changing serum homocysteine levels in rats. Conclusion: It may be concluded that hyperhomocysteinemia-induced myocardial remodeling is associated with increase in density of mast cells in heart. Moreover, rosiglitazone may have attenuated hyperhomocysteinemia-induced pathological cardiac hypertrophy by preventing the degranulation and increase in density of mast cells.

A150**EVALUATION OF NOVEL TOPICAL DRUG DELIVERY SYSTEMS OF COLCHICINE IN MONO SODIUM URATE (MSU) MODEL OF GOUT IN RATS**

Amrit Pal Singh, Hardevinder Pal Singh*, Anant Singh and Subheet Jain

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The gout characterized by deposition of urate crystals, agonizing pain and inflammation of joints, is a problem affecting world's adult population. Colchicine widely used drug for treatment of gout is associated with numerous side effects such as nausea, diarrhea, neuro-myopathy and bone marrow suppression. The topical drug delivery systems offer advantages such as by-passing hepatic metabolism, minimizing side effects and avoid drug degradation due to gastric pH. Hence, various formulations such as microspheres, elastic and non-elastic liposomes and niosomes were prepared and evaluated using mono sodium urate model of gout in rats. The subcutaneous air pouch was formed in anaesthetized rats by administering 10 mL of sterile air. The sterile air was re-injected in air pouch every 2-3 days to maintain pseudo-gout conditions. After 6 days, rats were divided into MSU control (without drug treatment), oral colchicine treatment and groups receiving colchicine entrapped in microspheres, niosomes, elastic and non-elastic liposomes (n=10-12/group). Rats from each group were sacrificed after 6, 12 and 24 hours of MSU administration and evaluated for exudate volume of air pouch, total leukocyte count (TLC). Moreover, haematoxylin-eosin staining was done for gross histology to observe extent of tissue

damage and collagen deposition considered as marker for fibrosis was assessed using picrosirius red staining. The MSU administration in air pouch induced extensive fluid accumulation, increased TLC, bizarre tissue and extensive fibrosis. The elastic liposomes claimed best efficacy followed by non elastic liposomes, niosomes, microspheres and oral administration of colchicine. Hence, our finding suggest that novel topical drug delivery systems especially elastic liposomes have the potential to used for sustained and site specific delivery of colchicine than oral therapy.

A151

GADOLINIUM DECREASES INFLAMMATION RELATED TO MYOCARDIAL ISCHEMIA AND REPERFUSION INJURY

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Gadolinium (Gd) protects the heart against infarction following ischemia and reperfusion (I/R). We determined the impact of Gd treatment on monocyte and neutrophil function during I/R. Rats (n=6/gp) were treated with saline or Gd (20 μ mol/kg) and subject to I/R. Sham rats were not subject to I/R. I/R resulted in a 2-3 fold increase in circulating monocytes and neutrophils, and increased myocardial GM-CSF, IL-1, IL-8, TNF- α , and myeloperoxidase (MPO) activity. Gd decreased the number of circulating monocytes and neutrophils after I/R to levels below those present prior to ischemia. Gd decreased the production of GM-CSF and IL-1 in the myocardium but not IL-8 and TNF- α after I/R. Furthermore, Gd decreased MPO activity after I/R to levels below those measured in the Control or Sham groups. Gd treatment prior to I/R decreases circulating monocytes and neutrophils, macrophage secreted cytokines, and neutrophil infiltration into injured myocardium. These results suggest Gd decreases leukocyte migration and activation may be a novel treatment for inflammation during ischemia and reperfusion.

A152

THE DISCOVERY OF A SERIES OF NOVEL SMALL MOLECULE MACROCYCLIC TNF- α ANTAGONISTS

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We have developed an integrated platform for the synthesis and screening of macrocyclic molecules (EnsemblinsTM) that can interact with protein-protein drug discovery targets. Tumor necrosis factor alpha (TNF-

α) has been linked to the pathogenesis of inflammatory disease, and agents that can prevent binding of TNF- α to its receptors have utility in the treatment of rheumatoid arthritis, Crohn's disease, psoriasis and ankylosing spondylitis. Currently marketed drugs are biologicals that target sequestration of TNF- α , and there are very few small molecule TNF- α antagonists at any stage of development. Ensemble Discovery has recently identified a series of selective and reversible small molecule macrocycles that competitively antagonize the activity of TNF- α on TNF receptors in both biochemical and cell-based assays. These compounds are currently in pre-clinical development.

A153

IDENTIFICATION OF FUNCTIONAL ROLES FOR BOTH IL-17RB AND IL-17RA IN MEDIATING IL-25 INDUCED ACTIVITIES

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IL-25 (IL-17E) is a unique IL-17 family ligand that promotes Th2-skewed inflammatory responses. Intranasal administration of IL-25 into naïve mice induces pulmonary inflammation similar to that seen in patients with allergic asthma, including increases in BALF eosinophils, BALF IL-5 and IL-13 concentrations, goblet cell hyperplasia, and increased airway hyperresponsiveness. IL-25 has been reported to bind and signal through IL-17RB (IL-17BR, IL-17Rh1). It has been demonstrated recently that IL-17A signals through a heteromeric receptor composed of IL-17RA and IL-17RC. We sought to determine whether other IL-17 family ligands also utilize heteromeric receptor complexes. The required receptor subunits for IL-25 biological activities were investigated *in vitro* and *in vivo* using a combination of knockout (KO) mice and antagonistic antibodies. Unlike wild-type mice, cultured splenocytes from either IL-17RB KO or IL-17RA KO mice did not produce IL-5 or IL-13 in response to IL-25 stimulation, and both IL-17RB KO and IL-17RA KO mice did not respond to intranasal (IN) administration of IL-25. Furthermore, treatment with antagonistic monoclonal antibodies to either IL-17RB or IL-17RA completely blocked IL-25 induced pulmonary inflammation and airway hyper-responsiveness (AHR) in naïve BALB/c mice, similar to the effects of an antagonistic antibody to IL-25. Finally, a goat blocking antibody to human IL-17RA prevented IL-25 activity in a primary human cell based assay. These data demonstrate for the first time that IL-25 mediated activities require both IL-17RB and IL-17RA, and provide another example of an IL-17 family ligand that utilizes a heteromeric receptor complex.

A154**THE ANTI-OBESITY EFFECT OF GYEONGSHINHAEGIHWAN T2 IS ASSOCIATED WITH A DECREASED NITRIC OXIDE SYNTHESIS**

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Gyeongshinhaegihwan T2 (GGT2) is a newly developed oriental medicine to help control weight. To evaluate the anti-obesity effect of GGT2, we investigated a possible effect of GGT2 on macrophage-related obesity reactions; nitric oxide production and cytokine secretion in mouse peritoneal macrophages. According to recent reports, macrophages are participated in fat accumulation and closely related with obesity. In this study, using mouse peritoneal macrophages, we have examined whether GGT2 affects the production of nitric oxide (NO), tumor necrosis factor- α (TNF- α), and interleukin (IL)-12 by interferon- γ and lipopolysaccharide (LPS). GGT2 inhibits LPS-induced NO production in a dose-dependent manner. The decrease in NO synthesis was reflected as a decreased amount of inducible NO synthase protein. We also found that GGT2 inhibits pro-inflammatory cytokines, TNF- α and IL-12 production. In mouse embryo preadipocyte 3T3-L1, GGT2 reduced the viability in a dose-dependent manner. These findings mean that GGT2 can be used in preventing and controlling adipogenesis and obesity.

A155**EFFECTIVE MECHANISM OF HERB COMPLEX PRESCRIPTION 'ANSSICHEGAMSAN' IN OBESITY**

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The Anssichegamsan (ACS) is a newly developed dietary product to help control weight. The aim of this study was to evaluate whether ACS combined with a high fat (HF) diet could influence body weight, fat accumulation and glucose level in blood. Rats were fed for 6 weeks with standard diet, HF diet, and HF + 10% ACS diet. Body weight was recorded weekly, and plasma levels of total cholesterol, triglyceride, and glucose were analyzed at the end of the study. Weight increases in the 10% ACS group were significantly less than in the HF diet group ($P < 0.05$). Plasma triglyceride level and total cholesterol level were significantly decreased by 15.0%, and 24.2% in ACS diet group. Glucose level also was decreased by 61.8% in ACS group compared with HF diet group. In addition, ACS inhibited obesity related cytokines, such as interleukin (IL)-6, tumor necrosis factor- α , and IL-1 β in rats' serum. *In vitro*, the author investigated ACS can prevent the differentiation of preadipocyte to adipocyte. Triglyceride contents of adipocytes were also inhibited by ACS significantly, and these effects were through inhibition of PPAR γ expression. These findings indicate that ACS may be beneficial in the reduction of HF diet-induced overweight.

A156**EXPLORATION OF THE MAP3K TAK1 AS A TARGET FOR MODULATING INFLAMMATORY ARTHRITIS**

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In various human fibroblasts, TGF- β -activated kinase-1 (TAK1) has been demonstrated as a pivotal upstream mediator of NF- κ B, p38 MAPK and JNK activation induced by IL-1, TNF α and TLR2/4 ligands. Therapeutic applicability of TAK1 as a target in synovial fibroblasts was tested using the small molecule inhibitor 11,12-dihydro-5Z-7-oxozeaenol (BRON). At a concentration of 1 μ M, a significant reduction was observed for IL-1 β -induced activation of the NF- κ B and C/EBP β -dependent SAA3 promoter. Using a lentiviral 5xNF- κ B-luciferase reporter it was shown that this reduction was not mediated through a diminished NF- κ B activation. Despite the lack of blocking NF- κ B, TAK1 inhibition proved very effective in preventing IL-1 β -induced upregulation of matrixmetalloproteinases (MMP-1,3,13) expression and markedly reduced the upregulation of chemokines (IL-8, MCP-1) and IL-6. Since synovial fibroblasts are the main producers of these genes in the inflamed joint, targeting TAK1 using small molecule inhibitors or gene therapy overexpressing a dominant-negative mutant of TAK1 hold promising potential in treatment of inflammatory arthritis.

A157**TREATMENT WITH ANTI-CD30 LIGAND PREVENTS DISEASE PROGRESSION IN MURINE SYSTEMIC LUPUS ERYTHEMATOSUS**

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The cell-surface glycoproteins CD30 and CD30 ligand (CD30L) are expressed primarily on activated immune cells, and their interactions regulate T cell responses. Systemic lupus erythematosus (SLE) is an autoimmune rheumatic disease exhibiting many immunologic abnormalities. Similar to human SLE, disease expression in NZB/W F1 mice requires activated T cells to stimulate B cells that secrete high affinity IgG autoantibodies. We used flow cytometry to determine the kinetics and cellular expression of CD30L during the course of lupus disease in untreated NZB/W F1 mice, and we compared the ability of CD30L-blocking/depleting and CD30L-blocking antibodies to prevent lupus disease progression in these mice. CD30L was expressed on subpopulations of leukocytes during the course of disease. The progression of lupus disease was prevented by treatment with CD30L antibodies. However, a CD30L blocking antibody with depleting properties was superior to simply blocking CD30/CD30L interactions. These data suggest that depletion of CD30L⁺ cells may be a viable treatment for human SLE patients.

A158**ELIMINATION OF TOLMETIN ULCERS BY ANISODAMINE (ANSA)**

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The efficacy of Tolmetin and other NSAIDs have been shown to be enhanced by acetaminophen (APAP). However APAP has no significant effect on the ulcerogenicity of NSAIDs (Wong & Gardocki, 1983). All NSAIDs, including Selective COX-2 Inhibitors, have ulcerogenic activity, leading the Food and Drug Administration (FDA) to withdraw Vioxx from the market and mandated warning labels added to all NSAID package inserts. Therefore it has become extremely important to find a non-ulcerogenic AI agent. Recent investigations by Healing Care, Ltd., discovered that the ulcerogenicity of NSAIDs were suppressed by Tropane alkaloids, atropine, scopolamine, and anisodamine (ANSA).

Careful studies, using a five-day rat ulcerogenicity assay, showed that Tolmetin ulcers could be eliminated by ANSA. The ID₅₀ and ID₁₀₀ values (53.7 and 148 mg/kg/day, p.o. respectively) were determined. Further studies with Ibuprofen, Naproxen and Piroxicam generated similar activity profiles.

Conclusion: "Ulcer induction by NSAIDs can be antagonized or eliminated by ANSA, a Tropane alkaloid".

A159**SMALL MOLECULE CXCR2 ANTAGONIST PREVENTS HYPEROXIA-INDUCED NEUTROPHIL ACCUMULATION IN THE LUNGS OF NEWBORN RATS**

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Recruitment of neutrophils is a normal physiological response to infection and tissue damage. However, excessive numbers of these cells can exacerbate tissue damage by releasing proteases, oxygen radicals, and other mediators contributing to the development and severity of conditions such as COPD and acute respiratory distress syndrome. CXC chemokines interact with CXCR1 & CXCR2 and are known to mediate, in part, the recruitment of neutrophils to areas of lung injury. A series of acylsulfamide derivatives were identified that function as competitive, reversible small molecule inhibitors targeting CXCR2. A lead compound in the series exhibited an IC₅₀ of 50nM against Eu-IL-8/CXCR2 membrane binding, inhibited IL-8-induced calcium flux in cells expressing hCXCR2 (IC₅₀ = 5nM) and inhibited rabbit neutrophil GRO α -driven chemotaxis. This compound also demonstrated human liver microsomal stability, low clearance (3.8 mL/min/kg with t_{1/2} = 2.7h) and excellent oral bioavailability (107%). In vivo, the compound was active at preventing neutrophil accumulation in the lungs of newborn rats exposed to hyperoxic conditions. These results support the continued evaluation of CXCR2 antagonists as therapeutic agents in diseases where neutrophil-mediated exacerbation is present.

A160**SYNERGISTIC INDUCTION OF IL-10 BY A TLR AGONIST AND A PHOSPHO-CERAMIDE ANALOG IS MEDIATED BY CAMP**

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Expression of the anti-inflammatory cytokine interleukin 10 (IL-10) can be induced either by toll-like receptor (TLR) agonists such as LPS, or by various endogenous stimuli, in particular those acting via a cAMP-dependent signaling pathway. Phospho-ceramide analog-1 (PCERA-1): 1-methyl-2-(3-methoxyphenyl)-2-(octanoylamino)ethyl-

disodium-phosphate, down-regulates LPS-induced production of TNF α in macrophages in a cAMP-dependent manner. The objective of this study was to evaluate the effect of PCERA-1 on IL-10 production, and to determine its mechanism. We show here that PCERA-1 induces IL-10 production in synergism with various TLR agonists. Cooperativity is evident both at the mRNA and protein levels. IL-10 production by LPS and/or PCERA-1 is mediated by PKA and the activity of PCERA-1 can be mimicked by a cell-permeable analog of cAMP. Furthermore, in the absence of PCERA-1, the residual IL-10 induction by LPS is completely blocked by the β -adrenergic receptor antagonist, propranolol. Our results thus indicate that basal cAMP is essential for IL-10 induction by LPS and that a co-stimulus by a TLR agonist and a cAMP-elevating agent results in synergistic IL-10 production.

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**DIFFERENTIAL MODULATORY EFFECTS OF TLR2 AND TLR4 ON T CELL BALANCE IN EXPERIMENTAL ARTHRITIS: POSSIBILITIES FOR NEW THERAPEUTIC STRATEGIES**

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Toll-like receptors (TLRs) might contribute to the progression of rheumatoid arthritis through recognition of microbial or host-derived ligands found in arthritic joints. We investigated the involvement of TLR2 and TLR4 in the progression of arthritis using IL-1 receptor antagonist-knockout (IL1Ra^{-/-}) mice, which spontaneously develop an autoimmune T cell-mediated arthritis. Clinical and histopathological evaluation of IL1Ra^{-/-} TLR2^{-/-} mice revealed more severe arthritis, characterized by reduced suppressive function of regulatory T cells (Tregs) and substantially increased IFN-gamma production by T cells. IL1Ra^{-/-} TLR4^{-/-} mice were, in contrast, protected against severe arthritis and had markedly lower numbers of Th17 cells and a reduced capacity to produce IL-23/IL-17. Therapeutic treatment of both IL-1Ra^{-/-} and collagen-induced arthritis models using a TLR4 antagonist prevented joint inflammation and cartilage and bone destruction.

These studies demonstrate distinct roles of TLR2 and TLR4 in the regulation of inflammatory cytokines and T cell balance, and indicate that TLR4 might be a potential therapeutic target in the treatment of RA.

VA02**THE ROLE OF CC CHEMOKINE RECEPTOR 7 DURING INVASIVE ASPERGILLOSIS**

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Aspergillus fumigatus is ubiquitously found in the environment and is easily cleared from the body in immunocompetent hosts. Invasive aspergillosis (IA) develops in immunocompromised patients, particularly in transplant recipients, and is a leading cause of death in patients who have undergone allogeneic hematopoietic stem cell transplantation (HSCT). Chemokine receptor 7 (CCR7) and its ligands, CCL19 and CCL21, are responsible for the migration of dendritic cells (DCs) from sites of infection and inflammation to secondary lymphoid organs. Thus, it has been suggested that CCR7 plays an important role in development of the adaptive immune response, while questions remain regarding the role of CCR7 during a primary immune response. We are currently investigating the role of CCR7 expression on DCs during an IA

infection using a murine HSCT model. Mice receiving CCR7 deficient stem cells have increased survival and decreased lung pathology compared to mice transplanted with wild type stem cells. Flow cytometric analysis of mice receiving wild type or CCR7 deficient stem cells, reveals an increase in the number of DC present in the lungs of the knockout animals following infection with *aspergillus* conidia. Additionally, RNA data shows an increase in IFN- γ , TNF- α , IP-10, and IDO in the mice receiving CCR7^{-/-} cells. Our results suggest that the absence of CCR7 provides protection from IA after myeloablation and stem cell reconstitution. This data reveals a potential role for CCR7 in a DC mediated primary immune response against *aspergillus fumigatus* during IA.

VA03**PRO-INFLAMMATORY CYTOKINES INHIBIT CHONDROGENESIS OF HUMAN MESENCHYMAL STEM CELLS THROUGH NF- κ B DEPENDENT PATHWAYS**

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The differentiation of mesenchymal stem cells (MSCs) into chondrocytes provides an attractive basis for the regeneration of articular cartilage, but chondrogenesis will often need to occur in the presence of inflammatory mediators produced in response to injury or disease. Here we examined the effect of two important inflammatory cytokines, interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α), on the chondrogenic behavior of human MSCs. Aggregate cultures of MSCs recovered from the femoral intermedullary canal were used. Chondrogenesis was evaluated by measuring proteoglycan and collagen synthesis at both the protein and message levels. The possible involvement of NF- κ B in mediating IL-1 β effects was assessed by adenoviral delivery of a dominant negative inhibitor of NF- κ B (srI κ B). Both IL-1 β and TNF- α inhibited hMSC chondrogenesis in a dose-dependent manner, which was associated with a marked activation of NF- κ B. Delivery of srI κ B abrogated the activation of NF- κ B and rescued the chondrogenic response. Strategies for enabling cell-based cartilage repair within inflamed joints include targeting not only individual pyrogens, such as IL-1 and TNF, but also important intracellular mediators, such as NF- κ B.

VA04**BZ-423 IMPROVES GVHD-ASSOCIATED TISSUE DAMAGE AND MORTALITY BY SPECIFICALLY TARGETING EFFECTOR T CELLS**

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Graft-versus host disease (GVHD) is the major complication of allogeneic bone marrow transplantation and causes systemic inflammation, which can be fatal if left untreated. The treatment of GVHD with High-dose corticosteroids results in complete responses in less than half of patients and often leads to severe immunosuppression and other serious consequences. We have investigated Bz-423, a novel therapeutic agent that targets the mitochondrial ATPase, in a model of acute GVHD. We found that Bz-423 treatment beginning on day 3 after transplant prevented GVHD-associated mortality and reduced donor T cell infiltration into the liver and bone marrow, two GVHD target organs. Importantly, Bz-423 did not impair donor T cell expansion or activation marker expression, indicating that, unlike High-dose corticosteroids, Bz-423 is not broadly immunosuppressive.

VA05**MAPPING THE BINDING OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) TO THE CHEMOKINE RECEPTOR CXCR4**

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The cytokine macrophage migration inhibitory factor (MIF) plays a critical role in many acute and inflammatory diseases. MIF acts as a major regulator of inflammatory cell recruitment and atherogenesis. These chemokine-like functions of MIF were recently shown to be mediated through interaction to the chemokine receptors CXCR4 and CXCR2. However, the molecular details of this interaction have not yet been determined. We describe herein a peptide derived from the N-terminal extracellular region of the CXCR4 receptor as a site of interaction with human MIF and *Leishmania major* MIF. From $^1\text{H}^{15}\text{N}$ chemical shift perturbation studies a direct binding interaction between MIF and the N-terminal 27 residues of CXCR4 is shown. Titration studies using steady-state fluorescence spectroscopy result in a dissociation constant of $3.1 \pm 0.8 \mu\text{M}$. Notably, MIF-triggered monocyte chemotaxis activity is ablated by this N-terminal CXCR4 peptide. Structural studies of the MIF/CXCR4 complex may aid in the design of new drugs targeting MIF related diseases.

Author Index

(* denotes presenting author)

Abdollahi-Roodsaz*, S. VA01
Abdollahi-Roodsaz, S. A122
Abdollahi-Roodsaz, S. A123
Abreu*, M.T. SA04
Adler*, K.B. SA24
Aggarwal, R. A116
Ahrens, E.T. A112
Akama, T. A139
Albers, R. A140
Ali, I. A101
Alley, M.R.K. A139
Alt*, C. A100
Amalfitano, A. A138
Amir*, M. A101
An, H-J. A119
An, H-J. A120
An, H-J. A154
An, H-J. A155
Anjum, S. A118
Antunes, J. A139
Appledorn, D.M. A138
Aran, J.M. A135
Arnett, H.A. SA01
Aslam, K. A147
Audoly, L. SA13
Audoly, L. A109
Avni, D. A160
Awan, S.I. A118

BaileyHealy, I. A103
Baillie*, R.A. A102
Baillie, R. A116
Baker, J.E. A151
Banfield, C. SA22
Baugh, J. A127
Bekkali, Y. A125
Bendele, A. A134
Bennett, B. A140
Benton, B. A152
Bentzien, J. A125
Berg, E.L. A132
Berson*, A.E. A103
Bhagwat, S. A140
Bhaskiaraj, D. A145
Bilter, G. A140
Bishop, G.A. A128
Boczon, L. A159
Bolognese, B.J. A136
Bond, J. A152
Bose*, S. A104
Brameld, K. A106
Brenner, D. A140

Briggs, T.F. A152
Brombacher, F. A113
Brovarney, M. A103
Brown, S.A. A106
Bryant*, S. A105
Bryant, S. A114
Bucala, R. A127
Budelsky, A.L. A153
Buhr, C. A140
Burgess*, L.E. A106
Burnes, L.A. SA11
Burnet, M. A121
Burnet, M. A142
Burnet, M. A143
Burnette*, B. A107

Calderwood, D. A114
Cao, Y. A117
Capper-Spudich, E.A. A136
Castaneda, J. A109
Castellino*, F. SA14
Chagnovich*, D. A108
Chantry, D. A106
Chao, Q. A140
Chen*, B. A109
Chen*, G.G. A110
Chen, C. A111
Chun, S.L. A110
Cibotti, R. A109
Collins*, D. A111
Comeau, M.R. A153
Cornicelli*, J. A112
Cory Hogaboam VA02
Côté, B. SA13
Cox*, G. SA23
Coyle, A.J. A109
Crandall, T. A141
Crysler, C. A159
Cutler, A. A113

D'Andrea, A. A100
D'Sidocky, N. A140
Dabbagh, K. A103
De Groot*, A.S. SA15
Denning, T.L. SA02
Devesa, I. A122
Devesa, I. A123
Devlin, J.P. A158
Donatelli, R. A159
Ducharme, Y. SA13
Dugo, L. A143
Dyer, R. D. A108

Ehlers*, S. A113
Evans, C.H. VA03

Fasciano, S. A126
Favaloro Jr., F. A152
Feeney, E. SA08
Fellows, J. A108
Ferrara, J.L.M. VA04
Ferri, R. A140
Foley, J.P. A136
Freund, Y. A139
Friedman, G. A140
Friesen, R.W. SA13
Fuentes, M.E. A103

Gaffen*, S.L. SA18
Gaffen, S.L. A146
Gan, L. A126
Garssen, J. A115
Getz*, G.S. SA17
Geurts, J. A156
Glick, G.D. VA04
Glodek, A. A109
Goldsmith, M. A160
Goudreau, R. A114
Grimshaw, C. A140
Groneberg, R.D. A106
Guse, J-H. A121

Hale, S. A152
Han, Y. SA13
Hart*, M. A114
Hart, M. A105
Hartigan*, A. VA02
Hartog*, A. A115
Havekes, L. A129
Heitmann, L. A113
Herbst, R. A109
Ho*, R. A116
Hoag, K.A. A138
Hodsdon, M.E. VA05
Holan, V. A143
Hölscher, C. A113
Hong, J-W. A119
Hong, J-W. A120
Hong, J-W. A154
Hong, J-W. A155
Hong, S-H. A119
Hong, S-H. A120
Hong, S-H. A154
Hong, S-H. A155
Horner, M. SA22

- Horrigan, S.K. A109
 Horstmann, R.D. A113
 Hsu, A. A151
 Hu, Y-L. A157
 Huang*, Y. A117
 Hunt, K.W. A106
 Hussain, Z. A144
 Hyland, D. A105
 Hyland, D. A114

 Iacomini*, J. SA07
 Ikeuchi, M. SA11

 Jain, S. A150
 Jawed*, H. A118
 Jawed, H. A144
 Jawed, H. A147
 Jeong, H-J. A119
 Jeong, H-J. A120
 Jeong, H-J. A154
 Jeong, H-J. A155
 Ji, L.L. A117
 Johnson, E. A143
 Johnson, Y. A110
 Jones, R. A142
 Joosten, L. VA01
 Joosten, L.A.B. A122
 Joosten, L.A.B. A123
 Jungbluth, G. A107

 Kaddurah-Daouk, R. A102
 Kahn, J. A125
 Kaimal, V. A112
 Karaoglu Hanzatian, D. A104
 Kaur, T. A149
 Kavathas, P. A127
 Kaymakcalan, Z. A104
 Kehlet, H. A133
 Khatchenko, O. A140
 Khurshid, S. A147
 Kiener, P.A. A109
 Kim*, H-M. A119
 Kim*, H-M. A120
 Kim, H-M. A154
 Kim, H-M. A155
 Kim, K-Y. A120
 Kimura, R. A139
 Koch*, P. A121
 Koch, K. A106
 Koch, K. A134
 Koenders*, M.I. A122
 Koenders*, M.I. A123
 Kohm, A. SA08
 Kois, A. A140
 Kolker, S.J. SA11
 Kou, J.P. A136
 Krupinski, J. A135
 Kugler, D. A153
 Kulkulka, A. J. A125
 Kunkel, E.J. A132
 Kurumbail, R. A107

 Lagerweij, T. A129
 Lagerweij, T. A131
 Laufer, S. A143

 Laufera, S. A121
 Laurence*, A. SA21
 Le*, Y. A124
 Leáñez, S. A137
 Lee*, T.W. A125
 Lee, J. A152
 Lee, P. A106
 Lee, P. A134
 Lee, S.J. A127
 Lees, M. A142
 Leimgruber, R.M. A107
 Leisten, J. A140
 Leith, A. A157
 Leong, L. A159
 Lew, W. A140
 Lewis, A. A140
 Li*, L. A126
 Li, N. A146
 Lin*, S-L. SA22
 Lin, M. A141
 Liu, D. A109
 Lolis, E. VA05
 Long, M. W. A108
 Lopez, L. A134
 Lu*, J. SA16
 Lubberts*, E. SA20
 Luo, X. SA08

 Maes, D. A111
 Mailliard, R.B. A112
 Maitra, U. A126
 Mancini, J. A142
 Mancini, J. SA13
 Manning*, T. SA26
 Manning, A. A140
 Mansfield, L.S. A138
 Manthey, C. A159
 Mao, C.P. A103
 Mao, S.H. A117
 Maples, K. A139
 Mareska, D.A. A106
 Marijnissen, R.J. A122
 Marijnissen, R.J. A123
 Martin, A. SA08
 Mathieu, S. A105
 Mazzulla, M. A159
 McAlonan, L. A159
 McCarrick, M. A140
 McCarthy, D. SA08
 McConville, P. A112
 McEvoy, J. A102
 McKenzie, A.N.J. A113
 McQueney, M.S. A136
 Melrose, J. A132
 Merk*, M. A127
 Merk, M. VA05
 Meyer, C.G. A113
 Miller*, S. D. SA08
 Mnich, S. A107
 Mogil*, J.S. SA10
 Molloy, C. A159
 Momin, D. A147
 Monahan, J. A107
 Monterosso, T. A112
 Moradi, V. A143

 Morris, C. A109
 Motiwala, A. A140
 Muir, J. A140
 Muizzuddin, N. A111
 Munroe*, M.E. A128
 Murphy, J. VA05
 Murray, L. A159
 Murtaza, A. A105
 Murtaza, A. A114

 Nabozny, G. A125
 Nadolny, L. A140
 Nagelkerken*, L. A129
 Nagelkerken*, L. A130
 Nagelkerken*, L. A131
 Naiman, B. A109
 Nair, R. SA02
 Nelson, A.D. A112
 Nguyen, D. A132
 Nicolosi, A.C. A151
 Nielsen, H.J. A133

 O'Leary, E. A140
 O'Mahony*, A. A132
 Olivar, R. A135
 Olson, L. A105
 Olson, L. A114
 Omholt, P. A140
 Oranje, A.P. A129

 Pai, S. A140
 Pal Singh, A. A150
 Pal Singh, H. A149
 Palmer, G.D. VA03
 Pavan, S. A131
 Pedersen, J.L. A133
 Persoon-Deen, C. A129
 Persoon-Deen, C. A130
 Persoon-Deen, C. A131
 Petersen*, L.J. A133
 Pheneger*, J. A134
 Pheneger, J. A106
 Picarella, D. A141
 Plantevin, V. A140
 Plattner, J. A139
 Plomp, A. A130
 Pluvinet*, R. A135
 Podolin*, P.L. A136
 Pol*, O. A137
 Polgar, W. A100
 Porter*, R.M. VA03
 Poy, N. A103
 Prasad, S. SA08
 Privat, S. A132
 Pulendran*, B. SA02

 Rajagopal, S. A145
 Ramachandran, U. A145
 Rathinam*, V.A.K. A138
 Ravindran, P. A103
 Raymon, H. A140
 Raymond, H. A159
 Raza Shah, M. A144
 Regan, J. A125
 Rickel, E.A. A153

- Riendeau, D. SA13
 Rim, H-K. A119
 Rim, H-K. A120
 Rim, H-K. A154
 Rim, H-K. A155
 Rock, F. A139
 Roitt, I. A142
 Rosler, E. A132
 Rottman, J. A153
 Rottman, J.B. A157
 Routhu, K.V. A151
 Ruan, L. A124
 Ryan, D. A159
- Saeed, S.A. A147
 Sahasrabudhe, K. A140
 Saifullah, M.K. A101
 Sakata, S. A140
 Salazar, R.M. SA02
 Sanders*, V. A139
 Sanjay, K.V. A145
 Sapienza, J. A140
 Satoh,* Y. A140
 Savinainen*, A. A141
 Schlachter, S.T. A106
 Schopf, L. A105
 Schopf, L. A114
 Schreiber, T. A113
 Seed*, M. A142
 Seed*, M. A143
 Selness, S. A107
 Shah*, S.U. A144
 Sharma*, G.V.R. A145
 Shen,* F. A146
 Shevach*, E.M. SA06
 Shevlin, G. A140
 Shew, K. A100
 Shirley, M.A. A140
 Siegel, L.A. A153
 Simjee*, S.U. A147
 Simjee, S.U. A118
 Simjee, S.U. A144
 Sims, G.P. A109
 Singh*, A. A148
 Singh*, A.P. A149
 Singh*, H.P. A150
 Singh, A. A148
 Singh, A. A149
- Singh, A. A150
 Singh, H. A148
 Skov, P.S. A133
 Sluka*, K.A. SA11
 Smith, C. SA08
 Smits, M. A130
 Soppet, D. A109
 Souza, D. A125
 Stolaki, M. A131
 Strande*, J.L. A151
 Subasinghe, N. A159
 Sukunath, N. A145
 Sumoy, L. A135
 Swanson, R.M. SA01
- Targan*, S. SA05
 Terrett*, N. A152
 Therien, A. SA13
 Thirunavakkarasu, S. A145
 Thomson, D. S. A125
 Thye, T. A113
 Tielen, F. A131
 Tocker *, J.E. A153
 Tocker, J. SA22
 Toll, L. A100
 Tomczuk, B. A159
 Tong, M.C.F. A110
 Torres, I. A137
 Tran, T.T. A100
 Turley, D.M. SA08
- Uehara, T. A140
 Um*, J-Y. A154
 Um*, J-Y. A155
 Um, J-Y. A120
 Um, J-Y. A119
- van de Loo*, F.A.J. A156
 van de Loo, F. VA01
 van den Berg, W.B. A156
 van den Berg, W.B. A122
 van den Berg, W.B. A123
 van den Berg, W.B. VA01
 van der Mark, K. A130
 van Hasselt, C.A. A110
 Veldman*, T. SA25
 Verbeek, R. A129
 Verzaal, P. A129
- Verzaal, P. A130
 Vincent, M. SA22
 Viney*, J.L. SA01
 Vlantis, A.C. A110
 Vo, D A152
- Waegell, W. A114
 Wahl*, D.R. VA04
 Walder, R.Y. SA11
 Wang, D. A126
 Wang, J. M. A124
 Weaver*, C.T. SA03
 Wehling, N. VA03
 Westwick, J. A140
 White*, F.A. SA12
 Wilder*, R.L. SA09
 Willis*, C.R. A157
 Wilson, C. A152
 Winkler, J. A134
 Winters, M. A159
 Wixted, W.E. A136
 Wong*, S. A158
 Wood, T A146
 Worms, N. A130
 Worms, N. A131
 Wright*, J. SA19
 Wright, D. A134
 Wright, J. A140
 Wu, C. A104
 Wu, L. A141
- Xu*, D. SA13
 Xu, L. A140
 Xu, W. A140
 Yi, C. A117
- Yoon, B-R.P. A153
 Yurkow*, E. A159
- Zaman, M.S. A101
 Zhao, S. A159
 Zhou, Y. A139
 Zierow*, S. VA05
 Zierow, S. A127
 Zong, Q. A109
 Zor*, T. A160
 Zuvela-Jelaska, L. A125