Welcome

The International Cytokine and Interferon Society 2014 Annual Meeting

Cytokines Down Under: From Bench to Beyond

26th October – 29th October, 2014

Melbourne Convention & Exhibition Centre

Dear colleagues,

On behalf of the Scientific Organizing Committee, it is with great pleasure to welcome you to Melbourne to attend the second annual meeting of the International Cytokine and Interferon Society (ICIS). Specific topics will include the latest aspects on the biology, signal transduction and gene regulation of cytokines, interferons and their receptors in innate and adaptive immunity, as well as pattern recognition receptors and their role in host-pathogen interactions, infectious diseases, inflammation, cancer, autoimmunity and metabolism. Sessions will include cutting edge basic science and clinical presentations in plenary and concurrent symposia, as well as eminent keynote presentations, and are strongly supported by poster sessions and trade displays.

The meeting promises to provide an outstanding forum for basic science and clinical researchers to present their latest data and exchange ideas relating to the broad role of cytokines and interferons in human disease, and applications to therapies. In addition, the meeting will provide strong networking opportunities for scientists in the biotechnology and pharmaceutical industries. We are pleased with the attendance from all over the globe by both established and new investigators and students –Thanks for your support. We thank the Society and all sponsors who have helped to make this happen. This broad attendance, will help assure a vibrant and exciting conference for all.

We also note that Australia, and Melbourne in particular, is a perfect location to visit at this time of year, being in the peak of Spring. Melbourne is a vibrant and multicultural city known as the “sporting capital of Australia”, being home to the Australian Tennis Open, Australian Formula 1 Grand Prix, and the Spring Racing Carnival featuring the Melbourne Cup (first Tuesday in November), one of the largest and most prestigious horse races in the world. Together with its renowned riverside atmosphere, café culture, nearby beaches and wineries, it is no surprise Melbourne has been voted the most liveable city in the world for the 4th year in a row, with other Australian capital cities Adelaide, Sydney and Perth also featuring in the top 10.

We trust you will have a most productive conference and memorable experience whilst in Australia.

Brendan Jenkins and Paul Hertzog
Co-convenors

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Keynote Speakers

Peter Doherty, University of Melbourne

Professor Peter Doherty shared the Nobel Prize in Physiology or Medicine in 1996 with Swiss colleague Rolf Zinkernagel, for their discovery of how the immune system recognises virus-infected cells. He was Australian of the Year in 1997, and has since been commuting between St Jude Children's Research Hospital in Memphis and the Department of Microbiology and Immunology at the University of Melbourne. His research is mainly in the area of defence against viruses. He regularly devotes time to delivering public lectures, writing articles for newspapers and magazines and participating in radio discussions.

Peter Doherty graduated from the University of Queensland in Veterinary Science and became a veterinary officer. Moving to Scotland, he received his PhD from the University of Edinburgh Medical School. He is the first person with a veterinary qualification to win a Nobel Prize. Peter is also the author of several books, including "A Light History of Hot Air", "The Beginners Guide to Winning the Nobel Prize" and "Sentinel Chickens: What birds tell us about our health and the world".

Nicos Nicola, Walter and Eliza Hall Institute of Medical Research

Dr. Nicola's laboratory has focused on the molecular regulation of haemopoietic cell production and function for over 30 years. A major emphasis has been on the cytokines that regulate the production and functional activation of granulocytes and macrophages, two types of white blood cells that co-ordinate innate immune responses to bacterial and viral infections. His work has led to the identification, purification and/or molecular cloning of several important cytokines (G-CSF, GM-CSF and leukaemia inhibitory factor (LIF)), the identification and molecular cloning of several cytokine receptors (including the GM-CSF, interleukin-11 and interleukin-13 receptors) and the identification of a new family of inducible, intracellular inhibitors of these cytokine/receptor signaling pathways. These suppressors of cytokine signaling (SOCS) proteins have been shown to have crucial roles in vivo in limiting the extent and duration of responses to various cytokines thus preventing excessive inflammatory and autoimmune responses. He is also investigating the role of cytokines and cytokine signaling pathways in the development and maintenance of leukaemic cell populations and the usefulness of cytokines or cytokine antagonists as therapies or adjunct therapies in cancer treatments.

Luke O'Neill, Trinity College Dublin

The major focus of the group is to provide a molecular understanding of innate immunity and inflammation. We are interested in receptors involved in innate immunity, such as Toll-like receptors (TLRs) and Nod-like receptors (NLRs – including Nlrp3), and also signals activated, including NF-kappaB, IRF family transcription factors and MAP kinases. The role played by this system in inflammatory diseases is also under investigation. We have several projects underway including – Role of the adapter Mal in the epithelial barrier in the gut, Control of TLRs and NLRs by microRNAs, Role of Nlrp3 in Type 2 diabetes, Novel proteins in the TLR and NLR systems, The role of Btk in TLR signalling, The control of trafficking of TLR4, Genetic variation in innate immune genes and inflammatory diseases, Role of IL-36 in inflammation.

Symposia Speakers

Shizuo Akira, Osaka University

Shizuo Akira is a director and professor of WPI Immunology Frontier Research Center, and also a professor in Institute for Microbial Diseases at Osaka University, Japan. He received his M.D. and Ph.D. from Osaka University. After two years of postdoctoral working in Department of Immunology, University of California at Berkeley, he started to study on IL-6 gene regulation and signaling in the Institute for Molecular and Cellular Biology, Osaka University, and cloned transcription factors, NF-IL6(C/EBP beta) and STAT3. He was a professor in Department of Biochemistry, Hyogo College of Medicine from 1996 to 1999, where he became involved in Toll-like receptors research. By generating TLR family knockout mice, he identified ligands of many TLR members. He also demonstrated that the difference in signaling pathway among TLRs is due to selective usage of adaptor molecules such as MyD88 and TRIF. He demonstrated that pathogen-derived RNA is recognized by cytoplasmic receptor family, besides TLRs, and clarified the molecular mechanism of antiviral response against RNA viruses. His current research interests are molecular mechanisms of innate immunity and inflammation, which are studied mainly by generating knockout mice.
Awards and Honors: 2004 Robert Koch Prize (Robert Koch Foundation, Germany), 2006 William B. Coley Award for Distinguished Research in Basic Immunology (Cancer Research Institute, USA), 2007 Imperial Prize and Japan Academy Prize (Japan Academy), 2009 National Academy of Sciences of USA, Foreign Associate, 2009 Person of Cultural Merit (Japanese Government), 2010 Keio International Medical Science Prize (Keio University), 2010 Avery-Landsteiner Prize (German Society for Immunology), 2010 EMBO Associate Member

Mark Ansel, University of California San Francisco

Lymphocyte lineage decisions are critical for the development of protective immunity against a great diversity of pathogens, but improper or exaggerated responses also contribute to the development and pathology of autoimmune diseases, chronic inflammation, allergy, and asthma. The Ansel lab's primary experimental system is the differentiation of the central coordinators of adaptive immune responses -- helper T cells. Their distinct cellular identities (Th1, Th2, Th17, etc.) and associated functions are defined by characteristic gene expression programs. We and many others have documented how these programs are controlled by transcription factors, the cis-regulatory DNA elements to which they bind, and epigenetic modifications that constrain chromatin accessibility at those sites.
Most of our current work focuses microRNAs (miRNA) regulation of helper T cell behavior and immune function, and how immunogenic stimuli regulate miRNAs homeostasis. Naive CD4+ T cells that cannot produce any miRNAs exhibit reduced cell division and survival in response to immune stimuli. Surprisingly, they also undergo rapid unrestrained differentiation into effector cells. One of the goals of our research is to determine which specific miRNAs regulate each of these T cell behaviors, and which protein coding mRNAs they target to exert their effects. In addition, we learned that T cells rapidly reset their miRNA repertoire upon activation. This rapid change in miRNA expression may be important to allow T cells to change their gene expression programs and develop effector functions.

Frances Balkwill, Barts Cancer Institute

My research is focused on the links between cancer and inflammation, being especially interested in translating knowledge of cancer biology into new biological treatments for cancer and in the role that inflammatory cytokines play in cancer promotion.
My key areas of interest are: The Tumour Microenvironment, Inflammatory cytokines and chemokines in the tumour microenvironment, 'Ovarian' cancer with a focus on high grade serous and clear cell subtypes, Public engagement with biomedical science and science policy.

Jeff Babon, Walter and Eliza Hall Institute for Medical Research

Maintenance of the blood system is largely controlled by the secretion of small glycoprotein messengers called cytokines. Cytokine exposure initiates an intracellular signalling cascade that is driven by activation of a family of receptor-bound tyrosine kinases known as JAKs (Janus Kinases). Under physiological conditions JAK activation and signalling is tightly regulated, in particular, by the SOCS (Suppressors of Cytokine Signalling) proteins.

However, in certain disease situations, mutant JAK overcomes its regulation by SOCS, leading to haematological malignancies. One example of this is the myeloproliferative disorders; a group of conditions that cause haematological cell types (platelets, white blood cells, and red blood cells) to grow abnormally in the bone marrow. Our laboratory uses the techniques of Structural Biology, Biochemistry and Biophysics to uncover the mechanism of JAK/STAT signalling and its regulation by the SOCS proteins.

Gabrielle Belz, Walter and Eliza Hall Institute for Medical research

Our specific research interests include:

Deciphering the genetic control of effector and memory T cell differentiation, Unravelling the transcriptional regulation of dendritic cell development and function, Determining the control of innate immune cell subsets and their contribution to protective immunity during infection, Transcriptional and molecular regulation of peripheral lymphocyte differentiation

We are currently studying the following research areas: Development and specialization of dendritic cells, Id and E protein regulation of effector and memory T cell differentiation in infection, Transcriptional networks controlling T cell differentiation, Development and function of innate immune cells.

Our research is focused on understanding how a subset of white blood cells, called cytotoxic T cells (CTL, or CD8 T cells), recognise and remove virally-infected cells from the body following infection. The aim is to identify specific factors that determine how virus-specific killer T cells develop during an infection and how they impact on the establishment of immune memory after virus infection.

Our laboratory uses a combination of cutting-edge cellular and molecular technologies to dissect and study virus-specific T cell responses to Influenza A and herpes viruses. These include techniques such as flow cytometry, cell sorting, PCR and imaging.

Andrew Brooks, University of Queensland

Dr Andrew Brooks is a Senior Research Officer in the Institute for Molecular Bioscience (University of Queensland, Australia) and joined the research group headed by Prof Michael Waters in 2006. Andrew completed his Honours research on Flaviviruses in 1996 at the Department of Microbiology and Immunology at James Cook University and then moved to the Department of Biochemistry to study Dengue Virus where he completed his PhD in 2002. He then moved to St Jude Children's Research Hospital in Memphis, TN, USA where he researched the role of Epstein-Barr Virus in oncogenesis. Andrew's research interests are in cell signalling, oncogenesis, and virology. His current research focus is on the molecular mechanism of Growth Hormone mediated signalling via the Growth Hormone Receptor (GHR), the role of GHR in oncogenesis, and the role of Growth Hormone signalling in liver regeneration. This research has led to publications in journals including Science, Nature Cell Biology, PNAS, and Molecular Endocrinology. He has been the recipient of over $1.5 million in research grant funding and has a number of national and international collaborations. Andrew is an Editorial Board member for the Journal JAK-STAT and has been a committee member of Australian Early-Mid Career Researchers Forum (AEMCRF) launched by the Australian Academy of Science.

Yinon Ben-Neriah, Hebrew University-Hadassah Medical School

Studying signaling pathways in animal models.

Cell growth and other processes associated with cancer are regulated by several major signaling pathways, among which are the NF-kB, Wnt and the p53 activation pathways, which are of major interests to us. We have elucidated some of the key steps in NF-kB activation, including the degradation of the NF-kB inhibitor IkB (Alkalay et al, PNAS 1995; Yaron et al, EMBO J, 1997 & Nature 1998) and identified CKIα as a key component in the β-catenin destruction machinery at the core of the Wnt signaling pathway (Amit et al, Genes & Development 2002, Elyada et al, Nature 2011). We learned that studying signaling pathways in animal models is far more rewarding than tissue culture studies, and a great deal of our lab work today is carried out in genetically engineered mouse models. We engineer many model mice by ourselves, breed them onto different mutated mice and examine the models for the purpose of identifying new disease mechanisms that explain complex human pathologies.

Much of our work it is done in collaboration with colleagues in Israel and abroad, Eli Pikarsky, Moshe Oren, Varda Rotter, Aaron Ciechanover and Kari Alitalo.

Zhijian James Chen, University of Texas Southwestern Medical Center

We are broadly interested in mechanisms of signal transduction, namely how a cell communicates with its surroundings and within itself. In particular, we are fascinated by how a cell detects harmful or foreign insults and mounts an appropriate response to restore homeostasis.

Our research revolves around the common theme of cell signaling and host defense: Ubiquitin signaling in the NF-κB pathway, Innate immune sensing and signaling of cytosolic nucleic acids.

Daniel Cua, Merck research Laboratories

Daniel Cua is the group leader in Pathways of Inflammation at Merck Research Laboratories in Palo Alto and was a previous Research Fellow at Schering Plough Biopharma. His work focuses on the discovery of novel biologic agents for the treatment of immune-mediated inflammatory diseases.

Since 2003, Cua also has contributed to scientific and medical publications, with more than 60 original articles published in prestigious journals such as Nature, Cell, Immunology, Journal of Experimental Medicine and Journal of Clinical Investigation. In addition, he is the co-inventor on 21 granted patents and applications.

Vojo Deretic, University of New Mexico

Vojo Deretic's main contributions to science come from studies by his team on the role of autophagy in infection and immunity. Autophagy, a cytoplasmic pathway for the removal of damaged or surplus organelles, has been previously implicated in cancer, neurodegeneration, development, and aging. Dr. Deretic's group is one of those that made the discovery that autophagic degradation is a major effector and aregulator of innate and adaptive immunity mechanisms for direct elimination of intracellular microbes (such as Mycobacterium tuberculosis). His current work is on the role of autophagy in immunity and inflammation.

Mark Febbraio, Baker Institute

Professor Mark Febbraio is a Senior Principal Research Fellow of the NHMRC, is the Head of the Cellular and Molecular Metabolism Laboratory and Head of the Division of Signalling & Metabolism at the Baker IDI Heart & Diabetes Institute. He is also the Chief Scientific Officer and on the Board of Directors of N-Gene Research Laboratories Inc., a USA based Biotechnology company. His research is focussed on understanding cellular and molecular mechanisms associated obesity and type 2 diabetes. He has authored over 200 peer reviewed papers in leading journals such as Nature, Nature Medicine, Cell, Cell Metabolism, Nature Immunology, The Journal of Clinical Investigation, PNAS and Diabetes. He has an H factor of 64 and over 11,000 career citations. He has won prizes at international, national and institutional levels including the A K McIntyre Prize for significant contributions to Australian Physiological Science (1999), the Colin I Johnson Lectureship by the High Blood Pressure Research Council of Australia (2006) the ESA/ADS Joint Plenary Lecture (2009) and the Sandford Skinner Oration (2011). He is on the Editorial Board of Diabetes, The American Journal of Physiology Endocrinology & Metabolism, and the Journal of Applied Physiology.
Professor Febbraio is also dedicated to health and fitness and continues to complete in running races and multi-sport events.

Richard Flavell, Yale University School of Medicine

Richard Flavell is co-discoverer of introns in cellular genes: he showed DNA methylation correlates inversely with, and prevents, gene expression. He was the first to develop reverse genetics as a postdoc with Weissmann and in his own lab continued in this field throughout his career; he is a pioneer in the use of this approach in vivo to study function. Dr. Flavell's laboratory studies the molecular and cellular basis of the immune response. He has been instrumental in discovering the molecular basis of T-cell differentiation from precursor cells into differentiated subsets. This work led to the discovery of GATA3 as a critical regulator of the Th2 response and the first example of such a molecule in Th cell differentiation. He went on to demonstrate the first case of regulation of gene expression in trans, via "chromosome kissing." Moreover his laboratory has elucidated the mechanisms of immunoregulation which prevent autoimmunity and overaggressive responses to pathogens. Specifically, Dr. Flavell's laboratory has elucidated the role of TGF-β in the regulation of immune response. This work is of relevance both to the control of autoimmune disease and the evasion of immune response by tumors.
Dr. Flavell's laboratory has discovered the role of several receptor families in the innate immune response, including the role of several Toll-like receptors and intracellular Nod-like receptor families (NLRs). This has recently led to the elucidation of function of Nod2 in inflammatory bowel diseases and Nlrp proteins in the production of IL-1. Most recently he has established a fascinating connection between inflammasomes, microbial homeostasis and chronic diseases. He showed that inflammasome dysfunction causes dysbiosis of the microbiota which, in conjunction with a susceptible diet, leads to IBD and Metabolic Syndrome, including Obesity, Fatty Liver disease and Type 2 diabetes.

Frederic Geissman, King's College

The CMCBI was recently established in a new laboratory space in King's College London (Division of Immunology, Infection and Inflammatory Diseases) with the support of Arthritis Research UK.
We aim to advance knowledge of the molecular and cellular mechanisms of inflammation, and to open roads to innovative treatment of inflammation and inflammatory diseases.
We use both molecular approaches and in vivo model systems, to investigate the molecular and cellular pathways and networks that control inflammation. Research teams work on basic models of inflammation, as well as on human diseases. Research teams develop extensive collaborations among themselves and with other groups in the DIIID, the Randall Division and with Researchers across the world. Tools available in the lab include intravital microscopy, flow cytometry and cell sorting, mouse husbandry, and a Fly lab.

Florent Ginhoux, Agency for Science, Technology and Research (A*STAR)

My laboratory focuses on the biology of dendritic cells, which are very unique and special cells of the immune system. They are crucial pathogen sensing and antigen presenting cells that basically control the initiation of our body immune responses to any invading microbes or vaccines that we receive. We are trying to understand where do these cells come from in both mice and humans, how do they work and how they are made in order to better manipulate them in vitro and in vivo.

Elizabeth Hartland, University of Melbourne

Elizabeth Hartland obtained her B.Sc. (Hons) majoring in Microbiology and Biochemistry and subsequently her Ph.D in Microbiology from the University of Melbourne. She has held a Royal Society/NHMRC Howard Florey Fellowship in the Department of Biochemistry, Imperial College London, Lecturer/Senior Lecturer positions in the Department of Microbiology, Monash University, Australia and an Australian Research Council Future Fellowship at the University of Melbourne. She is currently Professor of Microbiology and Head of the Department of Microbiology and Immunology at the University of Melbourne. Professor Hartland has a long-standing research interest in the pathogenesis of infections caused by Escherichia coli and Legionella, with a focus on mechanisms of bacterial colonization and immune evasion.

Veit Hornung, University of Bonn

In our research projects, we are trying to understand what mechanisms are employed by our innate immune system to distinguish self from non-self. Central to this complex task is a repertoire of pattern recognition receptors (PRRs) that have evolved to detect the presence of microorganisms. The ligands or targets of these PRRs are usually referred to as pathogen-associated molecular patterns (PAMPs). PAMPs are typically molecular structures that are unique to the physiological processes of microorganisms but not the host. In addition, PAMPs usually constitute products that are essential to microorganisms and thus cannot be changed or lost by the respective microorganism via adaptive evolution. In addition to the recognition of PAMPs, some PRRs can also detect endogenous danger and stress situations. Cell stress or tissue damage can lead to the release of normally compartmentalized molecules or the chemical modification of self-molecules. These and other endogenous inflammatory signals that appear after cellular damage or due to metabolic derangements are collectively known as danger-associated molecular patterns (DAMPs), and their ability to trigger inflammation is mediated by the PRRs of our innate immune system.

Zhengfan Jiang, Peking University

Innate immunity is the first line of defense against microbial invasions via a set of pattern-recognition molecules. We have established a powerful cDNA library screening system to search systematically for new genes essential in innate immunity. Using Yeast two hybrid screen, immuno-purification and Mass-spec-based protein identification techniques, we are also interested in identifying new genes in the signal complexes. Current projects in the lab focus on 1) Mechanism through which cells recognize dsDNA. 2) Function of ubiquitination in innate immunity. 3) Mechanisms leading to cytokine production and secretion after infection. 4) Malfunction of innate immunity and related diseases.

Simon Jones, Cardiff University

It is likely that transition from the recruitment of leukocytes typically associated with innate immunity to those important in acquired immunity facilitates resolution of inflammation, and the restoration of normal tissue architecture. These findings emphasise a central role for IL-6 in the regulation of this step, and as such may be important in facilitating successful resolution of acute inflammation. Paradoxically, IL-6 concentrations are increasingly being monitored as a clinical marker of severity during ongoing inflammation or as an indicator of disease outcome. Our studies illustrate that in more chronic conditions like rheumatoid arthritis, sIL-6R-signalling elicits more detrimental consequences, and we have found that blockade of sIL-6R activity using soluble gp130 (sgp130) as a natural sIL-6R antagonist suppresses development of antigen-induced arthritis [Nowell et al., 2003 Journal of Immunology 171:3202; Richards et al., 2006 Arthritis & Rheumatism, In press]. Consequently IL-6 responses depend either on the inflammatory condition or its activities become dysregulated during more chronic inflammatory episodes. Ultimately these questions form the rationale basis for future investigations. In particular, translational studies in association with Professor Nicholas Topley are currently exploring the novel concept of 'resolution therapeutic®'. This therapeutic strategy is based on supplementing host defence to re-synchronise the immune response and in so doing promote inflammatory resolution. With Wellcome Trust support we have developed an IL-6-sIL-6R chimeric fusion protein, which we propose will aid resolution of local bacterial infections by facilitating transition from innate to acquired immunity.

Thirumula-Devi Kanneganti, St Jude Children's Research Hospital

Our research aims to understand how the innate immune system recognize and respond to pathogens and how mutations in these sensing systems affect the development of infectious, inflammatory, and autoimmune diseases in humans. Our initial studies examined the roles of TLRs, NLRs and their adaptor proteins in response to certain microbial components (Nature 440(7081):233-36; Nature Immunology 7(6):576-82; JBC 281(48):36560-68; Journal of Immunology 178(12):8022-27; Immunity 26(4):433-43). Ongoing studies are investigating the sophisticated system governing activation of signaling cascades and the mechanisms involved (Mol Cell Proteomics 7(12):2350-63; Blood 113(12):2742-5; Immunity 32(3):379-91).

Eicke Latz, University of Bonn

The innate immune system responds to microbial products and host factors that arise during tissue damage and metabolic dysregulation. Innate immune activation is important for the control of infections and overactivation can lead to inflammatory disease states. An international group of scientists work together with the major aims of: elucidating how the innate immune system maintains health and under which circumstances it promotes diseases, using basic research to decipher the molecular mechanisms of innate immune activation, developing novel therapeutic approaches that target a multitude of inflammatory diseases, such as Alzheimer's disease, diabetes, and atherosclerosis.

Fabienne MacKay, Monash University

Prof. Mackay's laboratory has an interest in autoimmune diseases and mechanisms leading to loss of immune tolerance, in particular that of B-lymphocytes. Prof Mackay has spent years studying a cytokine from the TNF superfamily named BAFF/BLyS and demonstrated the role of this factor in B cell survival. Excess BAFF leads to autoimmunity in mice and is associated with human autoimmunity, in particular systemic lupus erythematosus (SLE) and Sjögren's syndrome. Inhibitors of BAFF have recently met the primary endpoints in a phase III clinical trial with SLE patients, validating in human patients the critical role of the BAFF system in SLE.
BAFF drives autoimmunity independently of T cells but this process requires the function of Toll-like receptors (TLR) and as such drives an unusual form of autoimmunity. BAFF signals through three receptors BAFF-R, TACI and BCMA. BAFF-R triggers survival but is also express on activated T cells and regulatory T cells on which the role of BAFF-R is still obscure. TACI is expressed on a subset of B cells, monocytes and dendritic cells, and its expression is regulated by TLR activation. TACI is required for T-independent antibody responses but also negatively regulate B cells. Its role as a negative regulator of B cells remains unclear. TACI appears to be activated by oligomeric forms of BAFF and APRIL crosslinked by Heparan Sulfate Proteoglycans (HSPG) but not by trimeric ligands. BCMA is expressed on plasma cells and is required for cell survival. The function of APRIL another ligand for TACI and BCMA versus BAFF is also not fully understood. APRIL is highly expressed by cancer cells and it may drive TACI-dependent immune mechanisms in tumors. All these issues are currently addressed in Prof. Mackay's laboratory.
Prof. Mackay's lab also works on the chemokine receptor CXCR7, which is important for heart formation and tissue remodelling. CXCR7 deficiency delays some autoimmune conditions in mouse models and its role in T cells will be addressed.
Finally, Prof Mackay has worked on the role of neuropeptide Y (released in response to psychological stress) and its receptors in suppressing immune functions and protection against cancers.

Kingston Mills, Trinity College

Kingston Mills is Professor of Experimental Immunology, School of Biochemistry and Immunology, Trinity College Dublin (TCD). He is Head of The Centre for the Study of Immunology at Trinity Biomedical Sciences Institute and Theme Champion for Immunology, Inflammation and Infection at TCD. He is a graduate of TCD and trained at as a Postdoctoral Fellow at University College London and the National Institute for Medical Research, Mill Hill, London, before joining the Scientific Staff of NIBSC, Herts, UK. He returned to Ireland in 1993 to take up an academic position at National University of Ireland, Maynooth. He was appointed to a Personal Chair at Trinity College Dublin in 2001 and was Head of the School of Biochemistry and Immunology from 2008-2011. He heads an active research team focusing on T cells in infection, autoimmunity and cancer. He is co-founder of Opsona Therapeutics and TriMod Therapeutics, biotech companies focusing on the development of immunotherapeutics for inflammatory diseases and cancer.

Denise Monack, Stanford University

The primary focus of our research is to understand the genetic and molecular mechanisms of intracellular bacterial pathogenesis. We use two model systems, Salmonella typhimurium and Francisella tularensis, to study the complex host-pathogen interactions.
Both of these organisms survive and multiply in macrophages, an important immune effector cell. Macrophages express Pattern Recognition Receptors on the surface as well as in the cytosol. Our laboratory focuses on the cytosolic recognition of bacteria that leads to Type I Interferon signaling and Inflammasome activation. We take both a genetic and biochemical approach to understand the molecular mechanisms involved in host recognition pathways leading to inflammation and pathogen evasion mechanisms.
Salmonella typhi causes the systemic disease typhoid fever and Francisella tularensis causes the systemic disease tularemia ("rabbit fever"). Utilizing mouse models of systemic salmonellosis and tularemia, we would like to understand how Salmonella persists within certain hosts for years in the face of a robust immune response and how F. tularensis, a stealth invader, can cause a rapid, lethal infection.

Masaaki Murakami, Osaka University

Professor, Division of Molecular Neuroimmunology, Institute for Genomic Medicine and Graduate School of Medicine, Hokkaido University.
Education: 1983-1989 Hokkaido University, School of Veterinary Medicine, 1989-1993 Osaka University, Graduate School of Medical.
Research and career: 1993-1999 Assistant Professor, Institute of Immunological Science, Hokkaido University, 1999-2001 JST fellow, HHMI, National Jewish Medical and Research Center, 2001-2003 Visiting Associate Professor, Colorado University, Integrated Department of Immunology, 2002-2013 Associate Professor, Graduate School of Medicine and Frontier Biosciences, Osaka University, 2014-present Professor, Institute for Genomic Medicine and Graduate School of Medicine, Hokkaido University.
Research field:Neuro-immune interaction for the development of inflammation

Gabriel Nunez, University of Michigan

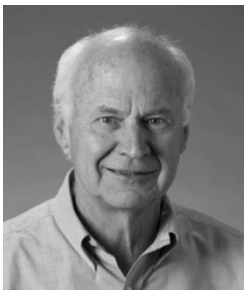
The Nuñez laboratory is interested in signaling pathways regulating innate immunity, the pathogenesis of inflammatory disease and cancer. Specifically, the research focuses on mechanistic studies to understand the role of proteins of the Nod-like receptor (NLR) and Toll-like receptor (TLR) families in the host immune response against microbial pathogens and endogenous danger signals. Several approaches that include analyses of genetically modified mutant mice, cell biology and biochemical studies are used to determine molecular mechanisms involved in the interaction between microbial/endogenous molecules and innate immune receptors. Several NLR proteins including Nod2 and Nlrp3/Nalp3 are mutated in patients with inflammatory diseases (Crohn's disease and autoinflammatory syndromes). Furthermore genetic variationin NLRs affect the susceptibility to asthma and sarcoidosis. Studies to understand how the NLR mutant proteins lead to disease are a major effort of the laboratory. Signaling pathways involved in inflammation and innate immunity are also known to play a role in cancer development particularly in the gastrointestinal tract. Understanding the mechanisms by which these pro-inflammatory pathways affect cancer initiation and/or progression in the intestinal tract is another interest of the laboratory.

Belinda Parker, La Trobe University

Our laboratory has a particular focus on determining the mechanisms of tumour cell spread to distant tissues and organs. In cancers such as breast and prostate, spread to distant organs can occur years after primary tumour removal and the treatments available for cancer patients with metastatic disease in tissues such as bone are rarely curative. For this reason, the focus of the Cancer Microenvironment and Immunology group is to determine the properties of tumour cells and interacting cells in the surrounding tissue that promote metastatic spread in clinically relevant models of breast and other cancers. Our ultimate goal is to predict patients that are likely to develop distant spread and to design new anti-metastatic therapies that block the invasion and growth of cancer cells in these distant tissues. Most models used to study bone metastasis involve injection of human cells into the blood stream of immunocompromised mice and do not allow interaction between tumour cells and stromal cells (other cells that interact with cancer cells in a tumour), nor permit analysis of the contribution of the immune system to metastasis. Using a model of breast cancer that mimics breast cancer metastasis and allows appropriate interaction between stromal cells and the immune system, our group has identified two pathways that are important in bone metastasis: A group of proteases called the cysteine cathepsins and their inhibitors that have an important role in tumour cells and surrounding stromal cells in invasion of breast cancer cells at the primary site and in metastatic tissues.
An immune defence pathway called the Type I IFN pathway that is produced by cancer cells and stimulates an immune response similar to the signals produced by infection. We have found that cancer cells suppress these signals to spread to the bone and that restoring the immune response has therapeutic value in breast cancer models.

Stefan Rose-John, University of Kiel

Since 25 years, my laboratory is focused on understanding the molecular biology of cytokines. Using structure-function analysis approaches, we have in the past defined the binding sites of Interleukin-6 and its two receptor subunits, Interleukin-6-receptor and gp130. This has enabled us to build the first molecular model of the Interleukin-6 receptor complex and was the starting point for the construction of various designer cytokines, which have considerably higher specific biologic activity than the natural cytokines. A second aspect of our work has started with the discovery of a naturally occurring proteolytic cleavage of the Interleukin-6 receptor, which leads to the generation of a soluble Interleukin-6 receptor. We have discovered that the complex of soluble Interleukin-6-receptor and Interleukin-6 stimulates cells, which express the signal transducing receptor subunit gp130 but not the ligand binding subunit Interleukin-6 receptor. In the absence of the sIL-6R such cells do not respond to IL-6. We have called this process 'transsignaling'.
We have shown in the past 10 years that 'trans-signaling' is important for the regulation of cellular differentiation and of apoptosis and has a prominent role in inflammation, neuronal survival, hematopoiesis, and tumor defense. We are currently developing the concept that the triggering of 'transsignaling' is an emergency reaction of the human immune system and that disruption of 'trans-signaling' can be used therapeutically for the treatment of chronic inflammatory diseases and cancer. Indeed, one cytokine antagonist, which – in animal models – has proven effective in blocking chronic inflammatory diseases such as Crohn's diseases, Rheumatoid Arthritis and inflammatory colon cancer, is now tested in Phase I clinic trials since June 2013.



Chuck Samuel, University of California Santa Barbara

The overall objective of the research in The Samuel Lab is to elucidate in molecular terms the mechanisms by which interferons exert their antiviral and cell growth control actions in mammalian cells. Present work includes biochemical and molecular genetic studies of two interferon-inducible enzymes, PKR and ADAR. PKR is a double-stranded RNA-dependent protein kinase induced by IFN, and activated by RNA-dependent autophosphorylation. PKR plays a major role in the regulation of translation of viral and cellular mRNAs and also modulates transcription and signaling. The ADAR1 deaminase is an RNA editing-enzyme that catalyzes the C-6 deamination of adenosine to yield inosine, thereby altering the genetic decoding and structure of RNAs. While PKR displays antiviral and proapoptic activities, ADAR1 is often proviral and antiapoptotic in virus-infected cells. Furthermore, PKR is not required for normal mouse embryogenesis, whereas ADAR1 is required.

Feng Shao, National Institute of Biological Sciences

Dr. Feng Shao is an investigator at National Institute of Biological Sciences (NIBS), Beijing, CHINA. He was a chemistry undergraduate of Peking University from 1991 to 1996 and received his master degree from Institute of Biophysics, Chinese Academy of Sciences in 1999. Dr. Shao was trained as a biochemist and obtained his PhD degree with Dr. Jack E. Dixon from University of Michigan in 2003. Prior to returning to China in 2005 to assume an assistant investigator and group leader position at NIBS, he was a Damon Runyon Postdoc Research Fellow at Harvard Medical School. Dr. Shao was promoted to be an associate investigator in 2009 and a full investigator in 2012 at NIBS. Dr. Shao's research focuses on biochemical mechanism of bacterial infection and host innate immune defense. His laboratory has discovered several novel post-translational modifications used by the bacteria to disrupt host immune defense as well as innate immune sensors for various bacterial molecules and toxins. Dr. Shao has authored 40 research articles in highly-regarded journals, including 10 in Nature, Science and Cell, and his work has been recognized by several renowned academic honors including the International Early Career Award from Howard Hughes Medical Institute and the Irving Sigal Young Investigator Award from the Protein Society. Dr. Shao also serves as journal editors for eLife and Cellular Microbiology.

Mark Smyth, Queensland Institute of Medical research

Professor Mark Smyth is regarded as an international leader in tumour immunology/immunotherapy and natural killer (NK) cell biology. He received his PhD from the University of Melbourne in 1988 and trained at the National Cancer Institute (USA) from 1988-1992. After 8 years at the Austin Research Institute in Melbourne, working on mechanisms of lymphocyte-mediated cytotoxicity, he relocated to the Peter Mac where his studies on effector molecules collectively rekindled world-wide interest in cancer immune surveillance. In recent years, Professor Smyth has: made key discoveries linking NK cells and adaptive T cell control of tumours; demonstrated a unique combination of pathways that can potently reject established cancers in mice; first defined immune-mediated dormancy of cancer; defined the role of host in chemotherapy responses; and defined adenosine and CD96 as new targets for cancer immunotherapy. A number of his discoveries have led to new clinical trials in cancer immunotherapy. In 2013, Professor Smyth relocated to the QIMR Berghofer in Brsibane as a Senior Scientist and NH&MRC Australia Fellow. He is a Senior Editor at Cancer Research, and a member of the Scientific Advisory Board of the Cancer Research Institute (USA).

Dale Umetsu, Genentech

Our lab focuses on the study of subpopulations of human and murine CD4+ T cells, which play a central role in the regulation of adaptive immunity and tolerance. The laboratory studies CD4+ T cells in humans and in mice, examining the function of CD4+ alphaß TCR T cells, antigen-specific regulatory T cells, as well as iNKT cells in regulating immune responses. We are interested in the cellular, molecular and genetic mechanisms that control the interaction of T cells with dendritic cells and that regulate cytokine synthesis in and the function of regulatory T cells and iNKT cells in the respiratory and gastrointestinal tracts. We are particularly interested in the function and immunology of the TIM family of genes, which we discovered several years ago, and which regulate the development of autoimmune and allergic diseases.

Uwe Vinkemeier, University of Nottingham

1992 - PhD in Biochemistry, Max-Planck-Institut für Biophysikalische Chemie in Göttingen; 1993-1999 - Post-doctoral Associate with James Darnell and John Kuriyan at the Rockefeller University in New York; 2000-2007 - Head of Junior Research Laboratory at the Leibniz-Institut für Molekulare Pharmakologie in Berlin; 2007 to date - Action Medical Research Chair of Cell Biology at The University of Nottingham.

Xiao-Fan Wang, Duke University

Xiao-Fan Wang was born in Wulumuqi, China. He entered Wuhan University in 1978 to receive his college education following the reform of the education system. In 1982, as one of the first group of Chinese students sent to study biology in the US, he started his graduate training in transcriptional regulation of immunoglobulin genes during B cell development with Dr. K. Calame at UCLA and received his Ph.D. in 1986. He then spent five years at Whitehead Institute and MIT as a postdoctoral fellow under the guidance of Dr. R. Weinberg. The main achievement during this period was the molecular cloning of transforming growth factor ß (TGF-ß) type II and type III receptors. In early 1992 he moved to Duke University Medical Center as an Assistant Professor in the Department of Pharmacology & Cancer Biology. He is currently Professor of Pharmacology and Cancer Biology, Donald and Elizabeth Cooke Professor of Experimental Oncology. His other academic activities include serving on the editorial boards of a number of scientific journals, such as an Associate Editor for the Journal of Biological Chemistry. The current research in the Wang laboratory mainly focuses on the elucidation of molecular nature and signaling mechanisms associated with tumor microenvironment that promotes tumor progression and metastasis. Particularly, the lab is interested in the roles of specific microRNAs as mediators of TGF-ß or hypoxia to affect the biological nature of tumor microenvironment, via the interactions with the immune system and the recruitment of various types of stromal cells, to enhance tumor metastasis. The lab is also studying the mechanisms underlying the phenomenon of cellular senescence.

Wolfgang Weninger, Centenary Institute

The laboratory is investigating how the cells of the innate immune system, the first responders, interact with pathogens in skin tissue. These researchers are challenging the tissue with different viruses, bacteria and parasites to test a variety of different models of infection. The studies have implications for the development of vaccines.

Hua Yu, Beckman research Institute

Dr. Hua Yu is Co-Leader of Cancer Immunotherapeutics Program, City of Hope Comprehensive Cancer Center and Associate Chair of the Department of Cancer Immunotherapeutics and Tumor Immunology. Dr. Yu is a noted expert and pioneer on the cancer-promoting protein STAT3 and was the first to uncover and define the protein's effect on the immune system. Dr. Yu's studies have laid the foundation for a new generation of molecular targeted cancer therapy approaches that disable both tumor cells and the tumor stromal cells, which are critical for tumor growth. She has developed a potentially paradigm-shifting new drug targeting STAT3 that will be among the first produced in City of Hope's new Chemical GMP Synthesis Facility.

Dr. Yu received her bachelor's and doctoral degrees from Columbia University. She completed fellowships with the American Cancer Society and the National Institutes of Health, and also was a faculty fellow at Columbia University. The fundamental discoveries from her laboratory have been well supported continuously by grants from the National Institutes of Health. Her recent studies have been published extensively in such prestigious biomedical/cancer research journals as Nature Medicine, Cancer Cell, Nature Biotechnology and Nature Reviews Cancer and Nature Reviews.

Elina I Zuniga, University of California San Diego

Viral persistence requires a long-term relationship between the host and the microbe that involves multiple layers of interactions from molecular to cellular to whole organisms. Our laboratory studies cellular and molecular aspects of virus-host interactions during acute versus chronic viral infections to determine general principles of viral immune-evasion, persistence and pathogenesis. The ultimate goal is to modulate or recover immune system functions to prevent or eradicate chronic viral infections. During the past years at UCSD, I have congregated a group of young scientists with diverse expertise and strong motivation to obtain solid answers for understanding immune responses during chronic viral infections, a topic of major biomedical relevance.

2014 Award Winners

Seymour and Vivian Milstein Award for Excellence in Interferon and Cytokine Research
Dr. Stefanie N. Vogel, University of Maryland School of Medicine, Baltimore, MD
Dr. Katherine A. Fitzgerald, University of Massachusetts Medical School, Worcester, MA

Honorary Lifetime Membership Award
Charles E. Samuel, University of California, Santa Barbara, Santa Barbara, CA
Amanda Proudfoot,

Milstein Young Investigator Award
Dr Dominic De Nardo, University of Bonn
Stacy Horner, Duke University Medical Center, Durham, NC
Maria Liaskos, MIMR-PHI Institute of Medical Research, Melbourne, Australia
Seth Masters, The Walter and Eliza Hall Institute, Victoria, Australia
Kate Schroder, The University of Queensland, Brisbane, Australia

Christina Fleischmann Award to Young Women Investigators
Sophie Broughton, St Vincents Institute For Medical Research, Victoria, Australia

ICIS Journal of Biological Chemistry / Herbert Tabor Award
Yeonseok Chung, Seoul National University, Seoul, South Korea

ICIS Sidney and Joan Pestka Award
Annie Bruns, Northwestern University, Evanston, IL

Postgraduate Award
Amanda Huber, University of Michigan, Ann Arbor, MI

Distinguished Service Award
Dr. Christine Czarniecki

ICIS & Melbourne Travel Award Winners
Ryann Guayasamin
Daniel Harari
Jeremy Hirota
Alan Hsu
Caleb Huang
Ian Humphreys
Iryna Kolosenko
Pankaj Kothavade
Andrew Larner
David Levy
Helene Minyi Liu
Yiliu Liu
Yueh-Ming Loo
Pei Ching (Regine) Low
Tao Lu
Pilvi Maliniemi
Isabelle Marie
Kazuya asuda
Mariko Matsui
Nontobeko Mvubu
Marcel Nold
Roza Nurieva
Olusegun Onabajo
Matthias Parrini
Hye-Lim Park
Sowmya Pattabhi
Leesa Pennell
Aradhana Rani
Mahesh Raundhal
Rebecca Coll
Nancy Reich
Fabiana Rizzo
Saleela Ruwanpura
Maria Sanchez-Aparicio
Marvin Sandoval
Karina Santiago
Smriti Sharma
Malcolm Starkey
Jason Twhohig
Lauren Vaughn
Nàdia Villacampa
Rui DanXie

Delegate Information

MEETING DATES
26 – 29 October, 2014

WEBSITE
www.cytokines2014.com

SECRETARIAT
ASN Events Pty Ltd
Unit 9
397 Smith Street
Fitzroy VIC 3065
Ph: +61 3 8658 9530
Fax: +61 3 8658 9531

VENUE
Melbourne Convention and Exhibition Centre
1 Convention Centre Place
South Wharf
VIC Australia 3006
Ph: +61 3 9235 8000

ORGANISER'S REGISTRATION DESK
The organiser's registration desk will be located in the main foyer. The desk will be attended at all times during the conference, see hours below. Delegates should collect their satchel, name tag and other conference material on arrival. A message board will be at the registration desk.

ON SITE REGISTRATION DESK CONTACT HOURS
During open hours of event only:
Sunday 26th: 14:00 – 18:30
Monday 27th: 07:30 – 18:30
Tuesday 28th: 07:30 – 18:30
Wednesday 29th: 07:00 – 18:30

REGISTRATION
Conference delegates receive the following as part of their registration:

- Access to all scientific sessions and the exhibition hall on day(s) of registration
- Sunday Welcome Function;
- Morning teas;
- Lunches;
- Afternoon teas;
- Poster & network drinks
- A satchel with a copy of the delegate handbook and abstracts
  **Not applicable for trade delegates unless supply allows*

Also included on the days of registration are:

- Lunches (Monday, Tuesday, Wednesday)
- Morning teas (Monday, Tuesday, Wednesday)
- Afternoon teas (Monday, Tuesday, Wednesday)
- The Welcome Function on Sunday evening
- Drinks during the poster sessions 6:00 – 7:30pm (Monday and Tuesday nights)

NAME TAGS
The Congress entrance will be manned by security and delegates are required to wear their name tags to all scientific and catered sessions.

INTERNET
Melbourne Convention and Exhibition Centre have access to free wireless internet. Please select the 'M Connect' wireless service on your device. Then, after opening your preferred internet browser, the M Connect page will appear for you to read the terms and conditions and confirm your connection by selecting 'Connect Now'.

ACCOMMODATION
Delegates are staying at a variety of hotels in the area. Most delegates are accommodated in the immediate vicinity of the Convention Centre. Standard hotel check in is 2:00pm and check out is 10:00am. Delegates seeking to vary this arrangement should contact the Hotel reception directly. Delegates are to settle their own accommodation account directly with the hotel upon departure.

Crowne Plaza Melbourne	
1-5 Spencer Street, Melbourne	+61 3 9648 2777
Rendezvous Grand Hotel Melbourne	
328 Flinders Street, Melbourne	+61 3 9250 1888
Hilton Melbourne South Wharf	
2 Convention Centre Place, Melbourne	+61 3 9027 2000
Melbourne Short Stay – MP Deluxe	
Corner of City Road & Moray Street	+61 3 8256 7500
Melbourne Short Stay – Southbank Deluxe	
63 Whiteman Street, Melbourne	+61 3 8256 7500
Vibe Hotel Savoy Melbourne	
630 Little Collins Street, Melbourne	+61 3 9622 8888
Holiday Inn Melbourne on Flinders	
575 Flinders Lane, Melbourne	+61 3 9629 4111

INSURANCE
The hosts and organisers are not responsible for personal accidents, any travel costs, or the loss of private property and will not be liable for any claims. Delegates requiring insurance should make their own arrangements.

DISCLAIMER
The hosts, organisers, venue and participating societies are not responsible for, or represented by, the opinions expressed by participants in either the sessions or their written abstracts. Responsibility for the literary and scientific content of abstracts accepted for publication remains with the authors and their sponsoring institutions. Acceptance by any of the participating societies for publication does not imply any acceptance by the Societies of responsibility.

SESSION LOCATIONS
The Plenary room is located on the Main Floor of the Convention Centre – near the registration desk. Some catering will be provided here. Concurrent Symposia room is located on Level 1. Also located on Level 1 is the trade hall, posters and more catering.

THE SPEAKER PREPARATION ROOM
The speaker preparation room is located on level 1 and is Speaker Room 101. As the program is large and complex, it is essential you load your talk to the conference network from the speaker preparation room (noted above) at least 2 hours in advance of your session start time. There will not be provision for people to use their own laptops. The conference presentation software is MS PowerPoint 2007. Those preparing on Macs should save for this output. Any issues should be resolved in the speaker preparation room beforehand and there will be a technician present to assist.

POSTER VIEWING
Delegates with posters can place their poster by finding the appropriate abstract number on the display panels in the exhibition area. The program provides instructions regarding the days which posters should be displayed and manned. Delegates with poster discussions on a particular day are encouraged be present at their poster during the breaks on that day to answer questions and meet colleagues with similar research interests. During the specific poster discussion sessions, presenters must be present talk discuss with delegates.

SMOKING
Smoking is not permitted in the venue.

MOBILE PHONES
Please ensure your mobile phone is turned off during any session you attend.

SOCIAL FUNCTIONS

- **Welcome Reception: Sunday 26th October, 7:30 - 9:00PM, Ground Level Foyer**

Delegates are invited to the Welcome Function following the opening symposium on the evening of Sunday October 26th, 7:30 – 9:00 PM. The registration desk will be open from 2:00pm. The opening formalities will be included and this function is included with full registration.

- **Poster Sessions: Monday 27th & Tuesday 28th October, 6:00 - 7:30 PM, Level 1 Foyer**

Each of these evenings, delegates are invited to view posters and socialise with the trade participants and other delegates for an hour at the end of the sessions. This is included in the registration of the day.

- **Networking session for students and ECR's: Monday 27th October, 7:30PM, The Boatbuilders Yard**

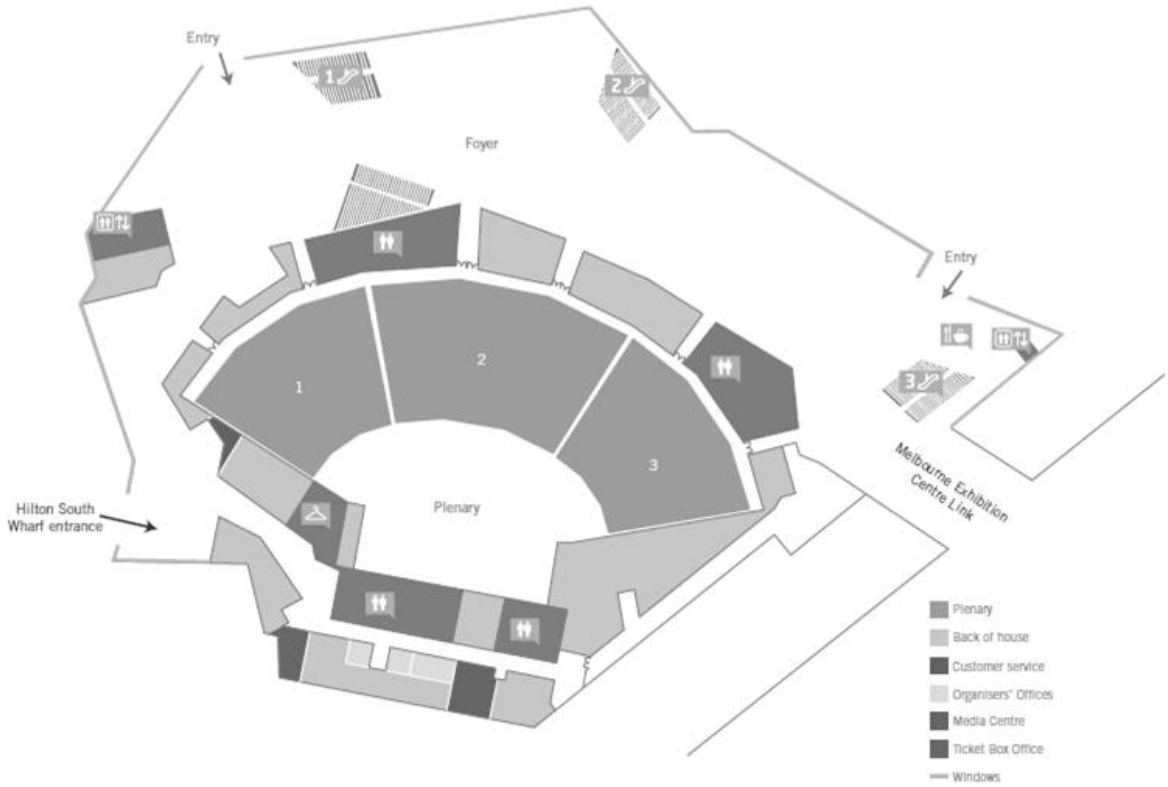
Optional social event for students and ECR's. Attendance includes 2 drink vouchers and nibbles.

- **Networking Dinner: Tuesday 28th October, 7:30 - 9:00PM, Melbourne Aquarium**

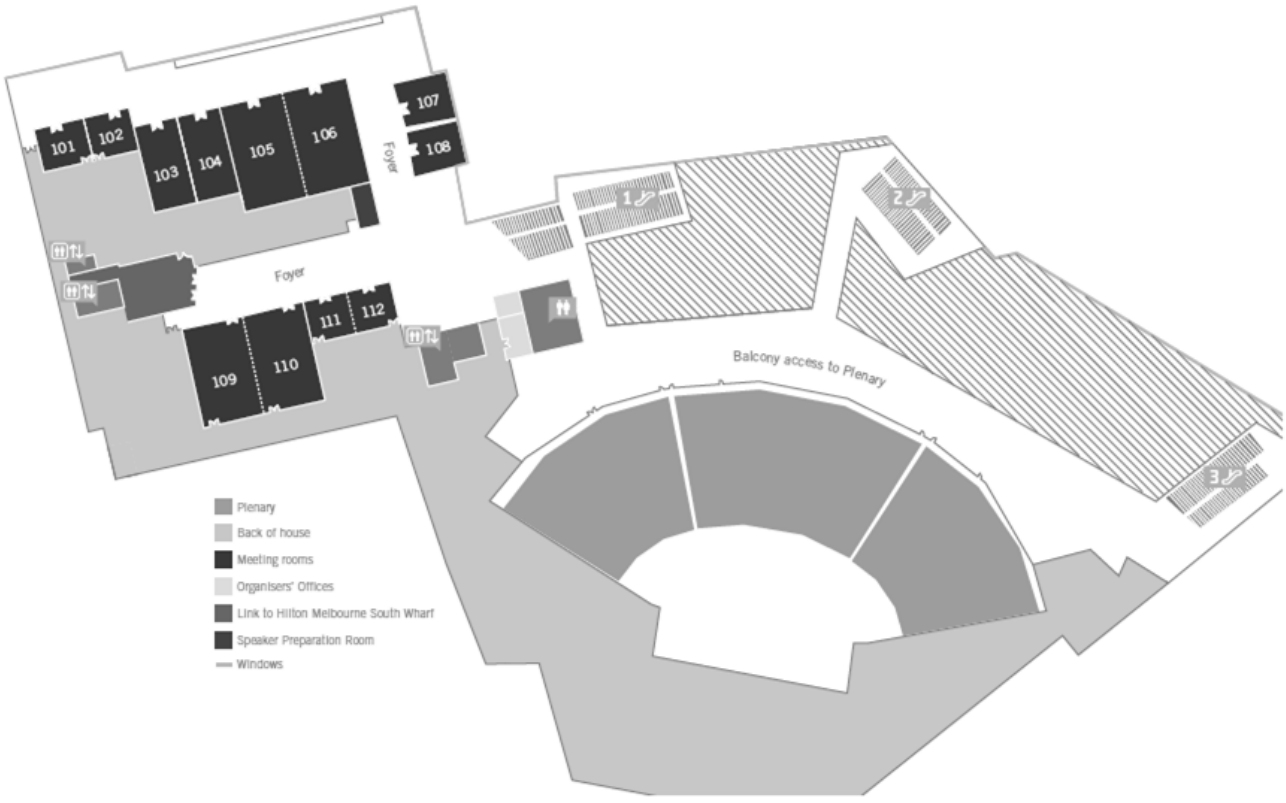
This Networking dinner will be held at the Melbourne Aquarium, King Street, Melbourne. If you wish to attend please visit the registration desk for availability.

Venue Map

Ground Floor



Level 1



Melbourne, Victoria

Melbourne is the Capital City of the State of Victoria. It has a population of approximately 3.5 million people. Melbourne is commonly referred to as the cultural capital of Australia. It is the pre-eminent centre for arts and culture, education, fine food and dining and shopping. Sport is also a city focus. Melbourne is home to many of the world's most famous sporting events F1 Grand Prix, Australian Tennis Open, AFL Grand Final and Melbourne Cup Horse Race.

BANKING AND CREDIT CARDS
Most banks are open from 9.00am to 4.30pm from Monday to Thursday, banks are usually open until 5.00pm on a Friday and some major banks are now open on a Saturday from 9.00am to 12.00pm. There are Automatic Teller Machines (ATMs) located around the city which can be used after hours. Most banks are able to cash travellers' cheques.
Major credit cards are widely accepted. Your credit card can also be used at an ATM with your Personal Identification Number.

CURRENCY EXCHANGE
There are many places to exchange currency in the CBD. Banks, travel agencies and dedicated exchange spots are readily available.

TIPPING
Melbourne (or anywhere in Australia) does not operate on a tipping culture. You may tip to show your appreciation for good service, but it is not a requirement.

TAXES IN MELBOURNE
A Goods and Services Tax of 10% tax is applied to most goods and services in Australia. All displayed prices for Goods and Services are required to include the GST.
If you leave Australia within 30 days of buying goods, you may be exempt from paying GST. You can take advantage of the Tourist Refund Scheme (TRS) to claim back the GST you have paid, and also claim back any Wine Equalisation Tax (WET) you paid. TRS only applies to goods that you can carry as hand luggage, and other conditions do apply.

CLIMATE
Spring is a mild period of year in Melbourne, the temperatures ranging from an average high of 20 degrees Celsius to an average low of 10 degrees Celsius. September to November is the most changeable time of the year for Melbourne as far as weather is concerned. The highest temperature on record has been beyond 25 degrees Celsius but there are lots of showers around and highs in temperature are rare.

TELEPHONE INFORMATION
If calling from a public telephone, the average price for a phone call to another landline within Australia is 50c. Phone cards can be purchased from post offices and newsagents, for local, interstate and international calls. Mobile (cell phone) calls are more expensive.
When making international phone calls, the following formula should be used: 0011 + country code + area code (if this has a '0' please omit) + telephone number.

DINING OUT
Meals' prices range anywhere from AU$10 at local cafes and small restaurants to AU$200 at world-leading restaurants.

THINGS TO SEE AND DO
Melbourne has a multitude of inner city and regional tourist options that are easily accessible from the city. From museums, plays, festivals and galleries to Federation Square, Crown Entertainment Complex and the Eureka Skydeck. Other attractions include the Melbourne Aquarium, Great Ocean Road, and Queen Victoria Markets. The following activities/tours are available for booking at the Registration Desk; Introduction to Melbourne walk, Bike Ride, Yoga, Running, Golf.

TRANSPORT
Melbourne public transport system comprises of train, tram, ferries and bus services stretching across the entire state of Victoria. The majority of Melbourne's innercity experiences are walking distance from the Convention Centre. Bike hire is also available throughout the inner city.

Sponsor Listing

Merck Serono **Gold Sponsor**

Website: *www.merck.com.au*

Merck Serono is the biopharmaceutical division of Merck. With headquarters in Darmstadt, Germany, Merck Serono offers leading brands in 150 countries to help patients with cancer, multiple sclerosis, infertility, endocrine and metabolic disorders as well as cardiovascular diseases. In the United States and Canada, EMD Serono operates as a separately incorporated subsidiary of Merck Serono.

Merck Serono discovers, develops, manufactures and markets prescription medicines of both chemical and biological origin in specialist indications. We have an enduring commitment to deliver novel therapies in our core focus areas of neurology, oncology, immuno-oncology and immunology.

Nexvet **Gold Sponsor**

Website: www.nexvet.com

Nexvet is a clinical-stage biopharmaceutical company focused on transforming therapeutics for companion animals, such as dogs and cats, by developing and commercializing innovative biologics to address their key unmet medical needs.

Nexvet's proprietary PETization™ platform is designed to rapidly translate monoclonal antibodies between species in a single step, enabling Nexvet to rapidly create "100% species-specific" therapeutics. Nexvet believes this substantially reduces the time, cost and clinical risks associated with the conventional development of biologics.

Through PETization, Nexvet has developed its lead biologic product candidates: the anti-nerve growth factor (NGF) monoclonal antibodies (mAbs) NV-01 and NV-02 for the treatment of osteoarthritic pain in dogs and osteoarthritic pain in cats, respectively. Further research has identified a tumor necrosis factor receptor (TNFr) fusion protein, NV-08, for the treatment of chronic inflammatory diseases in dogs. Nexvet's team members have international track records in the development of human biologics and veterinary drugs, and are committed to developing biologics to drive innovation in the global animal health therapeutic and vaccine market, and give companion animals longer, happier lives.

Genentech **Silver Sponsor**

Website: *www.gene.com*

Considered the founder of the industry, Genentech, now a member of the Roche Group, has been delivering on the promise of biotechnology for over 35 years.

At Genentech, we use human genetic information to discover, develop, manufacture and commercialize medicines to treat patients with serious or life-threatening medical conditions. Today, we are among the world's leading biotech companies, with multiple products on the market and a promising development pipeline.

MIMR-PHI Institute of Medical Research **Silver Sponsor**

Website: www.*mimr-phi.org*

With a combined 75 years of research experience, more than 400 leading research experts and postgraduate students and state-of-the-art research platforms and facilities, the Institute has taken its place as a leader in improving global wellbeing through excellence in medical research and clinical application.

As one of Melbourne's top medical research institutes and the research hub of the Monash Health Translation Precinct (MHTP), MIMR-PHI scientists and clinicians are at the forefront of discovery and translational research.

The Institute's specialist Research Centres tackle key health priorities in the following areas: Cancer, Genetic Disease, Immunity, Inflammation and Infectious Diseases, Reproductive Health, Fetal, Neonatal and Children's Health, Endocrinology and Metabolism.

Through our world-class research we strive to advance healthcare through an increased understanding of disease and its diagnosis, treatment and prevention.

Pfizer's Centers for Therapeutic Innovation **Silver Sponsor**

Website: *www.pfizer.com/research/rd_partnering/centers_for_therapeutic_innovation*

The Centers for Therapeutic Innovation (CTI) is a pioneering research and development network initiated by pharma that uses an open innovation model to bring great ideas to fruition. Part of Pfizer, we are an entrepreneurial group that partners with leading academic medical centers and disease foundations with the aim of translating promising science into clinical candidates.

Our work is based on authentic collaboration, reflected in shared decision making and aligned incentives. We are committed to bringing together cutting-edge academic and industry resources to develop medicines faster and more efficiently.
With locations in Massachusetts, New York and California, our facilities are conveniently located on or near academic campuses, allowing our partners to work closely with Pfizer scientists to translate research ideas into clinical applications, with the ultimate goal of moving a therapeutic hypothesis through Proof-of-Mechanism (PoM) in humans.
Through CTI, Pfizer is transforming the traditional model of drug discovery. By departing from the historical pharma model of R&D, we are working to solve key challenges – namely the high cost and substantial time investment – of drug discovery. CTI is rewiring the R&D ecosystem to offer new, targeted therapies to patients living with disease.

Australian Biosearch / Bio Legend **Bronze Sponsor**
Website: *www.aust-biosearch.com.au / www.biolegend.com*
BioLegend, with their Australian partner, Australian Biosearch, provide world-class antibodies, proteins, assays, and custom solutions. Cytokine products include a Multiplex solution with new LEGENDplex™ Multi-Analyte Flow Assay Kits, Recombinant Cytokines/Chemokines and LEAF™ antibodies for in vivo assays (Bulk pricing available). New LEGEND MAX™ ELISA Kits: TGF-β family (total, free active, latent, LAP, Soluble GARP); CCL8, Adiponectin, Free SCF, FGF-basic. New PE/Dazzle™ 594 conjugates for Multicolor Flow Cytometry and Alexa Fluor 594 conjugates for Multicolor Microscopy. Also available: LEGENDScreen™ Cell Screening Kits and Zombie dyes for live/dead cell discrimination. With our new Neuroscience R&D team and products, BioLegend is positioned to become a leader in neuroscience and immunology research.

Bio-Rad Laboratories **Bronze Sponsor**
Website: *www.bio-rad.com*
Bio-Rad Laboratories has been at the centre of scientific discovery for 60 years, developing products and solutions to advance healthcare and research.
Multiplex ELISA on magnetic bead xMAP technology enables the detection and measurement of up to 100 analytes in a single sample. Cytokines have great utility as biomarkers for disease including cancer and autoimmunity; therefore multiplexing gives you the power to understand complex relationships of proteins in immunological pathways. Bio-Rad's Bio-Plex assays are designed to quantitate proteins in diverse matrices such as serum, plasma and supernatant at small sample volumes. Disease state biomarker panels and pathways available include: Cancer, Apoptosis and Metabolism.
Visit the Bio-Rad booth to learn how this technology could be applied to your research.

Bio-Techne **Bronze Sponsor**
Website: *www.bio-techne.com*
Bio-Techne brings together the world class brands of R&D Systems, Novus Biologicals, and Tocris to better serve our customers and the community. R&D Systems cytokines defined the industry 30 years ago and continue to provide researchers with reliable research results. As the Bio-Techne portfolio grows, we are no longer just about consumables. We are adding innovative instruments to our portfolio in a way that allows us to leverage our gold standard reagents into complete solutions for both life science and diagnostic markets. Visit us at booth #8 to learn more about the entire Bio-Techne product portfolio.

eBioscience **Bronze Sponsor**
Website: *www.ebioscience.com*
eBioscience, an Affymetrix company, develops and manufactures over 11,000 antibodies, proteins, immunoassays and multiplex assays at ISO-certified facilities worldwide. Focused on accelerating scientific discovery in immunology and oncology, we provide innovative solutions to researchers and clinicians looking to answer questions driving today's life science communities. Partner with the industry leader of translational science.

PBL Assay Science **Bronze Sponsor**
Website: *www.pblassaysci.com*
PBL Assay Science, your trusted source for interferon ELISAs, proteins, antibodies, and assay services has expanded its human cell-expressed cytokine and growth factor line and ultrasensitive cytokine detection services. We invite you to visit with our scientists at Booth 7 in the ICIS exhibition space to find out how we can help you address your most pressing assay challenges. Whether you would like us to customize a

multiplex assay incorporating your key analytes, to serve as an outside laboratory to run samples in confirmatory assays, or to provide a novel human cell-expressed protein, PBL is here to serve your needs.

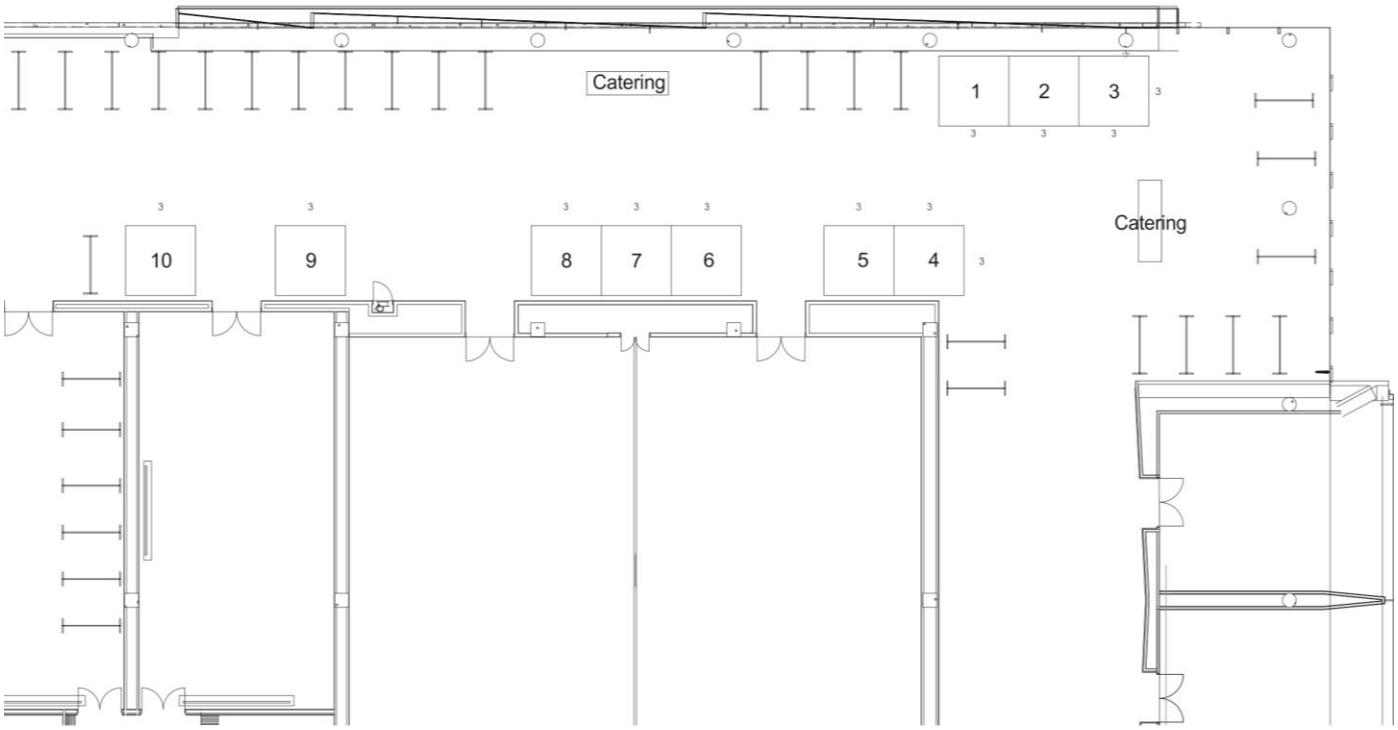
Elsevier **Sponsor**
Website: *www.elsevier.com*
Elsevier is a world-leading provider of information solutions that enhance the performance of science, health, and technology professionals. We publish nearly 2,200 journals, including a prestigious collection of immunology journals, The Lancet and Cell, and more than 25,000 book titles. Visit elsevier.com for more information.

Opsona Therapeutics **Session Sponsor**
Website: *www.opsona.com*
Opsona Therapeutics is a leading immunology drug development company, focused on novel therapeutic approaches to key targets of the innate immune system associated with a wide range of major human diseases, including autoimmune and inflammatory diseases, with specific focus on Solid Organ Transplantation and Oncology.
The company was founded in 2004 by three world-renowned immunologists at Trinity College in Dublin.
Opsona's lead product, a fully humanized monoclonal IgG4 antibody (OPN-305) targeting Toll-like-receptor-2 (TLR2) has demonstrated activity in a number of preclinical models and has been tested in a Phase I clinical trial in healthy volunteers. The company has initiated a three-part multi-centred, double blinded and placebo controlled Phase II clinical study to evaluate the safety, tolerability and efficacy of OPN-305 in renal transplant patients at high risk of Delayed Graft Function (DGF) as the first clinical indication for the development of OPN-305 of which the first part has been completed successfully. OPN-305 has obtained EMA and FDA orphan drug status in Solid Organ Transplantation.
Further information is available at: http://www.opsona.com/.

Mary Ann Liebert Inc. **Sponsor**
Website: *www.liebertpub.com/jicr*
Journal of Interferon and Cytokine Research (JICR), led by Co-Editors-in-Chief Ganes C. Sen and Thomas A. Hamilton, celebrates its 35th anniversary in 2015. Over the last three and a half decades the Journal has been a major source of new primary articles covering IFN-mediated anti-viral mechanisms and topical reviews covering important emerging areas by top contributors in the field of IFN and cytokine research. The relevance of such articles is reflected in our impact factor of 3.899, representing an 18% increase over last year and the 5th consecutive year of growth.

Walter & Eliza Hall Institute of Medical Research **Sponsor**
Website: *www.wehi.edu.au*
The Walter and Eliza Hall Institute is Australia’s oldest medical research institute and will celebrate its centenary in 2015. Affiliated with The University of Melbourne and The Royal Melbourne Hospital, the institute is home to more than 750 researchers working on cancers, infectious diseases and immune disorders.
The institute has more than 100 scientists working on cytokine signalling. The institute’s scientists were instrumental in the discovery of many cytokines, cytokine receptors and the suppressors of cytokine signalling (SOCS) proteins, and their functions. Current projects expand on this work, investigating the role of cytokines and their suppressors in many diseases including blood cancers, gastrointestinal cancer, rheumatoid arthritis and influenza.

Exhibitor Map & Listing



Abcam Australia **Booth 7**

Website: *www.abcam.com*

Abcam is a provider of high quality protein research tools. We are delighted to launch the direct service in Australia and New Zealand since August 2014. Researchers can benefit from:

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Australian Biosearch / BioLegend **Booth 9**

See Sponsorship Listing for details.

Bio-Rad Laboratories **Booth 6**

See Sponsorship Listing for details.

Bio-Techne **Booth 8**

See Sponsorship Listing for details.

eBioscience **Booth 4**

See Sponsorship Listing for details.

Lonza Australia **Booth 2**

Website: *www.lonza.com*

Lonza is a leading supplier of human normal and diseased cells, cell culture media, cell based assays and transfection devices and reagents and Endotoxin Detection devices, reagents and services to the pharmaceutical, healthcare and life science industries from research to final product manufacture.

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Miltenyi Biotec Australia **Booth 3**

Website: *www.miltenyibiotec.com*

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PBL Assay Science **Booth 5**

See Sponsorship Listing for details.

Peprotech **Booth 1**

Website: *www.peprotech.com*

Since 1988 PeproTech has been producing a wide range of recombinant proteins, mainly Cytokines, their related Antibodies and ELISA Kits; all of the highest quality and at the most competitive price. PeproTech appreciates the opportunity to support the ISCT 2014, and is keen to learn more about the scientific community and strengthen their level of cooperation.

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The PeproTech Australia office is managed by LONZA Australia, Ph.1300 657 508 or contact: Margret Schuller; 0430353301; mschuller@peprotechasia.com

Transnetyx **Booth 10**

Website: *www.transnetyx.com*

Transnetyx Automated Genotyping is fast, easy, and accurate and believes in making things simple. With more than 50 million successful reactions globally, sending your genotyping to Transnetyx is designed to eliminate the tedious process of extracting and testing DNA so researchers and labs can get back to what is most important - their research. Eliminate overcrowded cages and high cage costs with the efficiency and reliability of Transnetyx. Genotyping has evolved. Have you?

Program – Sunday 26th October 2014

11:00-17:00 **ICIS Council and Committee Meetings** **Rooms 101 and 102**

14:00-20:00 **REGISTRATION** **Level 1 Foyer / Main Foyer**

17:30-19:30 **OPENING SESSION**

Plenary 1

17:30-17:55 **Opening Remarks** (Brendan Jenkins, Co-convenor)

Award Presentations (Richard Flavell, President, ICIS)

17:55-18:20 **ICIS Honorary Lifetime Membership Awards**

Chair: Richard Flavell

Amanda Proudfoot, NovImmune, Switzerland

"Targeting chemokines: Pathogens can, why can't we?" abs# 318

Chuck Samuel *(presentation to be given in Concurrent Symposia 6)*

18:20-18:45 **Seymour and Vivian Milstein Award for Excellence in Interferon and Cytokine Research**

Chair: Richard Flavell

Kate Fitzgerald, University of Massachusetts Medical School, USA

"Regulation of the Interferon Response by the long non-coding RNA lincRNA-EPS" abs# 249

18:45-19:30 **Keynote lecture 1**

Chair: Paul Hertzog

Nicos Nicola, Walter and Eliza Hall Institute, Australia

"Old and new families of negative regulators of cytokine signalling" *abs# S-37*

19:30-21:00 **WELCOME RECEPTION** **Level 1 Foyer / Main Foyer**

Including indigenous dance performance

COMMITTED TO SCIENCE, MEDICAL EDUCATION AND PATIENT CARE

Merck Serono invests € 20 million in grants for innovative research and independent medical education:

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Program – Monday 27th October 2014

08:00-18:00 **REGISTRATION** **Level 1 Foyer / Main Foyer**

08:30-10:30 **PLENARY SESSION 1** **Plenary 1**

Cytokines in the control of innate and adaptive immunity

(Sponsored by Walter & Eliza Hall Institute of Medical Research, Australia)

Chairs: Matt Sweet, Dhan Kalvakolanu

08:30-09:00 **Zhijian James Chen,** University of Texas Southwestern Medical Center, USA

"Innate immune sensing and signaling of cytosolic DNA and RNA" abs# S-8

09:00-09:30 **Fabienne MacKay,** Monash University, Australia

"Cytokine-driven loss of plasmacytoid dendritic cell function in chronic lymphocytic leukemia" abs# S-15

09:30-10:00 **Zhengfan Jiang,** Peking University, China

"*Identification and Characterization of Phosphodiesterase V-cGAPs That Degrade 3'3'-Cyclic GMP-AMP in vibrio cholerae*" *abs# S-33*

10:00-10:15 **Julia Ellyard,** Australia National University, Australia

"Cytokine dysregulation in autoimmune disease pathogenesis" abs# 246

10:15-10:30 **Hong Tang**, Chinese Academy of Sciences, China

"Innate cell-like function of T cells in modulation of inflammatory response to infection and tissue injury" abs# 335

10:30-11:00 **MORNING TEA BREAK** **Level 1 Foyer / Main Foyer**

11:00-13:00 **CONCURRENT SYMPOSIA 1** **Rooms 105/106**

Cytokines and the regulation of tissue resident immune cells

(Sponsored by PBL Assay Science, Australia)

Chairs: Iain Campbell, Catarina Sacristan

11:00-11:30 **Frederic Geissman,** King's College, UK

"Origin, development and maintenance of tissue resident macrophages" abs# S-30

11:30-12:00 **Florent Ginhoux,** Singapore Immunology Network, Singapore

"Macrophage and dendritic cell ontogenuy" abs# S-31

12:00-12:15 **Dominic De Nardo,** Institute of Innate Immunity, Germany (ICIS Young Investigator Awardee)

"Modulation of innate immune responses by ATF3" abs# 36

12:15-12:30 **Margaret Hibbs,** Monash University, Australia
"G-CSF is a critical mediator of chronic inflammatory lung disease" abs# 264

12:30-12:45 **Cathleen Pfefferkorn,** University Medical Center Freiburg, Germany
"Identification of interferon-ß-producing cells in the virus-infected brain" abs# 148

12:45-13:00 **Ian Humphreys,** Cardiff University, UK
"Neutrophils recruited by IL-22 in peripheral tissues function as TRAIL-dependent antiviral effectors against MCMV" abs# 87

11:00-13:00 CONCURRENT SYMPOSIA 2 **Plenary 1**

The microbiome and mucosal immune responses
(Sponsored by e-Bioscience, an Affymetrix Company, Australia)
Chairs: Richard Ferrero, *Thirumula-Devi Kanneganti*

11:00-11:30 **Richard Flavell,** Yale University School of Medicine, USA
"Inflammasomes in health, dysbiosis, and disease" abs# S-11

11:30-12:00 **Gabriel Nunez,** University of Michigan, USA
"Linking pathogen virulence, immunity and the microbiota" abs# S-18

12:00-12:30 **Elizabeth Hartland**, University of Melbourne, Australia
"Death receptors and bacterial diarrhoea" abs# S-12

12:30-12:45 **Phil Hansbro,** University of Newcastle, Australia
"Shifts in the microbiome during experimental chronic obstructive pulmonary disease (COPD)" abs# 261

12:45-13:00 **Tomonori Kamiya,** Tokyo University of Science, Japan
"Dectin-1 modifies colonic microflora by inducing cytokine-dependent antimicrobial peptide secretion from intestinal epithelial cells" abs# 275

13:00-14:00 LUNCH BREAK (lunch provided) **Level 1 Foyer / Main Foyer**

14:00-15:30 **CONCURRENT MINI-SYMPOSIUM 1** **Plenary 1**

Chairs: Claudia Nold, Rose Ffrench

14:00-14:15 **Stacy Horner,** Duke University Medical Center, USA (ICIS Young Investigator Awardee)

"Proteomic analysis of mitochondrial-associated ER membranes during RNA virus infection reveals dynamic changes in protein and organelle trafficking" abs# 267

14:15-14:30 **Florian Wiede,** Monash University, Australia

"The role of PTPN2 in early thymocyte development and JAK/STAT signaling"

14:30-14:45 **Andrew Larner,** Virginia Commonwealth University, USA *abs# 351*

"STAT3 interacts with Cyclophilin D in cancer cells to regulate the mitochondrial permeability transition pore" abs# 107

14:45-15:00 **Saleela Ruwanpura,** MIMR-PHI Institute, Australia

"Novel role for interleukin-6 trans-signaling in the pathogenesis of pulmonary inflammation and emphysema" abs# 161

15:00-15:15 **Rebecca Coll,** University of Queensland, Australia

"MCC950 is a potent and specific inhibitor of the NLRP3 inflammasome and a novel potential therapeutic for NLRP3 driven diseases" abs# 30

15:15-15:30 **Amanda Huber,** University of Michigan, USA

***(Sidney & Joan Pestka Post-Graduate Award in Interferon & Cytokine Research Sponsored by PBL Interferon Source*)**

"The Type I Interferon axis suppresses Th-17 driven autoimmune inflammation in a mouse model of Multiple Sclerosis via Interferon Regulatory Factor-7" abs# 86

14:00-15:30 **CONCURRENT MINI-SYMPOSIUM 2** **Rooms 105/106**

Chairs: Niamh Mangam, Zoltan Fehervari

14:00-14:15 **Yeonseok Chung,** Seoul National University, South Korea (Journal of Biological Chemistry/Herbert Tabor Young Investigator Award) *abs# 233*

"Proatherogenic Condition Promotes Autoimmune Th17 Cell Responses"

14:15-14:30 **Laura Dagley,** Walter and Eliza Hall Institute, Australia

"A mass spectrometry-based approach for studying kinase activity" abs# 33

14:30-14:45 **Sonja Best,** *NIAID/NIH, USA*

"Viral antagonism of type I interferon responses reveals prolidase as a regulator of IFNAR1 trafficking and expression" abs# 220

14:45-15:00 **Sukanya Raghaven,** University of Gothenburg, Sweden
"The role of IL-17A and IFNγ in vaccine-induced protection against Helicobacter pylori infection" abs# 154

15:00-15:15 **Betsy Barnes,** Rutgers Biomedical and Health Sciences, USA
"Intrinsic and extrinsic mechanisms of metastatic inhibition by IRF5 in human ductal carcinoma" abs# 218

15:15-15:30 **Michael White,** Walter and Eliza Hall Institute, Australia
"The apoptotic caspase cascade suppresses mitochondrial DNA-induced STING-mediated type I IFN production by dying cells" abs# 197

15:30-16:00 AFTERNOON TEA BREAK Level 1 Foyer / Main Foyer

16:00-18:00 CONCURRENT SYMPOSIA 3 Plenary 1
Inflammasomes and the IL-1 cytokine family
(Sponsored by BioLegend/Australian Bioresearch, Australia)
Chairs: Ashley Mansell, Betsy Barnes

16:00-16:30 ***Thirumala-Devi Kanneganti,*** St Jude Children's Research Hospital, USA
"IL-1 regulation in inflammatory disease" abs# S-34

16:30-17:00 **Denise Monack,** Stanford University, USA
"Host recognition of intracellular bacterial pathogens" abs# S-17

17:00-17:30 **Veit Hornung,** University of Bonn, Germany
"Cytosolic recognition of nucleic acids by the innate immune system" abs# S-32

17:30-17:45 **Seth Masters,** Walter and Eliza Hall Institute, Australia (ICIS Young Investigator Awardee)
"Inflammasome activation due to polymerized actin triggers an autoinflammatory disease that is dependent on IL-18, not IL-1β" abs# 126

17:45-18:00 **Kate Schroder,** University of Queensland, Australia (ICIS Young Investigator Awardee)
"The neutrophil NLRC4 inflammasome selectively promotes IL-1β maturation without pyroptosis during acute Salmonella challenge" abs# 165

16:00-18:00 **CONCURRENT SYMPOSIA 4** **Rooms 105/106**

Cytokine signalling mechanisms

(Sponsored by Nexvet Biopharma, Australia)

Chairs: Sandra Nicholson, George Stark

16:00-16:30 **Uwe Vinkemeier**, University of Nottingham, UK

"STAT protein phase transitions in cytokine signalling" abs# S-26

16:30-17:00 **Jeff Babon,** Walter and Eliza Hall Institute, Australia

"*Inhibition of IL-6 family cytokines by SOCS3: characterizing the mode of inhibition and the basis of its specificity" abs# S-3*

17:00-17:30 **Andrew Brooks,** University of Queensland, Australia

"A new cytokine receptor activation paradigm: activation of JAK2 by the growth hormone receptor" abs# S-7

17:30-17:45 **Sophie Broughton**, St Vincent's Institute, Australia (Christina Fleischmann Award to Young Women Investigators)

"Structural and functional analysis of the GM-CSF:GM-CSF receptor alpha chain binary complex provide new insights into signalling of the GM-CSF ternary complex" abs# 17

17:45-18:00 **Marcel Nold,** MIMR-PHI Institute, Australia

"IL-37 requires IL-18Rα and SIGIRR to carry out its multi-faceted anti-inflammatory program on innate signal transduction" abs# 308

18:00-19:30 **POSTER SESSION 1** **Level 1 Foyer / Main Foyer**

Including trade/exhibitor display with drinks and cheese

19:30-21:00 Networking mixer for students and ECR's **The Boatbuilders Yard**

Program – Tuesday 28th October 2014

08:00-18:00 **REGISTRATION** **Level 1 Foyer / Main Foyer**

08:30-10:30 **PLENARY SESSION 2** **Plenary 1**

Cytokines and interferons in cancer

(Sponsored by Merck Group, Germany)

Chairs: Brendan Jenkins, Tracy Putoczki

08:30-09:00 **Frances Balkwill,** Barts Cancer Institute, UK

"Targeting inflammatory cytokines and chemokines in cancer" abs# S-4

09:00-09:30 **Mark Smyth,** Queensland Institute of Medical Research, Australia

"Checkpoints and interferons in tumor control" abs# S-24

09:30-10:00 **Belinda Parker,** La Trobe University, Australia

"*Exploiting the type-1 interferon pathway as a biomarker and therapeutic target for metastatic cancer" abs# S-20*

10:00-10:30 **Masaaki Murakami,** Osaka University, Japan

"The inflammation amplification loop in non-immune cells, which is regulated by cytokines and neural activation" abs# S-36

10:30-11:00 **MORNING TEA BREAK** **Level 1 Foyer / Main Foyer**

11:00-13:00 **CONCURRENT SYMPOSIA 5** **Rooms 105/106**

Innate lymphoid cells and cytokine secretory mechanisms

(Sponsored by Bio-Rad Laboratories, Australia)

Chairs: Meredith O'Keefe, Howard Young

11:00-11:30 **Dale Umetsu,** Genentech, USA

"A role for ILC2s, ILC3s and the NLRP3 inflammasome in different forms of asthma" abs# S-25

11:30-12:00 **Gabrielle Belz,** Walter and Eliza Hall Institute, Australia

"Critical mucosal protection by the complex innate lymphoid cell network" abs# S-5

12:00-12:30 **Wolfgang Weninger,** Centenary Institute, Australia

"Regulation of inflammation by perivascular macrophages" abs# S-40

12:30-12:45 **Elena Vigano,** Singapore Immunology Network, Singapore
"A novel player for the regulation of IL-1α/β release in human monocytes" abs# 189

12:45-13:00 **Gregory Bouchaud,** French National Institute for Agricultural Research, France
"Previous food allergy aggravates allergic markers and intestinal damages in a mouse model of asthma" abs# 14

11:00-13:00 CONCURRENT SYMPOSIA 6 **Plenary 1**
Mary Ann Liebert, Inc. Symposium in Honour of Philip I. Marcus
Chairs: David Levy, Paula Pitha-Rowe

11:00-11:45 **Chuck Samuel,** University of California Santa Barbara, USA
ICIS Honorary Lifetime Membership Awardee
"RNA adenosine deaminase ADAR1 and RNA-dependent protein kinase PKR: opposing modulators of antiviral innate immunity" abs# S-22

11:45-12:15 **Peter Staehli,** Institute for Virology, Germany
"Epithelial IFN-λ and IFN-α/β constitute a compartmentalized mucosal defense system that restricts virus infection of the intestinal and respiratory tract" abs# 333

12:15-12:30 **Annie Bruns,** Northwestern University, USA
(Sidney & Joan Pestka Graduate Award in Interferon & Cytokine Research Sponsored by PBL Interferon Source)
"The innate immune sensor LGP2 activates antiviral signaling by regulating MDA5-RNA interaction and filament assembly" abs# 229

12:30-12:45 **Julia Heinrich,** Institute for Experimental Infection Research, Germany
"Kinetics of Type I Interferon responses determine either establishment of antiviral memory or no induction of adaptive immunity" abs# 77

12:45-13:00 **Nathalie Grandvaux,** University of Montreal, Canada
"*Identification of two distinct mechanisms that control the duration of the Interferon-mediated antiviral response" abs# 64*

13:00-16:00 LUNCH BREAK (lunch provided; also Melbourne City Tours available)

16:00-18:00 **CONCURRENT SYMPOSIA 7** **Plenary 1**

Genomic regulation of cytokine and interferon responses

Chairs: Chuck Samuel, Kate Fitzgerald

16:00-16:30 **K Mark Ansel,** University of California San Francisco, USA
"MicroRNA regulation of T cell cytokine responses" abs# S-2

16:30-17:00 **Xia-Fang Wang,** Duke University, USA
"Tumor microenvironment: microRNAs and metastasis" abs# S-39

17:00-17:15 **Colleen Elso,** St Vincent's Institute, Australia
"Apics deficiency reveals a role for a long noncoding RNA in dendritic cell function and autoimmunity" abs# 42

17:15-17:30 **Isabelle Marie,** NYU School of Medicine, USA
"Positive and Negative Epigenetic Regulatory Steps During IFN-Stimulated Transcriptional Initiation and Elongation" abs# 297

17:30-17:45 **Michael Gantier,** MIMR-PHI Institute, Australia
"Master regulation of transcriptional responses by microRNAs" abs# 253

17:45-18:00 **Kasia Blaszczyk,** Mickiewicz University, Poland (Melbourne Travel Awardee)
"IFNα engages a sustained antiviral response depending on STAT2/IRF9 but not ISGF3" abs# 223

16:00-18:00 **CONCURRENT SYMPOSIA 8** **Rooms 105/106**

Metabolic homeostasis and autophagy

Chairs: Bryan Williams, Carolyn Geczy

16:00-16:30 **Vojo Deretic,** University of New Mexico, USA
"Immunological manifestations of autophagy" abs# S-29

16:30-17:00 **Mark Febbraio,** Baker Institute, Australia
"Fatty acid-induced inflammation in immune cells: implications for metabolic disease" abs# S-10

17:00-17:15 **Maria Kaparakis-Liaskos,** MIMR-PHI Institute, Australia (ICIS Young Investigator Awardee)
"Identification of the intracellular location and mechanisms of NOD1-dependent inflammatory responses" abs# 93

17:15-17:30 **Eleanor Fish**, University of Toronto, Canada
"IFN-ß regulation of glucose metabolism is PI3K/Akt dependent and important for antiviral activity against coxsackievirus B3" abs# 55

17:30-17:45 **Dhan Kalvakolanu,** University of Maryland Baltimore, USA
"Interferon-induced autophagy and antibacterial defenses occur through the Apoptosis stimulating kinase-1" abs# 273

17:45-18:00 **James Harris,** Monash University, Australia
"Autophagic Regulation of Pro-inflammatory Cytokines" abs# 262

18:00-19:30 POSTER SESSION 2 Level 1 Foyer / Main Foyer
Including trade/exhibitor display with drinks and cheese

19:30-21:30 NETWORKING FUNCTION Melbourne Aquarium

Program – Wednesday 29th October 2014

08:00-17:30 **REGISTRATION** **Level 1 Foyer / Main Foyer**

08:30-10:30 **PLENARY SESSION 3** **Plenary 1**

Cytokines and interferons in the inflammatory response

(Sponsored by MIMR-PHI Institute of Medical Research, Australia)

Chairs: John Hamilton, Kate Stacy

08:30-09:00 **Yinon Ben-Neriah,** The Hebrew University of Jerusalem, Israel

"Parainflammation in cancer"abs# S-6

09:00-09:30 **Elina I Zuniga,** University of California San Diego, USA

"Dynamics of cytokine responses during chronic viral infection" abs# S-35

09:30-10:00 **Simon Jones,** Cardiff University, UK

"Interleukin-6 drives tissue fibrosis in response to repeat acute inflammation" abs# S-13

10:00-10:30 **Kingston Mills,** Trinity College, Ireland

"Function and regulation of IL-17 cytokine family in infection and autoimmunity" abs# S-16

10:30-11:00 **MORNING TEA BREAK** **Level 1 Foyer / Main Foyer**

11:00-12:00 **CONCURRENT MINI-SYMPOSIUM 3** **Plenary 1**

Chairs: Lisa Mielke, Robert Silverman

11:00-11:15 **Helene Minyi Liu,** National Taiwan University, Taiwan (Melbourne Travel Awardee)

"*Deacetylation-dependent regulation of RIG-I activation by the HDAc6 deacetylase mediates innate anti-viral immunity*" *abs# 113*

11:15-11:30 **Cristina Bergamaschi,** National Cancer Institute at Frederick, USA

"Heterodimeric IL-15 promotes tumor control through the regulation of the balance of effector and regulatory cells via an IL-2 deprivation mechanism" abs# 8

11:30-11:45 **Tim Hercus,** Centre for Cancer Biology, Australia

"Insights into the function of an anti-leukaemia antibody: structural studies of CSL362 bound to soluble CD123" abs# 78

11:45-12:00 **Tao Lu,** Indiana University, USA

"PRMT5 dimethylates R30 of the p65 subunit to activate NF-κB" abs# 120

11:00-12:00 CONCURRENT MINI-SYMPOSIUM 4 Rooms 105/106

Chairs: Patricia Fitzgerald-Bocarsly, Benjamin Kile

11:00-11:15 **Matthias Parrini,** University of Veterinary Medicine, Vienna, Austria (Melbourne Travel Awardee)

"STAT1 Isoforms in Transcriptional Control – Distinct Traits" abs# 144

11:15-11:30 **Daniel Gough,** MIMR-PHI Institute, Australia

"STAT3 supports experimental K-RasG12D-induced murine myeloproliferative neoplasms dependent on serine phosphorylation" abs# 63

11:30-11:45 **Nancy Reich,** Stony Brook University, USA

"Gammaherpesvirus requires STAT3 to establish B cell viral latency in animals" abs# 158

11:45-12:00 **Howard Young,** National Cancer Institute, USA

"Inhibiting cytokine activity – the use of modified DNA aptamers as an alternative to neutralizing antibodies" abs# 208

12:00-13:00 LUNCH BREAK (lunch provided)

13:00-15:00 CONCURRENT SYMPOSIA 9 Plenary 1

Pattern recognition receptors and responses to pathogens

(Sponsored by bio-Techne, Australia)

Chairs: Scott Durum, Eleanor Fish

13:00-13:30 **Shizuo Akira,** Osaka University, Japan

"Regnase-1, a ribonuclease involved in the inflammatory and immune responses" abs# S-1

13:30-14:00 **Eicke Latz,** University of Bonn, Germany

"Inflammasomes have extracellular activities that propagate inflammation" abs# S-14

14:00-14:30 **Feng Shao,** National Institute of Biological Sciences, China

"Innate immune sensing of bacteria and bacterial virulence by the cytosolic inflammasome complex" abs# S-23

14:30-14:45 **Adam Wall,** University of Queensland, Australia

"Regulation of inflammatory cytokine responses by a new TLR4 regulator complex of Rab8a/PI3Kγ" abs# 347

14:45-15:00 **Ashley Mansell,** MIMR-PHI Institute, Australia
"*PB1-F2 from H7N9 Influenza A virus activates the NLRP3 inflammasome to induce inflammation*" *abs# 296*

13:00-15:00 CONCURRENT SYMPOSIA 10 Rooms 105/106

Translatable outcomes targeting cytokines: the lessons learned

(Sponsored by Nexvet Biopharma, Australia)

Chairs: Amanda Proudfoot, Simon Jones

13:00-13:30 **Daniel Cua,** Merck Research Laboratories, USA
"*IL-23 regulation of innate and adaptive immunity" abs# S-28*

13:30-14:00 **Stefan Rose-John,** University of Kiel, Germany
"Blocking only the bad side of Interleukin-6" abs# S-21

14:00-14:30 **Hua Yu,** Beckman Research Institute, USA
"STAT3: from fundamental discoveries to the clinic" abs# S-27

14:30-14:45 **Walter Ferlin,** NovImmune, Switzerland
"Exploiting receptor clustering enhances inhibitory effects of a therapeutic monoclonal antibody to TLR4 revealing a novel mechanism of action driving the pathology of rheumatoid arthritis" abs# 51

14:45-15:00 **Scott Durum,** National Cancer Institute, USA
"IL-7R: a target in ALL and autoimmunity" abs# 245

15:00-15:30 AFTERNOON TEA BREAK Level 1 Foyer / Main Foyer

15:30-17:15	**CLOSING SESSION**	**Plenary 1**
	(Sponsored by Opsona Therapeutics, Ireland)	
15:30-16:15	**Keynote lecture 2**	
	Chair: Elizabeth Hartland	
	Peter Doherty, University of Melbourne, Australia	
	"Cytokine profiles in experimental and human influenza" abs# S-9	
16:15-17:00	**Keynote lecture 3**	
	Chair: Paul Hertzog	
	Luke O'Neill, Trinity College, Ireland	
	"A lifelong love affair with IL-1 and inflammation" abs# S-38	
17:00-17:15	**Closing remarks** (Paul Hertzog, Co-convenor)	

Abstracts

Additional Abstracts:
Please refer to “Cytokine”, provided in your satchel, for speaker abstracts S-1 – S-27 and meeting abstracts 1 – 212. Abstracts are numbered in alphabetical order by the presenting author surname.

Withdrawn:

Abs#1
Abs#40
Abs#56
Abs#57
Abs#58
Abs#61
Abs#111
Abs#114
Abs#134
Abs#137
Abs#155
Abs#183
Abs#192
Abs#215

Author Index

S-28

IL-23 regulation of innate and adaptive immunity

Daniel Cua

Following the discovery of IL-23-dependent T cell immunity, the past decade has witnessed a major revision of the T-helper subset paradigm and substantial progress has been made in deciphering the molecular mechanisms of T cell lineage commitment and function. I will present our recent work on the transcriptional control of TH17 cell development and highlight the protective vs. pathogenic roles of IL-17 and IL-22 in mucosal tissues. I will also discuss the emerging clinical data showing that antibody-mediated neutralization of IL-17 and IL-23 are remarkably effective for treating immune-mediated inflammatory diseases.

Immunological manifestations of autophagy

Vojo Deretic[1]

1. UNM School of Medicine, Albuquerque, Mexico

The broad immunological roles of autophagy span innate and adaptive immunity and are often manifested in inflammatory diseases. The immune effects of autophagy partially overlap with the hub function of autophagy in metabolism and cytoplasmic quality control but typically expand further afield to encompass unique immunological adaptations. One of the best-appreciated manifestations of autophagy is protection against microbial invasion, but this is by no means limited to direct elimination of intracellular pathogens and includes a stratified array of nearly all principal immunological processes. This presentation will provide a broad background to immunological roles of autophagy, highlight a few model examples, and report on the newest development demonstrating that the TRIM family of proteins act as cellular organizers of the core autophagy regulatory machinery and as receptors for selective autophagic elimination of microbial and endogenous cellular targets.

Origin, Development and Maintenance of Tissue Resident Macrophages

Frederic Geissmann[1]

1. King's College London, London, United Kingdom

Macrophages, Monocyte, and Dendritic Cells (DCs) are myeloid cells, phagocytes, and effectors cells of the innate immune system. Despite the identification of a number of phenotypic subsets, our understanding of the functions of these cells in vivo remains largely incomplete. Recent fate-mapping studies from our lab have identified a dual developmental origin for macrophages, monocytes and DCs. Monocytes, some tissue monocyte/macrophage subsets, such as in the adult gut, classical DCs and plasmacytoid DCs, develop and renew *in vivo* from *c-Myb*-dependent Hematopoietic Stem and Progenitors Cells (HSPCs) and require require the transcription factor for its development and express *Csf1R* and *Flt3*. In contrast a large number of 'resident' tissue macrophage networks, including liver Kuppfer cells, epidermal Langerhans cells, brain microglia, alveolar macrophages, and a proportion of splenic red pulp and kidney macrophages, develop during embryogenesis and early post-natal life from *Csf1R*+ Yolk-Sac erythro-myeloid progenitors, that do not express *Flt3*- and develop in the absence of *c-Myb*-. The Yolk-Sac erythro-myeloid progenitors colonize the fetal liver after E9, expand and give rise to the transient fetal liver hematopoiesis, and to definitive resident macrophage networks. These c-*Myb*-independent macrophages persist in adult mice, independently of HSPCs and c-*Myb*. We propose that differentiation of macrophages should be studied in the context of the development of macrophage networks.

Dendritic Cell and Macrophage Ontogeny

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Dendritic cells (DCs), monocytes and macrophages play crucial and distinct roles in tissue homeostasis and immunity, but also contribute to a broad spectrum of pathologies and are thus attractive therapeutic targets. Potential intervention strategies aiming at manipulation of these cells will require in-depth insights of their origins and the mechanisms that govern their homeostasis.

DCs and monocytes arise from common bone marrow (BM) precursor named macrophage-dendritic cell precursors (MDP), branching into exclusively DC- or monocyte-committed progenitors named common dendritic cell progenitors (CDPs) or common monocyte progenitor (cMoPs) respectively. CDPs give rise to plasmacytoid DC and migratory DC precursors termed pre-DCs. Pre-DCs seed tissues where they differentiate into the two major functionally specialized DC lineages, CD8α+/CD103+ DCs and CD11b+ DCs.

Recent evidence from our laboratory and others have showed that monocytes do not substantially contribute to all tissue macrophage populations in steady state and inflammatory conditions. Rather certain tissue macrophages in mice are derived from embryonic precursors, are seeded before birth and maintain themselves in adults by self-renewal. In addition, we now provide evidence that commitment to CD8α+/CD103+ DC or CD11b+ DC subsets is imprinted early in the BM. Combining single cell sequencing with conventional transcriptomic analysis, Cytometry by Time-Of-Flight mass spectrometry (CyTOF) and intra-femoral transfer, we identified for the first time DC subset-specific precursors in the BM as well as previously unknown molecular checkpoints for DC lineage commitment as early as the CDP stage.

These new insights into the origins of DCs, monocytes and macrophages should aid the rational design of therapies aimed at harnessing the functions of these cells in homeostasis and inflammation and will allow efficient targeting and manipulation during health and disease.

Cytosolic DNA detection by the inflammasome and beyond

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Inflammasomes are large multi-protein complexes that trigger the activation of so called inflammatory caspases, most importantly caspase-1. Upon activation, caspase-1 cleaves various substrates, including the pro-inflammatory cytokines IL-1β and IL-18, which are rendered active upon proteolytic maturation. Beyond these members of the IL-1 family, multiple additional caspase-1 targets have been identified, yet the physiological role of these cleavage events remains to be elucidated. Moreover, caspase-1 activation leads to a certain type of cell death, named pyroptosis, that can also trigger inflammation in trans. Various sensor proteins have been identified that can trigger the formation of inflammasome platforms. These inflammasome-forming PRRs, except for the DNA sensor AIM2, belong to the Nod-like receptor (NLR) family. NLRs are cytosolic PRRs with a tripartite domain architecture comprising of C-terminal leucine-rich repeats (LRRs) that are thought to sense microbial molecules or endogenous stress mediators; a central NACHT nucleoside triphosphatase domain that mediates NLR oligomerization and formation of the core structure of the inflammasome; and an N-terminal effector domain required for signal transduction. AIM2 is an exception to this rule, as it contains a unique C-terminal ligand binding domain, the HIN200 domain. While AIM2 plays a non-redundant role in sensing the presence of cytosolic DNA leading to ASC-dependent inflammasome activation, other cytosolic DNA sensing mechanisms have been identified that are required to drive pro-inflammatory gene expression. Among these, the recently identified cGAS/STING axis plays the predominant role. In this talk an update is given on our recent progress on the characterization of cytosolic DNA sensing pathways and their interconnection in the context of infectious and sterile inflammatory conditions.

Identification and Characterization of Phosphodiesterase V-cGAPs That Degrade 3’3’-Cyclic GMP-AMP in vibrio cholerae

Zhengfan Jiang

Not available at time of printing

IL-1 regulation in inflammatory disease

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Inflammation plays vital roles in protective responses against pathogens and tissue repair, however, improper resolution of inflammatory networks is centrally involved in the pathogenesis of many acute and chronic diseases. Extensive advances have been made in recent years to define the inflammatory processes that are required for pathogen clearance, however, in comparison, less is known about the regulation of inflammation in sterile settings. Our recent studies describe the cellular events and molecular signalling pathways regulated by the cytosolic innate immune receptors NLRs and IL-1 that govern inflammation in inflammatory disease.

Dynamics Of Cytokine Responses During Chronic Viral Infection

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Our laboratory is interested in understanding the cellular and molecular mechanisms underlying the regulation of the immune system during viral infections, particularly infections with persistent viruses, which represent a serious health problem. To overcome the lack of small animal hosts for human persistent viruses, we use models of chronic viral infections in their natural murine hosts. In particular, by using chronic lymphocytic choriomeningitis virus (LCMV) infection in mice we have evaluated the dynamics of several cytokines (including interleukin-6 and transforming growth factor-b) and investigated their regulatory function on immune cells and their impact on the outcome of the infection at different times after viral challenge. By doing in-vivo studies at the whole organism level we are able to assess the impact of selected cytokine signaling in the context of the persistent infectious environment in which the immune cells are navigating. I will be presenting recent discoveries in this research area.

Masaaki Murakami[1]

1. *Hokkaido University, Japan*

Our laboratory is interested in understanding the cellular and molecular mechanisms underlying the regulation of the immune system during viral infections, particularly infections with persistent viruses, which represent a serious health problem. To overcome the lack of small animal hosts for human persistent viruses, we use models of chronic viral infections in their natural murine hosts. In particular, by using chronic lymphocytic choriomeningitis virus (LCMV) infection in mice we have evaluated the dynamics of several cytokines (including interleukin-6 and transforming growth factor-b) and investigated their regulatory function on immune cells and their impact on the outcome of the infection at different times after viral challenge. By doing in-vivo studies at the whole organism level we are able to assess the impact of selected cytokine signaling in the context of the persistent infectious environment in which the immune cells are navigating. I will be presenting recent discoveries in this research area.

Old and new families of negative regulators of cytokine signalling

Nicos Nicola[1], Donald Metcalf[1], Warren S Alexander[1], Douglas J Hilton[1], Sandra E Nicholson[1], Jian-Guo Zhang[1], Andrew W Roberts[1], Alan Ching[1], Jose Villadangos[1], Dina Stockwell[1], Nadia J Kershaw[1], Jeff J Babon[1]

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Our discovery of a family of negative regulators of cytokine signalling (the Suppressors of Cytokine Signalling, SOCS) was based on a screen that allowed the M1 myeloid leukaemic cell line to continue to grow in the presence of interleukin 6.

The expression of various SOCS proteins is induced by cytokine activation of the JAK/STAT pathway and they then act as feedback inhibitors to terminate the pathway. Biochemical studies have revealed that the SH2 domains of SOCS proteins bind phosphorylated tyrosines on selected substrates and that the C-terminal SOCS box recruits elonginsB/C and cullin 5 to form an E3 ubiquitin ligase that ubiquitinates the bound substrates and targets them for proteasomal degradation.

Two members of the family, SOCS1 and SOCS3, display an additional inhibitory mechanism that involves direct inhibition of the kinase activity of JAKs. Recent structural and biochemical studies have revealed the precise mechanism by which this occurs. Genetic deletion studies in mice have revealed how overactivity of cytokine action and specificity of action are regulated by SOCS proteins and how these relate to the molecular mechanism of action. Very recently we have used the same M1 assay described above to discover a second family of inhibitors of cytokine signalling- the MARCH proteins. These proteins are integral membrane proteins that also display E3 ubiquitin ligase activity and have been previously shown to regulate the expression at the cell surface of a number of immunoregulatory receptors. We now show that some of these proteins also specifically down-regulate the cell surface expression of cytokine receptors like those for interleukin 6 and render cells unresponsive to the cytokine.

A lifelong love affair with IL-1 and inflammation

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IL-1 was one of the first cytokines to be described, first as a bioactivity that mediated inflammation in many different disease contexts, and then as a family of proteins – IL-1alpha, IL-1beta and the IL1 receptor antagonist. By the late 1980s IL-1 was known to induce the expression of a large number of immune and inflammatory genes and a key mystery was how it signals. Great detail into the signalling pathways leading to NF-kappaB and p38 MAP kinase then emerged. The IL-1 receptor was the founder member of the IL-1R/TLR superfamily and was first defined in detail 15 years ago. Since then, there has been remarkable progress in our understanding of both branches of the superfamily. Ligands have been described for most receptors. Within the IL-1R subfamily, notable examples include IL33 for ST2 and IL-36 for IL-1Rrp2.

The role of TLRs in the sensing of microbial products led to a renaissance of interest in innate immune mechanisms and represented one of the biggest advances in our understanding of the immune system over the past 50 years. For investigators interested in signal transduction, the area has proved very fruitful in terms of the discovery of new signalling pathways and processes, notably the MyD88 family of adapter proteins and the IRAK family, involving a complex series of ubiquitination and phosphorylation reactions.

Studies on how the-pro form of IL-1beta is processed led the description of inflammasomes, multiprotein complexes that activate caspase-1. Inflammasomes can also trigger a type of cell death dubbed pyroptosis. The best understood inflammasome comprises Nlrp3, and this protein has been implicated in all of the pathologies suggested to involve IL-1 in the 1980s which even then included gout, atherosclerosis, Alzheimer's disease and Type 2 diabetes.

We therefore have a love story beginning as an ill-defined pro-inflammatory factor in supernatants from LPS-activated macrophags, to a revolution in innate immunity, and the real prospect of limiting the IL-1 system for substantial clinical benefit.

The Paradox roles of IL-18 Signaling in Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is the fifth most common cancer and third leading cause of cancer-related mortality world-wide. This cancer almost always develops upon the background of chronic pathological inflammation and resultant fibrosis, which regulate both the development and progression of this cancer. Interleukin 18 (IL-18), a member of the IL-1 family of cytokines, has been reported to be elevated in serum of patients with liver disease and HCC, however, its mechanistic significance remains poorly understood.

To explore this question, we evaluated patient serum and tissue samples, and found that while IL-18 was indeed elevated in comparison to healthy individuals, and its elevation correlated to worse survival of patients, its levels were decreased inside tumor compared to non-tumor tissue from the same patient. Utilizing an immune-competent mouse model with orthotopic tumor implantation into a fibrotic liver, we found that both knocking down IL-18 expression in mouse HCC cells implanted into wild-type mice and inoculating mouse HCC cells in an IL-18 receptor-deficient mouse resulted in enhanced tumor growth. Post-implantation, CD8+ T-cells were reduced and CD4+ T-cells mildly increased in tumors in the IL-18 receptor-deficient mice compared to wild-type controls, with a resultant robust change in CD8:CD4 T-cell ratio, while other IL-18-responsive cell types, such as NK cells, were unchanged. A chemically-induced carcinogenesis model also displayed increased tumor burden in IL-18 receptor-deficient mice compared to wild-type controls. Re-examination of patient samples revealed that magnitude of the difference in IL-18 expression between tumor and non-tumor liver correlated with patient prognosis, with worse prognosis in patients with less IL-18 inside the tumor compared with non-tumor liver tissues. These results suggest a possible biphasic role for IL-18 in the pathological process of this disease, and demonstrate a complex role for IL-18 in HCC tumorigenesis and progression.

The role of perivascular macrophages in neutrophil recruitment into the skin

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Neutrophils are important defenders against bacterial infections. Upon bacterial entry into the skin, neutrophils migrate rapidly into the inflamed dermis. We found that perivascular macrophages (PVM) are essential regulators of neutrophil homing following infection with the pathogen *Staphylococcus aureus* (*S. aureus*). PVM are juxtaposed to post-capillary venules in the dermis, and are capable of taking up molecules from plasma. Using real-time multiphoton intravital microscopy, we show that neutrophils extravasate from inflamed venules in close contact with PVM. The latter are a major source of the neutrophil chemo-attractants CXCL1 and 2. Strikingly, the *S. aureus* virulence factor alpha-hemolysin targets macrophages, resulting in their lysis. Consequently, neutrophil recruitment is delayed leading to increased bacterial survival and tissue necrosis. We further demonstrate that macrophages express high levels of the hemolysin receptor ADAM-10 and that pharmacologic blocking of ADAM-10 interferes with macrophage killing by hemolysin in vitro. In summary, we reveal the thus far unknown guidance of blood-borne cells by perivascular cells resulting in extravasation. We propose the existence of an integrated multi-cellular unit, termed the perivascular extravasation unit (PVEU), which, besides endothelial cells and pericytes, includes PVM. Together, PVEU components regulate leukocyte recruitment into the skin. Finally, the killing of PVM by a bacterial product provides insight into the mechanisms of immuno-evasion by a human pathogen.

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Characterisation of the function of the Interleukin-11 signaling complex in human disease

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Interleukin (IL)-11 is an under-characterised member of the IL-6 family of cytokines that signals through a cell-type specific a-subunit receptor, IL-11Ra, and a ubiquitously expressed transmembrane b-subunit receptor, GP130. The formation of the active hexameric IL-11/IL-11Ra/GP130 (2:2:2) signaling complex leads to the recruitment of intracellular Janus Kinases (JAKs) which phosphorylate the cytoplasmic tail of GP130 providing docking sites for the signal transducer and activator of transcription (STAT) proteins. Once phosphorylated, STATs form active dimers that translocate to the nucleus where they regulate the expression of numerous genes involved in cell survival and proliferation. Recently, mutations in components of the IL-11 signaling complex have been linked to a range of human disorders including craniosynostosis.

Our current understanding of the structural mechanism of IL-11 signaling has been based on homology models using IL-6, despite low sequence similarity (~20%). IL-6, signals through a hexameric IL-6/IL-6Ra/GP130 (2:2:2) signaling complex, for which high resolution crystal structures are available. We have recently solved the first crystal structure for human IL-11, which suggests important differences between these two cytokines including how they engage GP130 and trigger the downstream signaling cascade. In order to understand how mutations in components of the IL-11 signaling complex identified in human disease impact on signal transduction, we have performed site-directed mutagenesis of key residues at the IL-11/IL-11Ra and IL-11Ra/GP130 interface. We characterised the impact of each mutation on the phosphorylation of STAT proteins in BA/F3 cells, which otherwise lack IL-11/IL-11Ra/GP130 components. Our results identify regions of the IL-11 signaling complex that are crucial for signaling, and represent druggable target sites to agonise or antagonise this signaling pathway.

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HIV infection is associated with altered monocyte subset basal cytokine levels and responsiveness to LPS stimulation.

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Introduction: HIV+ individuals are at increased risk of inflammatory age-related disease despite effective suppression of viremia with combination antiretroviral therapy (cART). Lipopolysaccharide (LPS) is elevated in these individuals and stimulates monocytes via the Toll-like receptor 4 pathway producing IL-6 and TNF. We hypothesise that chronic LPS exposure enhances monocyte response to LPS in HIV+ individuals that exacerbates chronic inflammation and the risk of developing age-related disease.

Methods: The inflammatory state of the three monocyte subsets (classical, intermediate and non-classical) from cART naïve, viremic (n=10) and virologically supressed (VS) HIV+ donors (n=10) was evaluated by measuring basal and LPS-induced (10 ng/mL, 4 h) levels of IL-6 and TNF by whole blood intracellular cytokine staining via flow cytometry and mRNA gene expression and results compared to age-matched HIV- controls (n=10). Soluble signalling components (LPS, LBP), inflammatory markers (CXCL-10) and surface TLR-4 were also measured.

Results: All monocyte subsets from viremic and VS HIV+ individuals showed increased basal levels of intracellular IL-6 and TNF in comparison to HIV- individuals ($p<0.001$ for all comparisons). Furthermore, these groups showed enhanced IL-6 and TNF production when stimulated with LPS ($p<0.05$ for all comparisons). While plasma LPS and LBP levels were elevated in HIV+ individuals in comparison to controls ($p<0.05$ for both), mixing serum from HIV+ individuals with control monocytes did not enhance response to LPS challenge *ex vivo* suggesting enhanced monocyte response to LPS in HIV+ individuals is governed by mechanisms intrinsic to the monocyte. This is supported by increased surface TLR-4 expression on all monocytes subsets for viremic HIV+ individuals ($p<0.05$).

Conclusions: Monocytes from HIV+ individuals, regardless of therapy status, show increased basal intracellular IL-6 and TNF levels and a heightened response to LPS which may contribute to chronic inflammation and related inflammatory diseases.

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Characterization of human-restricted TLR4 responses identifies a novel ubiquitin ligase required for NLRP3 inflammasome responses in human macrophages

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We previously characterized conservation and divergence in Toll-like Receptor (TLR)4-dependent transcriptional responses in human versus mouse macrophages. In this study, we focused on orthologous human and mouse genes uniquely regulated by lipopolysaccharide (LPS) in primary human macrophages. We confirmed “human-specific” LPS regulation of these genes by examining expression in primary human macrophages versus a panel of different mouse macrophage populations. In investigating potential functions, we found that one of these genes (a novel ubiquitin ligase) was required for NLRP3 inflammasome responses, as demonstrated by gene knock-down in both primary human macrophages and PMA-differentiated THP-1 cells. The NLRP3 inflammasome is a multi-protein complex containing NLRP3, ASC and caspase-1. Activation of the NLRP3 inflammasome results in the processing and release of the pro-inflammatory cytokines IL-1beta and IL-18, as well as pyroptotic cell death. NLRP3 assembly requires both a priming signal such as that delivered by the TLR4 agonist LPS, as well as a triggering signal such as damage-associated host molecules (e.g. ATP) or pathogen-associated stimuli. Recent studies have demonstrated that direct deubiquitination of NLRP3 is required for triggering of the NLRP3 inflammasome. Our findings now suggest that one of the mechanisms by which LPS primes inflammasome responses in human macrophages is via the upregulation of a specific ubiquitin ligase. We provide evidence that this ubiquitin ligase affects LPS priming of inflammasomes in human macrophages by promoting ubiquitination-dependent degradation of p21(Waf1/Cip1), which has previously been shown to inhibit LPS-inducible IL-1beta expression independently of its cell-cycle inhibitory role. Current studies are aimed at further exploring the dynamics of p21 protein regulation by the ubiquitin ligase, as well as p21 regulation of IL-1beta synthesis. These findings provide new insights into mechanisms affecting pathological inflammatory processes and provide direction for potential treatment strategies.

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IL-10 is critical for the expansion of peritoneal and splenic CD5$^+$ B cells

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Aims: Interleukin-10 (IL-10) is a pleiotropic cytokine with immunosuppressive properties that has been shown to support B cell survival. Recent studies have identified a subset of regulatory B cells that can suppress the progression of autoimmunity by production of IL-10. Previous work from our laboratory has shown that the introgression of a chromosome 4 interval spanning 32 to 150Mb from autoimmune prone New Zealand Black mice expands two innate-like lymphocyte populations, CD5$^+$ B cells and Natural Killer T cells. Additional work on these mice identified the expanded splenic and peritoneal CD5$^+$ B cell population as highly IL-10 competent and regulatory in nature. The aim of this study was to identify a possible role of IL-10 in the development or homeostatic expansion of NKT and CD5$^+$ B cells.

Methods: IL-10 knockout mice, obtained from Jackson laboratories, were bred onto our chromosome 4 congenic background. Congenic knockout mice and controls were aged to 18 - 23 weeks. Splenic and peritoneal cell populations were identified de novo by flow cytometry.

Results: Similar to previous studies, the IL-10 knockout reduced the proportion of splenic Natural Killer cells on both the B6 and chromosome 4 backgrounds when compared to IL-10 sufficient controls. Interestingly, IL-10 knockout had no impact on the expansion of splenic NKT cells, suggesting that IL-10 is not important in the expansion of this population. Surprisingly, IL-10 knockout prevented the expansion of both splenic and peritoneal CD5$^+$ B cells in chromosome 4 congenic mice and greatly expanded the splenic marginal zone compartment.

Conclusions: Taken together, these data indicate that IL-10 may play a unique and critical role in sustaining the genetic expansion of CD5$^+$ B cells in the splenic and peritoneal compartments. Unlike previous reports, this study suggests that the expansion of regulatory B cells may be impacted by the loss of IL-10.

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Intrinsic and extrinsic mechanisms of metastatic inhibition by IRF5 in human ductal carcinoma

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Metastasis of primary breast cancer to distant sites and recurrence to incurable disease are the main causes of breast cancer fatalities. While migration of breast cancer cells out of a duct or lobule is a prerequisite for invasion and metastasis, the ability of these cells to migrate at all is due to intrinsic intratumoral and extrinsic microenvironment changes. Little is known of the factors that regulate these intrinsic and extrinsic functions. Previous work has shown that expression of the transcription factor interferon regulatory factor 5 (IRF5) is significantly decreased as a breast lesion progresses from a non-malignant stage to ductal carcinoma *in situ* and is eventually lost in ~80% of invasive ductal carcinomas examined. Human *in vitro* and murine *in vivo* models of invasive breast cancer cell growth confirm an important role for IRF5 in regulating breast cancer cell migration, invasion and metastasis. We recently identified the protein domain necessary for the intrinsic migratory function of IRF5 and found that this function is cytoplasmic and transcription-independent. Given that IRF5 is also a key transcriptional immune regulator, we examined cytokine/chemokine expression in IRF5(+) and (-) breast cancers grown in 3D culture and found that IRF5 positively regulates the expression of key cytokines/chemokines, such as CXCL13, that define a pro- or anti-tumor immune microenvironment. We found that anti-tumorigenic primary T cell subsets, including Th1, Treg and Tfh cells, were specifically recruited to IRF5(+) tumors as compared with IRF5(-) tumors. We also found that CD19+CXCR5+ B cells were specifically recruited to IRF5(+) tumors. This finding is intriguing since CXCR5+ B and T cells (Tfh) are essential for the formation of tertiary lymphoid structures (TLS). Together, these data support that IRF5 controls intrinsic mammary epithelial cell migration and directly regulates a network of genes that shapes a tumor immune response.

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Development of new automated technology for the detection and analysis of pathogenic autoantibodies in autoimmune disease

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Anti-nuclear antibodies (ANA) are considered a hallmark of autoimmune rheumatic diseases. An ANA test is routinely performed on serum from patients presenting with symptoms of autoimmune diseases such as systemic lupus erythematosus (SLE), autoimmune hepatitis, rheumatoid arthritis, polymyositis/dermatomyositis, mixed connective tissue disease, Sjögren's syndrome, and systemic sclerosis. The standard method for ANA detection is the indirect immunofluorescence (IIF) assay on human epithelial-2 (HEp-2) cells (ANA-HEp-2 test). This assay is a slide-based microscopic analysis that allows for the detection of clinically relevant autoantibodies generated against cytoplasmic and nuclear antigens in patient serum. One of the major issues with the ANA-HEp-2 test lies in the subjective evaluation of HEp-2 slides that complicates standardization and reproducibility. We have developed a new high-throughput process of ANA testing that eliminates the subjectivity of data interpretation, and capitalizes on the properties of imaging flow cytometry to accurately localize, detect and quantify multiple autoantibodies in a single run. The method was initially developed using anti-nuclear antibody (ANA) reference sera, prepared by the Arthritis Foundation and the Centers for Disease Control, that contains nuclear components useful in the diagnosis and classification of autoimmune diseases. Samples were run on an Amnis Imagestream and algorithms that distinguish between individual staining patterns using masks and features provided in Amnis IDEAS® software were generated. The method was then tested using complex sera from patients with SLE and data compared to the clinical standard ANA-HEp-2 test. We obtained 100% replication of the standard ANA-HEp-2 assay with much greater sensitivity and detection of potentially new pathogenic autoantibodies. The detection of such new and known autoantibodies is of significant clinical diagnostic value since it allows for not only the identification of severe autoimmune disorders but also helps to make predictions about their course and prognosis.

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Viral antagonism of type I interferon responses reveals prolidase as a regulator of IFNAR1 trafficking and expression.

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Flaviviruses, including tick-borne encephalitis virus (TBEV), West Nile virus (WNV) and dengue virus, cause severe disease in humans on a global scale. All flaviviruses antagonize type I interferon (IFN-I)-dependent activation of the JAK-STAT signaling cascade, suggesting that this pathway is an essential target for flavivirus evasion of innate immunity. However, despite nearly 10 years of research, the molecular mechanism of inhibition utilized by TBEV and WNV is largely unknown. IFN-I signals through two receptor subunits, IFNAR1 and IFNAR2. TBEV and WNV antagonize IFN-I responses at a point proximal to the receptor as all signaling events downstream of this are inhibited in virus-infected cells. Replication of WNV (strain NY99) is associated with degradation of IFNAR1 via lysosomes, implicating IFNAR1 as the target of viral interference (Evans et al. 2011. *Viral Immunol.* 24:253). Here we show that TBEV and WNV downregulate IFNAR1 expression at the protein level. The viral protein responsible is NS5, the major flavivirus IFN-I antagonist. We show that NS5-mediated loss of IFNAR1 is dependent on binding to a host cell dipeptidase called prolidase (PEPD). Knockdown of PEPD expression prevented IFNAR1 maturation (glycosylation), increased the ubiquitination of IFNAR1, and promoted degradation of immature (partially glycosylated) IFNAR1 via the lysosome. In humans, genetic prolidase deficiency (PD) is associated with recurrent infections although the reasons for immune dysfunction are not known. Our results suggest that IFN-I signaling may be compromised, which was confirmed in primary fibroblasts from PD patients that expressed low IFNAR1 associated with reduced STAT1/2 phosphorylation and ISG expression following IFNβ stimulation. Thus, by understanding flavivirus antagonism of IFN-I signaling, we have uncovered PEPD as a central regulator of cellular responses to IFN-I humans and newly identified PD as a primary immune deficiency. Manipulation of IFNAR1 stability by therapeutic targeting of PEPD may represent new treatments for both viral and autoimmune diseases.

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Correlation and kinetics of IFN-gamma and TNF-alpha transcription and translation in lymphocyte subsets using the FlowRNA assay and intracellular antibody staining

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Aims:

Intracellular staining and flow cytometric analysis of lymphocytes is commonly used to assess cytokine production at the single-cell level in heterogeneous samples. Here, we describe a novel fluorescence *in situ* hybridization (FISH)-based flow cytometry assay (PrimeFlow RNA Assay) used in combination with intracellular antibody staining to study the kinetics of the transcription and translation of IFN-gamma and TNF-alpha in lymphocytes.

Methods:

Normal human peripheral blood mononuclear cells were stimulated with PMA and Ionomycin for 0-5 hours. Cells were fixed, permeabilized, and intracellularly stained with antibodies for CD8, IFN-gamma, and TNF-alpha. Next, cells underwent a series of hybridization steps to label mRNA for IFN-gamma and TNF-alpha. Data were collected on an LSRFortessa and analyzed using FlowJo software.

Results:

IFN-gamma mRNA was upregulated in CD8+ and CD8- lymphocytes within 1 hour after stimulation, while protein levels were not detected until 2 hours, after which both mRNA and protein were maintained for the next 3-4 hours. In contrast, TNF-alpha mRNA and protein were both upregulated within 1 hour after stimulation and expression was maintained in CD8+ cells while expression in CD8- cells peaked between 1-2 hours and then decreased over the next 4 hours, with the decrease in mRNA preceding the decrease in protein.

Conclusions

Using the PrimeFlow RNA Assay, we found that induction of IFN-gamma and TNF-alpha mRNA and protein exhibit unique kinetics and that TNF-alpha protein and mRNA are differentially regulated in CD8+ and CD8- lymphocytes. This new PrimeFlow RNA Assay enables the study of gene expression at the single-cell level in heterogeneous samples without the need for sorting specific subsets and the ability to compare and contrast the kinetics of mRNA and protein induction.

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Autocrine CXCL13-CXCR5 signaling potently induces EMTof breast cancer cells via RANKL-Src-PI3Kp110α during lymph node metastasis

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Background

Epithelial to mesenchymal transition (EMT) is an important hallmark of metastasis. Increased EMT is associated with lymph node metastasis (LNM). We investigated EMT inducing potential of chemokine CXCL13 in breast cancer (BC) cell lines and CXCL13-CXCR5 signaling in primary breast tumors in association with different epithelial and mesenchymal markers and EMT inducers.

Methods

Chemokine CXCL13 and and its sole receptor CXCR5 were evaluated in 98 primary breast tumor samples from breast cancer patients with infiltrating duct carcinoma (IDC). BC cell lines were transduced with CXCR5, treated with recombinant CXCL13 and assessed for cell migration, EMT markers and activation of intracellular signaling pathways.

Results

A total of 56 patients were LNM positive and 42 were LNM negative. LNM positivity was positively associated with co-expression of CXCL13 and CXCR5. Increased expression of various mesenchymal molecular markers and regulators and decreased expression of epithelial marker E-cadherin was found in CXCL13-stimulated BC cells and tumors from CXCL13-CXCR5 co-expressing patients. Experiments with protein kinase inhibitors demonstrated that CXCL13 stimulates EMT and expression of matrix metalloproteinase (MMP9) via RANKL-Src axis.

Conclusion

This study demonstrated the prognostic value of CXCL13-CXCR5 co-expression in primary BC. Moreover, it showed the EMT inducing potential of CXCL13. RANKL-Src axis may present a therapeutic target in LNM positive BC patients.

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IFNα engages a sustained antiviral response depending on STAT2/IRF9 but not ISGF3

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Evidence is accumulating for the existence of a STAT2/IRF9-dependent, STAT1-independent IFNα signaling pathway. However, no detailed insight exists in the genome-wide transcriptional regulation and the biological implications of STAT2/IRF9 dependent IFNα signaling as compared to ISGF3. In STAT1 KO cells overexpressing STAT2 we observed that the IFNα-induced expression of classical interferon stimulated genes (ISGs) such as human OAS2 and mouse Ifit1 correlated with the kinetics of STAT2 phosphorylation, and the presence of a STAT2/IRF9 complex requiring STAT2 phosphorylation and the STAT2 transactivation domain.

Subsequent microarray analysis of IFNα treated WT and STAT1 KO cells over-expressing STAT2 extended our observations and identified around 120 known antiviral ISRE-containing ISGs commonly up-regulated by STAT2/IRF9 and ISGF3. The STAT2/IRF9 directed expression profile of these ISGs was prolonged as compared to the early and transient response mediated by ISGF3. ChIP-seq analysis confirmed binding of STAT2 to the promoter of a selection of commonly up-regulated ISGs, in an IFNα-dependent manner in the absence of STAT1.

In addition, we identified a group of “STAT2/IRF9-specific” ISGs, whose response to IFNα was ISGF3-independent. Finally, STAT2/IRF9 was able to generate a sustained antiviral response upon EMCV and VSV.

Our results further prove that IFNα-activated STAT2/IRF9 induces a prolonged ISGF3-like transcriptome and generates an antiviral response in the absence of STAT1. Moreover, the existence of “STAT2/IRF9-specific” target genes predicts a novel role of STAT2 in IFNα signaling.

Possibly, in cells with prolonged STAT2 phosphorylation kinetics this could provide a level of redundancy to ensure effective induction of an antiviral state and be beneficial for example against viruses that directly block STAT1 and impair the formation of ISGF3.

Preliminary results imply that this situation is very likely to be cell-type specific, where both complexes seem to be involved in different stages of the antiviral response; ISGF3 stimulating a rapid and transient antiviral response whereas STAT2/IRF9 being responsible for a more prolonged antiviral response.

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The rs12971860 C/T SNP in the interferon lambda3 (IL28B) gene influences the expression of thymidine phosphoryase and cytidine deaminase, two enzymatic markers of inflammation

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Background

Some chronic viral infections are responsive to treatment with interferon alpha (IFN-a), but outcomes are variable and unpredictable. In the case of chronic hepatitis C virus (HCV) infection, single nucleotide polymorphisms (SNPs) affecting the gene for interferon IFN-L3, (alias interleukin-28B (IL28B), affect response to IFN-a-based treatments. The most studied SNP is C/T at rs12971860 in the IFN-L3 gene's CpG rich promoter region, with the ranked probability of favourable response (viral clearance) being CC>CT>TT. The biological mechanisms responsible for this phenomenon are not known. One hypothesis to explain the observed ranking proposes that the T allele lowers the threshold for immune activation, consequently inducing a refractory state by raising the threshold and reducing the dynamic range available for response to additional immunogenic stimuli from exogenous cytokines. Thymidine phosphorylase (TP) and cytidine deaminase (CDA) are evolutionarily conserved enzymes of pyrimidine metabolism that are inducible directly and indirectly (respectively) by pro-inflammatory cytokines. Increases in TP and CDA activity are therefore indicative of immune activation.
Hypothesis and Aim

If the IFN-L3 rs12971860 T allele is associated with increased immune activation, it may increase the activities of TP and CDA, which are easily measurable in peripheral blood.

Methods

A simple HPLC-based assay for whole blood TP and CDA activity was devised and used to measure enzyme activities in >200 blood samples obtained from individuals whose IFNL3 genotype was determined by a PCR-based method.

Results and Conclusions

Remarkably, concentrations of thymidine and cytidine close to a million-fold greater than their normal (submicromolar) plasma concentrations were required to saturate blood TP and CDA. A very large (~10-fold) range of both activities was found. Correlation with IFNL3 genotyping results revealed a ranked difference in enzyme activities in the order TT>CT>CC, supporting the hypothesis that the rs1291860 T allele is associated with increased pro-inflammatory activity.

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Spontaneous mutation in TNF in a new model of rheumatoid arthritis

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We have discovered mice with a genetic mutation that predisposes to rheumatoid arthritis. The trait is autosomal dominant and fully penetrant. The affected mice present with a severe symmetrical, erosive chronic poly-arthritis, similar to human rheumatoid arthritis (RA). It mostly affects the peripheral joints (the ankles, metatarsal and inter-phalangeal joints) with less severe damage in the central joints (knees and shoulders). Inter-vertebral joints, especially between T9 and T13, are also affected. The bone marrow shows unusual B lymphoid aggregates, associated with osteolytic lesions, and bronchus associated lymphoid tissue is present in all affected mice. Homozygozity dramatically increases the severity of the disease. The phenotype is independent of T and B cells, and still develops in the absence of Myd88 or GM-CSF.

We found that the mutation is a spontaneous insertion of a transposon in the 3'UTR of the TNF gene, and causes a dramatic increase in the expression of this cytokine. Accordingly, loss of TNFR1 completely prevents all aspects of the phenotype.
We are presently in the process of identifying new regulators of TNF expression.

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Alterations in inflammatory cytokine network in diseased conditions associated with cognitive impairments

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Background. Numerous findings suggest implication of upregulated inflammation in defects of neuronal plasticity and synaptic connectivity leading to cognitive impairments in schizophrenia, posttraumatic stress disorder (PTSD) and ischemic stroke (IS). However, molecular pathomechanisms responsible for development of deleterious inflammatory reactions in these diseased conditions are only beginning to be understood. Aim. This report provides a brief overview of our recent studies on functional state of the major inflammatory mediators, proinflammatory and chemotactic cytokines and their receptors, in etiopathogenesis of schizophrenia, PTSD, and IS. Methods. Experiments were performed on gene, transcription, and protein levels using genomic DNA, mRNA, and blood plasma samples of diseased and healthy individuals (250 subjects in each group). Single specific primer-polymerase chain reaction (SSP-PCR), *real-time PCR* and enzyme-linked immunosorbent assay were applied. Data were analyzed using a wide range of methods including parametric and nonparametric statistical tests, multiple comparisons corrections, and correlation analysis. Results. The results obtained indicated association of polymorphic variations in genes encoding a number of proinflammatory and chemotactic cytokines and their receptors with schizophrenia, PTSD and IS. Different types of associations were found for each disorder. In addition disease-specific changes in gene expression levels as well as in plasma concentration for many proinflammatory and chemotactic cytokines and their receptors were detected. Some of these changes correlate with frequency and intensity of clinical signs, symptoms, and severity of diseased conditions as well as other clinically significant characteristics of patients such as age of the disease onset, etc. Also, relationships between genotypes and protein expression levels were found for many cytokines. Conclusions. Schizophrenia, PTSD, and IS are accompanied with changes in functional activities of proinflammatory and chemotactic cytokines and their receptors. Each disease is characterized by specific combination of single nucleotide variations, changes in mRNA and protein expression patterns of the inflammatory cytokine network components.

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F-actin is required for negative regulation of NLRP3 inflammasome activity by Flightless-I and LRRFIP2

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The NLRP3 inflammasome interacts with the adaptor ASC to activate caspase-1 for the maturation of pro-IL-1β into active IL-1β. The formation and activity of NLRP3 inflammasome is regulated through mechanisms not fully elucidated. It was recently demonstrated that leucine-rich repeat Flightless-I-interaction protein 2 (LRRFIP2) and Flightless-I (FliI) negatively regulate NLRP3 inflammasome activity. It was also showed that FliI is a pseudosubstrate and inhibitor of caspase-1. Since FliI is an actin-remodeling protein, we addressed the question of a role of actin in the regulation NLRP3 inflammasome in human macrophages (THP-1). The depolymerization of filamentous actin (F-actin) in globular actin (G-actin) significantly increased the production of mature IL-1β while the stabilization of F-actin decreased IL-1β production. These results suggest that activation of NLRP3 inflammasome depends on the actin polymerization state but not on the active polymerization process. The silencing of LRRFIP2 and FliI by lentiviral-based shRNA transduction showed that LRRFIP2 and FliI were required for co-localization of NLRP3 on F-actin in response to ATP or Nigericin. Thus, FliI-mediated caspase-1 inhibition involves the localization of NLRP3 inflammasome on F-actin leading to the diminution of caspase-1 activation and in turn to decreased IL-1β production. The present data demonstrate that in addition to directly inhibiting caspase-1, FliI together with LRRFIP2 allow the location of the NLRP3 inflammasome on F-actin, further inhibiting caspase-1 activation and IL-1β production. Our results unveil a new function of actin and a dual function of FliI in the regulation of NLRP3 inflammasome activity strengthening the importance of cytoskeleton in the regulation of inflammation.

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Super-resolution microscopy reveals important roles for microtubule bundling by rabies virus P3 protein in immune evasion and disease

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Rabies virus (RABV) is the causative agent of rabies, a severe neurological disease with a c.100% case-fatality rate that results in >55,000 human deaths per year. The high lethality of RABV is thought to be dependent on its ability to inhibit host interferon (IFN)-mediated antiviral immune signalling via the association of its IFN-antagonist P3 protein and STAT immune signalling proteins. *In vitro* studies have indicated that P3 association with the microtubule (MT) cytoskeleton is important to STAT signalling inhibition but the molecular details of the P3-STAT-MT interaction and its roles in disease *in vivo* are currently unresolved.

Using a unique RABV pathogenicity model, viral reverse genetics, immune signalling assays and a novel, quantitative *in vivo* MT tracing assay using live 3D confocal laser scanning microscopy (CLSM), we identified a specific mutation (N_{226}-H) that can impair the MT-association, as detected by CLSM, and IFN-antagonistic function of P3. Introduction of the N_{226}-H mutation into an invariably lethal strain of RABV strongly impaired disease progression *in vivo* in mice. Importantly, analysis utilising super-resolution dSTORM (**d**irect**ST**ochastic **O**ptical **R**econstruction **M**icroscopy) revealed that P3 induces significant MT bundling, and that N_{226}-H is defective in this respect. However, N_{226}-H does not affect the physical interaction of P3 with MT fractions or tubulin, indicating that the mutation specifically impacts P3's capacity to modify MT structure. This indicates a vital link between MT bundling and inhibition of immune signalling by P3 and this mechanism is crucial to RABV pathogenicity, a novel discovery among viruses.

This data indicates that RABV and potentially other pathogenic viruses exploit/modulate dynamic MT functions to manipulate host cell biology. This study has clear implications for our understanding of viral disease mechanisms and their potential targeting for vaccine/therapeutic development and highlights the power of super-resolution microscopic approaches to probe molecular events at the host-pathogen interface. Ongoing research aims to characterise in detail the molecular events at the MT interface of RABV, Ebola and HIV-1.

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The Innate Immune Sensor LGP2 Activates Antiviral Signaling by Regulating MDA5-RNA Interaction and Filament Assembly

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Cytoplasmic pattern recognition receptors detect non-self RNAs during virus infections and initiate antiviral signaling. One receptor, MDA5, possesses essential signaling domains, but weak RNA binding. A second receptor, LGP2, rapidly detects diverse dsRNA species, but lacks signaling domains. Accumulating evidence suggests LGP2 and MDA5 work together to detect viral RNA and generate a complete antiviral response, but the basis for their cooperation has been elusive. Experiments presented here address this gap in antiviral signaling, revealing that LGP2 assists MDA5-RNA interactions leading to enhanced MDA5-mediated antiviral signaling. LGP2 increases the initial rate of MDA5-RNA interaction and regulates MDA5 filament assembly, resulting in the formation of more numerous, shorter MDA5 filaments that are shown to generate equivalent or greater signaling activity in vivo than the longer filaments containing only MDA5. These findings provide a mechanism for LGP2 coactivation of MDA5 and a biological context for MDA5-RNA filaments in antiviral responses.

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An elucidation of the regulation of the Interferon pathway using the *Interferome* and *Enrich* bioinformatics resources.

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Aims

Interferons play a vital role in mammalian responses to bacterial and viral infections and to tumor development. The Interferon response following disease challenge is highly modulated. The host response must be sufficient to protect the host, but excessive responses can cause toxicity and even death. Furthermore, the Interferon response follows a highly structured temporal response, with stimulation activating an organised sequence of transcriptomic events. Here we present two bioinformatics tools that can be used to analyse the transcriptional regulation of the Interferon response: 1) the *Interferome* v2.0 database (http://interferome.its.monash.edu.au/interferome/home.jspx), which identifies genes regulated by Interferons from mouse and human systems, and 2) the *Enrich* custom software, which is powerful and intuitive tool for identifying transcription factors involved in regulating co-expressed gene sets and thus elucidating signaling pathways.

Methods

The *Interferome* database was queried for genes that were up-regulated in murine cells over discrete time periods following stimulation by type I Interferons. Transcription factors that were significantly enriched (p<0.05) among each gene set were identified using the *Enrich* custom bioinformatics tool.

Results

Interrogation of the *Interferome* database identified hundreds of genes that are up-regulated following stimulation by type I Interferon at different time points. Following analysis of the associated promoters using *Enrich*, we have been able to identify clear patterns in the transcription factors associated with temporally co-expressed genes. Many of the transcription factors identified are already known to be involved in the Interferon pathway, such as STATs and IRFs. However, the analysis also revealed the involvement of further additional transcription factors which might provide new insights into Interferon signalling.

Conclusion

By integrating the *Interferome* and *Enrich* bioinformatics resources, it has been possible to explore the transcription factors that regulate different phases of the interferon response. The analysis has identified new regulatory elements for experimental validation.

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Interleukin-37 – An Anti-inflammatory Cytokine for Necrotizing Enterocolitis?

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Introduction: Necrotizing enterocolitis (NEC) is sometimes dubbed the spectre of neonatal intensive care units (NICUs), as its onset is insidiously non-specific and once NEC becomes manifest, the damage inflicted on the premature baby's intestine and other organs is often disastrous. It remains unknown which participants of the immune system initiate the cascade of events that lead to NEC; consequently, no specific therapy exists. We hypothesize that an immature and inappropriate inflammatory response towards bacterial colonization and/or food components plays a pivotal role in the initiation and perpetuation of NEC. Hence, we investigated the therapeutic potential of the anti-inflammatory cytokine interleukin-37 (IL-37) in a murine model of NEC.

Methods: Newborn C57BL6J mice transgenic for IL-37 (IL-37tg) and wild-type (WT) controls (n=8 for both) were collected and separated from dams at birth. Mice were formula-fed every 3hrs using the Yajima style Hoshiba nipple and subjected to asphyxia (100% nitrogen gas for 45s) followed by cold stress (4°C, 5mins) twice daily to induce NEC. At the experimental endpoint (72hrs), all pups were culled and intestinal samples collected. Littermates were dam-fed and kept as controls (n=16).

Results: On a 0-3 scale (no to severe pathology), clinical scores were 1.8 (WT) vs 1.2 (IL-37tg) for ileus, 0.9 vs 0.1 for hematochezia and 1.5 vs 0.6 for diarrhea. Tissue histological injury scores assessing epithelial vacuolation and mucosal disintegration were 1.8 (WT) vs 0.8 (IL-37tg) in the duodenum, 1.4 vs 0.6 in the jejunum and 1.4 vs 0.8 in the ileum. This marked reduction of NEC severity in IL-37tg mice was associated with a decrease in IL-1β.

Conclusion: Our results demonstrate that IL-37 protects newborn mice against NEC, indicating that excessive inflammation contributes to disease progression. Blocking this inappropriate inflammatory response may represent a promising approach to improve the outlook of babies suffering from this devastating disease.

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Undefined region of ectodomain plays a role in determining the activity of TLR8

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Toll-like receptors play important roles in detecting pathogen-associated molecular patterns for initiation of immune responses against microbial infections. Toll-like receptor 8 (TLR8) belongs to a subfamily comprising TLR7, TLR8 and TLR9, and recognizes viral single-stranded RNA and small molecular weight agonists to activate anti-viral immune responses. TLR8s from different species have distinct ligand recognitions. TLR8s from several non-rodent species including cat, horse, sheep, and bovine are activated by small molecular TLR8 agonists, whereas TLR8s from mouse and rat, two rodent species, are activated only in the presence of PolyT-ODN. The lack of a five-amino-acid motif in the undefined region following the leucine-rich repeat 14 of the mouse and rat TLR8 ectodomains has been suggested to be a reason for the weak activities of these two rodent TLR8s. To further investigate the activation of TLR8, in this study we cloned rabbit TLR8 (rabTLR8) cDNA. The rabTLR8 has a longer undefined region in its ectodomain than the TLR8 from other species. In cell-based assay, this rabTLR8 is activated by TLR8 ligand in the absence of PolyT-ODN. Nevertheless, upon stimulation the rabTLR8 had a lower activation compared to the activation of TLR8 from other species, except the mouse and rat TLR8s. Using different deletion and human-rabbit chimeric TLR8 expressing constructs, we showed that the extra peptide in the undefined region played a role in determining the activity of rabTLR8. These results suggest that the undefined region plays a role in determining the activity of rabTLR8.

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Proatherogenic Condition Promotes Autoimmune Th17 Cell Responses.

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Patients with systemic autoimmune diseases show increased incidence of atherosclerosis. However, the contribution of proatherogenic factors to autoimmunity remains unclear. We found that atherogenic mice (herein referred to as LDb mice) exhibited increased serum interleukin-17, which was associated with increased numbers of T helper 17 (Th17) cells in secondary lymphoid organs. The environment within LDb mice was substantially favorable for Th17 cell polarization of autoreactive T cells during homeostatic proliferation, which was considerably inhibited by antibodies directed against oxidized low-density lipoprotein (oxLDL). Moreover, the uptake of oxLDL induced dendritic-cell-mediated Th17 cell polarization by triggering IL-6 production in a process dependent on TLR4, CD36, and MyD88. Furthermore, self-reactive CD4+ T cells that expanded in the presence of oxLDL induced more profound experimental autoimmune encephalomyelitis. These findings demonstrate that proatherogenic factors promote the polarization and inflammatory function of autoimmune Th17 cells, which could be critical for the pathogenesis of atherosclerosis and other related autoimmune diseases.

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West Nile virus sfRNA prevents incorporation of anti-viral molecules into exosomes to facilitate virus spread

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Exosomes and microvesicles are small 40 - 100 nm extracellular vesicles, which act as carriers for intercellular signalling. In particular, these vesicles are packed with small RNAs, such as microRNAs, as well as proteins, and mRNAs. In this study, we show that microvesicles derived from A549 cells stimulated with interferon (IFN) are potent inhibitors of West Nile virus (WNV) replication in cells treated with these extracellular vesicles. siRNA knockdown of cellular RNases Xrn1, Dicer, Drosha, and RNaseL in IFN-stimulated cells reduced the anti-WNV activity of extracellular vesicles. Anti-WNV activity was also observed for vesicles derived from cells infected with WNV mutant IRAΔCS3 deficient in generation of subgeneric flavivirus RNA (sfRNA) but not for vesicles derived from wild type WNV-infected cells. We have previously shown that WNV sfRNA inhibits cellular RNA decay pathway by inactivating Xrn1 and RNAi pathway by inhibiting Dicer. Therefore, we propose that WNV sfRNA inhibits generation of anti-viral RNA decay intermediates and/or miRNAs and their subsequent incorporation into microvesicles and exosomes in order to facilitate virus spread. To test the hypothesis we are undertaking RNAseq to identify exosomal RNAs from WT and IRAΔCS3 infected cells and will present the data at the meeting.

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Combined siRNA and TLR-activating therapy for the improved treatment of viral infections.

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RNA interference is one of the fastest developing fields in biological science and has been demonstrated as a promising therapy for a wide range of viral infections. This highly specific suppression can be achieved by the introduction of short interfering (si) RNAs into mammalian systems, resulting in the efficient reduction of viral load *in vitro*. However, this activity can have the unfortunate consequence of non-specifically activating several innate immune sensors including toll-like receptor (TLR) 7/8 and stimulate the expression of interferon, which can mask the true specific effects of the siRNA. While often not desired, the localised activation of TLR pathways in combination with specific anti-viral activity of the siRNA may have a beneficial role in providing a more potent control of viral infection, especially in reducing transmission between hosts. Here we show that specific TLR agonists are able to induce cytokines of the innate immune response and improve the anti-viral response when used in tandem with siRNAs targeting specific viral sequences in a Semliki Forest Virus model. Furthermore, individual pre-treatment of HeLa cells with poly(I:C) or viral nucleocapsid siRNA before Hendra virus (HeV) infection resulted in a 1.5 log (>95%) reduction while the combination resulted in a 2.5 log (>99.9%) reduction in titre, indicating a trend for the two pre-treatments to act simultaneously to cause additional suppression of HeV. This foundation will allow further investigation into *in vivo* systems and once matched with efficient delivery mechanisms may result in better viral control and a new treatment modality for acute viral infections.

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Cleavage and Secretion of Interleukin-1b in the Absence of Cell Death.

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Interleukin-1b (IL-1b) is an important signalling molecule in inflammation and auto-immunity. Specific host, environmental, and pathogen derived danger molecules activate inflammasome protein complexes. These in turn activate caspase-1, which processes IL-1b into its mature biologically active form. Active caspase-1 also induces lytic cell death known as pyroptosis. Whether pyroptosis and IL-1b maturation and secretion are separable events remains unclear.

To study caspase-1 function in the absence of inflammasome signalling we created a lentiviral flag-tagged caspase-1-bacterial DNA-gyrase-GFP fusion construct (FC1GG). Expression of the caspase-1 fusion protein is induced through addition of doxycycline. The dimeric antibiotic coumermycin causes the gyrase domains to dimerise and caspase-1 to auto-activate.

Mouse embryonic fibroblast (MEF) cell lines engineered to constitutively express inactive precursor IL-1b were stably infected with the inducible FC1GG construct. We observed that these cells, which do not express other inflammasome components, secreted IL-1b upon caspase-1 induction and dimerisation. Notably, caspase-1 dependent IL-1b cleavage and secretion occurred in the absence of caspase-1-mediated cell death.

This novel system allows the study of caspase-1-mediated IL-1b secretion without the activation of upstream signalling pathways, or caspase-1 mediated pyroptosis. Consequently, we are currently using it to identify the cellular machinery required for IL-1b secretion via mass-spectrometry based approaches.

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MCC950 is a potent and specific inhibitor of the NLRP3 inflammasome and a novel therapeutic for NLRP3 driven diseases

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NLRP3 is a critical component of the inflammatory process and its aberrant activation is pathogenic in inherited disorders such as the cryopyrin associated periodic syndromes and complex diseases such as multiple sclerosis, type II diabetes and atherosclerosis. We demonstrate that a small molecule, MCC950, specifically blocks NLRP3 activation in response to numerous stimuli. MCC950 potently inhibits ASC oligomerisation, caspase-1 activation and IL-1ß secretion in response to NLRP3 but not AIM2, NLRC4 or NLRP1 inflammasome activation. MCC950 can block IL-1ß *in vivo* and attenuates the course of the NLRP3 dependent, IL-1ß driven disease model of multiple sclerosis, experimental autoimmune encephalomyelitis. Furthermore, MCC950 treatment rescues neonatal lethality in a murine model of cryopyrin associated periodic syndromes and is active in *ex vivo* samples from patients with Muckle-Wells syndrome. MCC950 is thus a novel potential therapeutic for NLRP3 associated syndromes and a useful tool for the further study of NLRP3 in human health and disease.

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Interferon-α-induced IL-6 improves immune and clinical benefits of regulatory T cell depletion as ovarian cancer immunotherapy

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Tregs blunt anti-tumor immunity. Their depletion is effective treatment in mouse cancer models. Treg depletion in human trials is only partially effective, however. In our phase II ovarian cancer trial, denileukin diftitox (DT) depleted Tregs significantly in blood and the tumor microenvironment but without significant clinical efficacy. Interferon-α alone does not treat ovarian cancer effectively, but we now show that it significantly boosts immune and clinical effects of DT-mediated Treg depletion in human ovarian cancer. In the ID8 mouse ovarian cancer model, DT improved anti-tumor immunity and survival modestly. Interferon-α alone did not affect Treg function or numbers, but improved $CD8^+$ T cell anti-tumor immunity. Adding interferon-α to DT significantly increased mouse survival versus either agent alone. Using type I $IFNR^{-/-}$ mice that are unable to mediate interferon-α signals, we showed that interferon-α boosted $CD8^+$ T cell function independent of $CD4^+$ T cell help through direct action on $CD8^+$ T cells. In combination with DT, interferon-α reduced Treg function (without further reducing their numbers) through indirect effects on dendritic cells *in vivo*. *In vitro* we determined that interferon-α reduced Treg suppression and increased IL-6 in Treg suppression assays only when conventional dendritic cells were present. Anti-IL-6 antibody reversed the effect, and recombinant IL-6 recapitulated the effect in the absence of dendritic cells. When three ovarian cancer patients failed DT alone in the phase II trial, two experienced immunologic and clinical benefit by adding weekly pegylated interferon-α2a at 180 μg subcutaneously, with manageable toxicities. There was a trend for pegylated interferon-α2a to reduce Treg function and increase serum IL-6 when combined with DT, versus effects of DT alone in these 3 patients. IL-6 is generally detrimental to anti-tumor immunity and promotes carcinogenesis, but in specific instances it could be beneficial. These studies demonstrate novel immune and clinical interferon-α anti-cancer benefits that augment Treg depletion using approved agents for rapid clinical translation.

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Pathogenic potential of interferon αβ in acute influenza infection

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Severe influenza virus-induced disease is characterised by acute lung injury, associated with a vigorous inflammatory response. However, disease severity varies between individuals and this cannot be entirely explained by differences in influenza strain virulence, or an individual's pre-existing adaptive immunity or health status. Host specific genetic factors must therefore contribute to susceptibility. Type I interferon (IFNαβ) is known to have antiviral function in-vitro, yet its role in influenza infection in-vivo is less clear. **Aims:** To identify host specific determinants of susceptibility to influenza-induced disease. **Methods:** We assessed susceptibility of inbred mouse strains to influenza-induced disease where viral load was comparable during early infection. Influenza-induced gene expression, cytokine production and cellular recruitment, activation and death in the pulmonary environment were compared between susceptible and resistant mouse strains. **Results:** Influenza infected 129 and DBA strains showed dramatically increased morbidity, mortality and lung damage, yet higher levels of pulmonary IFNαβ, compared to the more resistant C57BL/6 or BALB/C mice. Ablation of IFNαβR signalling in 129 mice markedly reduced mortality, lung damage, proinflammatory cytokines and lung-infiltrating inflammatory cells such as inflammatory monocytes (IMs). Furthermore, IFNα treatment of influenza-infected C57BL/6 mice increased morbidity. Plasmacytoid dendritic cells (pDCs), which are potent IFN producers, were recruited in higher numbers to lungs of infected 129 mice. pDC derived IFNαβ was demonstrated to be upstream of the robust proinflammatory response. Significantly, IFNαβ signalling induced the upregulation of Tumour necrosis factor-Related Apoptosis-Inducing Ligand (TRAIL) on IMs and its receptor: Death Receptor 5 (DR5) on lung epithelia. TRAIL-DR5 interaction is required for epithelial apoptosis, lung damage and mortality of 129 mice. **Conclusion:** Host-intrinsic differences can determine the severity of influenza-induced disease. pDC frequency and responsiveness to IFNαβ signalling is a host-specific determinant with protective or pathogenic potential. This study has important implications for the prediction and treatment of severe influenza-induced disease.

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Development and characterization of a cell-penetrant IRF5 inhibitor

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The transcription factor interferon regulatory factor 5 (IRF5) has previously been implicated in the onset of the autoimmune disorder systemic lupus erythrematosus (SLE). Elevated levels of inflammatory cytokines are a common characteristic of SLE, and are believed to contribute to both autoantibody production and wide spread inflammation. Upon activation, cytoplasmic IRF5 translocates to the nucleus to initiate pro-inflammatory gene transcription. To achieve nuclear translocation, IRF5 relies on two nuclear localization signals located in the N' and C' termini of the protein. To investigate the therapeutic potential of IRF5 inhibition, we have developed two unique cell-penetrating peptides. Upon treatment with these inhibitors, we show IRF5 is excluded from the nucleus, while IRF7 and NFkB nuclear translocation were unaffected following activation. We have investigated the impact of IRF5 inhibition in a variety of cell lines as well as primary peripheral blood mononuclear cells. The inhibitors show no impact on cell cycle, viability, or IRF5 protein levels. As IRF5 has previously been linked to expression of IgG subtypes in mice, we utilized the IRF5 inhibitors to examine the impact of IRF5 inhibition in the Ramos B cell line. Interestingly, we found no impact of the inhibitors on surface IgG expression. In THP1 cells, however, a marked reduction in inflammatory cytokine expression was seen following stimulation with LPS and IFNg. These data highlight the potential of targeting IRF5 in order to reduce the inflammatory signature characteristic of SLE patients.

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Characterizing the role of IRF5 in human B cell development and function

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The transition of naïve B cells to effector B cells is dependent on a large transcription factor network, which mediates both effector B cell differentiation and function. The full repertoire of transcription factors involved in this process is not known, yet dysregulation of this transcription factor network can result in altered B cell function and autoimmunity. Work from our lab, as well as others, has suggested that the transcription factor, interferon regulatory factor 5 (IRF5), is involved in the development of effector B cells. *Irf5*$^{-/-}$mice have previously been reported to have reduced plasma B cells, as well as reduced serum IgG subtypes. It remains unclear, however, what role IRF5 may play in human B cell development and function. We find significant levels of IRF5 in B cells translocate to the nucleus following stimulation with anti-IgM antibody and CpG. In order to characterize the role of IRF5 in human B cells, we have performed IRF5 ChIP-Seq in both primary naïve B cells and Ramos B cells either mock or anti-IgM and CpG treated. Genes associated with plasma B cell development were significantly enriched following activation, suggesting IRF5 plays a critical role in the differentiation of plasma B cells. To further characterize the role of IRF5 in primary human B cells, we have been able to successfully perform siRNA-mediated knockdown of IRF5. Knockdown of IRF5 did not show significant impact on cell viability, however, reduced inflammatory cytokine expression was seen. These data highlight the multi-functional role of IRF5 in regulating both human B cell differentiation and function.

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Role of an IFNAR1-IFNβ mediated signalling axis in immune cells

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Type I interferons (IFNs) are an important family of cytokines which enable the immune system to fight viral infections and cancer, and modulate the immune response. Type I IFNs are unique amongst cytokines since multiple ligands can signal through the same heterodimeric receptor composed of low (IFNAR1) and high (IFNAR2) affinity components. Despite sharing a receptor, discernible differences result from receptor engagement by different IFN subtypes. We recently demonstrated a molecular basis for the unique functional characteristics exhibited by IFNβ; it can bind and signal via IFNAR1 in the absence of IFNAR2, inducing a novel signaling axis that contributes to lethality in a mouse model of sepsis. Having determined the crystal structure of IFNβ bound to full length extracellular domain (ECD) of IFNAR1, we are validating the interface to identify key residues mediating this interaction, and further characterising the signaling pathway. The novel signaling axis we identified by microarray analysis of induced ISGs after *in vivo* IFNβ treatment of IFNAR2$^{-/-}$mice includes genes encoding proteins with known roles in sepsis. We now demonstrate that this treatment also induces phosphorylation of AKT in cells of the peritoneal cavity. Furthermore, we observed reduced levels of B220+CD11c- leukocytes in the peritoneal cavity and increased presentation of TREM1 on the surface of Gr1+ peritoneal cells in an IFNAR1-IFNβ dependent manner. That TREM1 is known to amplify the lethal response associated with sepsis gives some mechanistic insight into how the IFNAR1-IFNβ signaling axis may be contributing to lethality in this disease. Our results further demonstrate the importance of IFNβ and IFNAR1 in the transmission of signals and in influencing cell numbers and increased surface expression of inflammatory mediators. Understanding how to abrogate IFNβ binding to IFNAR1, and the down stream effects of the IFNAR1-IFNβ signaling axis may aid in development of targeted therapeutics in IFNβ-mediated inflammation.

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Targeting placental leukemia inhibitory factor in vivo with a unique inhibitor as a novel treatment strategy for ectopic pregnancy.

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Ectopic pregnancy is unique to humans and a leading cause of maternal morbidity and mortality. The etiology remains unknown however factors regulating embryo implantation likely contribute. Leukemia inhibitory factor (LIF) has roles in extravillous trophoblast adhesion and invasion during normal placental development and is also present in ectopic implantation sites. We hypothesised that LIF facilitates blastocyst adhesion/invasion in the Fallopian tube, contributing to ectopic pregnancy. LIF blockade could serve as a potential treatment strategy.

LIF receptor (R) was immunolocalised in tubal ectopic pregnancy implantation sites (N=5). An oviduct cell line (OE-E6/E7) and a trophoblast cell line (HTR-8/SVneo; spheroid culture) were used to model blastocyst attachment to the Fallopian tube. LIF signaling pathways in OE-E6/E7 were examined by Western blot. The effect of LIF and LIF inhibition (using PEGylated LIF antagonist; PEGLA) on first-trimester placental outgrowth was determined. To demonstrate that LIF blockade could reverse trophoblast invasion *in vivo,*pregnant C57BL/6J mice were IP administered with PEGLA (500µg/application PEGLA or PEG control) at 1000h and 1600h on days (D)8-10 or 10-13 of pregnancy (D0: day of plug). Implantation sites collected at D10 or 13 were stained with cytokeratin (trophoblast marker) isolectin-B4 and α-SMA (vascular markers).

LIFR localised to villous and extravillous trophoblast and Fallopian tube epithelium in ectopic pregnancy. LIF activated STAT3 but not the ERK pathway in OE-E6/E7 cells. In vitro, PEGLA inhibited LIF stimulated HTR-8/SVneo spheroid adhesion to OE-E6/E7 cells and explant outgrowth. LIF inhibition in pregnant mice impaired decidualization and placental spongiotrophoblast and labyrinth formation.

Our data suggests LIF facilitates the development of ectopic pregnancy by stimulating blastocyst adhesion and trophoblast outgrowth from placental explants. In mice, LIF inhibition dramatically altered placental development and trophoblast structure. Ectopic pregnancy is usually diagnosed after 6 weeks of pregnancy, therefore PEGLA may be useful as a treatment for ectopic pregnancy.

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Induction of IL-6 expression through TLR4 by oxidised phospholipids involves the selective induction of Arid5a but not Regnase-1 expression.

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Pathology following influenza infection develops through the induction of host-derived oxidized phospholipids (OxPLs), which stimulate TLR4-dependent inflammation and macrophage-IL6-dependent acute lung injury. Interestingly, through TLR4 stimulation in alveolar macrophages, lipopolysaccharide (LPS) induces expression of the pro-inflammatory cytokines IL-6 and TNF-α, however OxPLs induce only IL-6 expression.

The mechanistic basis for the selective induction of IL-6 by OxPLs (compared to LPS) is not known, but is clearly of importance to understanding the pathogenesis of influenza-induced lung pathology.

Recently, we identified an LPS-TLR4-induced protein called Arid5a, which selectively stabilizes IL-6 (but not TNF-α) mRNA and counteracts the function of the LPS-TLR4-induced RNAse Regnase-1 which degrades IL-6 mRNA. In the current study, using cultured peritoneal macrophages, we have found that through TLR4 signaling, OxPLs induce Arid5a, but do not induce Regnase-1 expression. We also observed that treatment with OxPLs inhibit the basal mRNA levels of TNF-α but not IL-6. Interestingly, in co-stimulation experiments, OxPLs inhibited the expression levels of TNF-α (but not IL-6) mRNA following stimulation of peritoneal macrophages with either poly I:C (TLR3 ligand) or CpG-a (TLR9 ligand).

Taken together, these observations indicate that OxPLs selectively induce IL-6 expression through TLR4 through two biological properties (i) promoting IL-6 mRNA stability through induction of Arid5a but not Regnase-1 and (ii) by inhibiting TLR4-TNF-α mRNA expression levels. The molecular mechanisms underlying these observations and their involvement in lung pathology, are currently under investigation.

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IL-7R: a target in ALL and autoimmunity

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T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological malignancy resulting from leukemic transformation of T-cell progenitors in the thymus. It accounts for approximately 15% of ALL cases in childhood and 20-25% in adults and is a leading cause of death in children. IL-7 and its receptor (IL-7R) play a critical role in normal T-cell development and homeostasis. Mutations in IL-7R were identified in 9% of pediatric T-ALL patients. These mutations usually involved insertions of three amino acids including cysteine and proline in the extracellular juxtamembrane region. WT or mutant forms of the human IL-7R (hIL-7R) from patients were retrovirally transfected into an IL-7-dependent murine thymic cell line D1. Mutant hIL-7Rs induced ligand-independent activation of the Jak-Stat and PI3K pathways, cell survival and proliferation. Constitutive signaling required homodimerization via cysteines in the inserted sequences and downstream Jak1 activation, and was IL-7, gc and Jak3-independent. Mutant hIL-7R-expressing D1 cells formed subcutaneous tumors in Rag1-/- mice, with substantial infiltration into various organs that are normally affected in advanced stages of T-ALL, such as bone marrow, liver, lymph nodes and spleen. Janus kinase inhibitors effectively killed these cells in vitro and in vivo. The hotspot for insertions lies in exon 6 in precisely the same region as a coding polymorphism regulating risk for MS and other autoimmune diseases, and we observe that this polymorphism affects strength of signaling. Our findings indicate that IL-7R mutations drive T-ALL, whereas polymorphisms that increase signaling promote autoimmunity, implicating IL-7R and Jak1 as therapeutic targets in these diseases.

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Cytokine dysregulation in autoimmune disease pathogenesis

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There are more than 80 different autoimmune diseases, and collectively they affect 3-5% of the population. They are chronic, incurable, and cause substantial morbidity and mortality. The fundamental lack of understanding of autoimmune disease pathogenesis means that available treatments – most causing general immune suppression - are non-specific, often fail to treat the most serious disease manifestations, and cause serious side-effects. Our research over the past decade first focused on “mouse-to-human” approaches to understand autoimmunity. Random mouse mutagenesis led to our discovery of the Roquin family revealing a pathway in which accumulation of Tfh cells caused by Roquin mutations leads to rogue positive B cell selection and autoantibody-mediated autoimmune disease. Roquin and its paralogue Roquin2 act in T cells and myeloid cells to repress mRNAs posttranscriptionally. The key pathogenic pathways leading to autoimmunity and inflammation when Roquin is mutated consist on excessive IFN-g production that promote Tfh and GC B cell accumulation, and increased myeloid cell-derived TNF. More recently, we have undertaken a “human-mouse-human” approach to focus our efforts on illuminating the mechanism of disease in each individual patient and elucidating the biological pathways that connect human genetic variation to disease. This has led to identification of rare gene variants contributing strong effects to autoimmune susceptibility. Amongst these is a case of childhood cerebral SLE manifesting in the first years of life with severe clinical manifestations including stroke. A novel homozygous variant in TREX1 leading to a significant reduction in the protein’s exonuclease activity causes increased type I interferon production, likely to be at the root of the autoimmune phenotype. This human-centered approach is expected to help stratify what are notoriously heterogeneous diseases, and illuminate targeted therapies based on the affected molecular pathways.

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Small tumor necrosis factor-α receptor (TNFR) biologics selectively inhibit the TNF-p38 signalling axis and inflammation

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INTRODUCTION: Tumor necrosis factor (TNF) neutralising biologics, antibodies and soluble TNF receptors (TNFR), have revolutionised treatment of diseases such as rheumatoid arthritis. However, targeting a cytokine with a key role in immunity to infection and malignancy continues to raise serious concerns. Attempts to target the pro-inflammatory p38 MAP kinase, downstream of TNFR, have ceased owing to poor efficacy and unacceptable toxicity. The aim of this research was to develop biologics that could selectively inhibit the TNFR-p38 signalling axis and inflammation.

METHODS: Two TNF-derived peptides, TNF70-80 and TNF132-150 were investigated for their effects on neutrophil activation (lucigenin-dependent chemiluminescence assay), tumour cell cytotoxicity (MTT assay) and activation of MAP kinases (western blot). A pre-B cell line transfected with truncated TNFRI mutants was used to identify the region on TNFRI which binds TNF70-80. Peptides to this region of TNFRI were made and investigated in mice for their ability to inhibit LPS-induced peritoneal inflammation, carrageenan- and antigen-induce paw swelling/thickness and respiratory syncytial virus -induced lung inflammation, by assessing cell types and number and cytokines present at the inflammatory sites.

RESULTS: We have previously demonstrated that, TNF70-80 and, TNF132-150 had distinct biological properties but which were collectively representative of the parent TNF. Our data show that these differences reflect the types of signalling pathways they stimulate via TNFRI. Of interest was that TNF70-80 selectively stimulated p38 activation. Using this peptide and truncated TNFR mutants we identified the sequence in TNFRI which is coupled selectively to p38 activation. Peptides of this TNFRI sequence bind TNF/TNF70-80 and selectively inhibited their ability to induced p38 activation, TNF-induced neutrophil stimulation and inflammation in all four experimental models.

CONCLUSIONS: A TNFRI peptide which binds to TNF and selectively inhibits the TNFRI-p38 signalling axis and inflammation has been developed as potentially a new approach to targeting TNF in inflammatory diseases.

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Regulation of the Interferon Response by the long non-coding RNA lincRNA-EPS

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Details not available at time of print...

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Imidazoquinolines signal through TLR7 and IRF-5 for the production of type I and III interferon in primary human plasmacytoid dendritic cells

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Human pDC recognize Influenza virus, HIV-1 and small molecules from the imidazoquinoline family through TLR7 and HSV and CpGA through TLR9, resulting in production of type I and III IFN and pro-inflammatory cytokines. Imidazoquinolines have been used for anti-viral therapy and as immune adjuvants and are often considered to be prototypical TLR7 agonists. However, there have been reports of these molecules inhibiting virus-induced cytokine production. We investigated the mechanisms by which the imidazoquinoline, 3M003, induces type I and III IFN production in pDCs while inhibiting virus-stimulated cytokine production. 3M003 induced Type I and III IFN and TNF-α much more rapidly than Flu or HSV, but at much lower levels and for shorter duration; actin polymerization and endosomal acidification were required for this activation. The TLR7 inhibitor IRS661 effectively inhibited Flu and 3M003-induced IFN production. 3M003 inhibited Flu, HSV, HIV and CpGA induced IFN production in pDC when used together with these TLR7/9 agonists. This inhibitory effect of 3M003 was observed when it was added any time in the first four hours of virus stimulation. Similar to Flu and HSV, 3M003 enhanced IRF7 serine 477/479 phosphorylation but, surprisingly, did not translocate IRF7 to the nucleus as shown by imaging flow cytometry. Instead, 3M003 and the related molecule, R848, strongly and rapidly (within 30 minutes) induced IRF5 nuclear translocation in pDC, while HSV induced strong IRF7 and to a similar extent, IRF5 translocation, but later. Co-culture of pDC with 3M003 and virus yielded the high IRF5/low IRF7 translocation phenotype. HSV-1- but not 3M003-induced IFN was inhibited by the PI3K inhibitor, LY294-002. Taken together, these results indicate a novel signaling pathway in pDCs wherein 3M003 or 3M003 plus virus preferentially activates IRF-5 in a PI3K-independent manner, while HSV activates both IRF-5 and -7, as well as PI3K, resulting in the different levels and kinetics of IFN production.

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Comprehensive Integration of RNA and HITS-CLIP Sequencing for fine scale mapping of the Type 1 Interferon Response

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The innate immune response of an organism is the primary response to challenges including inflammatory stimuli, infectious agents or the presence of cancer cells. The important protective role of IFN activation and signalling is a key component of this response. While IFN signalling is essential for host survival, fundamental to vaccine responses, can be activated therapeutically and is often the target for pathogen evasion strategies uncontrolled or aberrant responses can be toxic and result in disease. It is therefore critical to balance signaling pathways to attain benefit while minimizing toxicity. While there is considerable knowledge of transcriptional regulation and role of protein coding transcripts in the IFN response equivalent knowledge of non-coding responses and their regulatory mechanism remains an active research area. In this context, an understanding of the role of small non-coding RNA, particularly miRNA, is rapidly being gained with important insights in many areas of biology.

High throughput sequencing technologies have unlocked the ability to measure the complete transcriptional landscape of a cell ultimately revolutionizing our ability to understand these complex systems. Combined with HITS-CLIP sequencing, a recently developed experimental sequencing based approach capable of detecting microRNA regulatory interaction with target genes can provide important regulatory insights into this system. We present the results of parallel IFNβ time course stimulation of small, ribosomal depleted total RNA and HITS-CLIP sequencing complemented with miRNA expression and target network identification and experimental validation in murine bone marrow derived macrophages. Analysis of this data has resulted in comprehensive network level understanding of the complete transcriptional response to IFNβ including the identification of almost 100, IFNβ inducible putative novel miRNA and associated characterization of potential targets. Novel computational algorithms and analysis approaches within our custom designed RNA-eXpress framework have been developed and applied resulting in the generation of predictive target networks. Results of experimental validation of these related miRNA pathways will also be presented.

This systems level interpretation of IFN mediated signaling has the potential to provide a basis for novel insights into both host-pathogen interactions and host response critical in the efficient development of effectively targeted therapeutics.

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Modulation of RIG-I signalling by OASL

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The innate immune system is the first line of defense against invading pathogens such as viruses. A fast and efficient innate immune response depends upon an early recognition of the pathogen by so-called pattern-recognition receptors (PRRs). One of the most important PRRs in terms of recognition of viral RNAs in the cytoplasm is the retinoic acid inducible gene I (RIG-I). Following viral infection, RIG-I predominantly recognizes short double-stranded RNAs (dsRNA) containing triphosphate at their 5' end and triggers a distinct signal transduction pathway leading to induction of cytokines such as interferon (IFN) as well as other antiviral proteins. Activation of RIG-I is regulated at several levels by cellular proteins in order to both prevent undue activation of the innate immune response and to increase the efficiency of RIG-I-mediated signaling. For example, following recognition of viral RNA, RIG-I binds free unanchored K63-linked polyubiquitin chains and this interaction with polyubiquitin is required for its activation. However, our collaborator Saumendra N. Sarkar from the University of Pittsburgh Cancer Institute, USA, has recently discovered that another IFN-stimulated protein known as the oligoadenylate synthetase-like protein (OASL) can act as a substitute for polyubiquitin in activation of RIG-I. Through its two C-terminal ubiquitin-like repeats, OASL interacts with the RIG-I caspase recruitment domains (CARD) and thus mimics the role of polyubiquitin. However, how the remaining N-terminal part of OASL contributes to the activation of RIG-I is not clear although it was shown to interact with RIG-I as well. Here, we show that the N-terminal domain of OASL binds dsRNA and that this binding is required in order for OASL to promote RIG-I activation. This suggests that OASL acts not only as a cofactor for RIG-I activation but also as a PRR.

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Master regulation of transcriptional responses by microRNAs

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microRNAs (miRNAs) are critical to most cellular processes and are essential modulators of responses to infection. Following our identification of miR-19 as a positive regulator of NF-κB signalling, we investigated the role of this miRNA in the modulation of NF-κB-dependent transcriptional responses. We identified a converging activity of miR-19 and NF-κB targeting, suggesting that their coordinate activity regulates gene induction upon infection. Genome-wide microarray analyses following overexpression or repression of more than 60 miRNAs confirmed that in ~75% of cases, miRNA targets could be associated with the activity of a single over-represented transcription factor. Collectively, our research demonstrates that miRNAs are master regulators of global transcriptional responses, acting to dampen transcriptional variation or enforce transcriptional restriction. In addition to developing our latest findings on this topic, I will discuss the far-reaching implications of these results pertaining to the field of interferon-driven transcriptional responses.

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Do G-CSF and Neutrophils Contribute to the Pathophysiology of Rheumatoid Arthritis?

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Rheumatoid arthritis (RA) is characterised by a persistent, but poorly understood interplay between innate and adaptive immunity. Neutrophils are the predominant cell type in inflamed RA synovial fluid (SF). Granulocyte colony-stimulating factor (G-CSF) is a key regulator of neutrophil production, function and survival. In this study peripheral blood (PB) neutrophil phenotype and function were analysed according to disease activity (DAS28 scores and other clinical parameters) in RA patients (n=50-60). We found significant correlations between disease activity and PB neutrophil percentage ($r=0.26$, $p\leq0.05$), as well as between neutrophil activation state (CD62L, CD11b) and the expression of receptors for factors regulating neutrophil production and function. For example, CD62L vs G-CSF-R ($r=0.58$, $p\leq0.0001$); CD62L vs CD35 ($r=-0.37$, $p\leq0.01$); CD62L vs CXCR2 ($r=0.42$, $p\leq0.01$); CD62L vs CXCR1 ($r=0.46$, $p\leq0.001$). To further explore the role of neutrophils and G-CSF in RA, transcriptional profiling using RNASeq was performed comparing: PB neutrophils isolated from healthy donors (HD) and RA patients (n=5); neutrophils isolated from paired PB and SF samples of RA patients (n=3); neutrophils or white blood cells from HDs stimulated with G-CSF *in vitro* (n=4). 194 genes were differentially expressed (DE) in RA neutrophils (logFC≥1; adjusted p≤0.05) when compared to HDs. Over 50 of those genes were also differentially expressed when neutrophils were stimulated with G-CSF *in vitro.* There were 1724 DE genes (logFC≥1; adjusted p≤0.05) when comparing PB and SF neutrophils from RA patients. Bioinformatic interrogation using Ingenuity Pathway Analysis software demonstrated that G-CSF was a likely regulator ($p=9.83\times10^{-21}$) of these differences. These data provide evidence that neutrophils and G-CSF contribute to the pathogenesis of RA. G-CSF may therefore represent a potential therapeutic target in the treatment of RA and other inflammatory diseases where there is a pathogenic contribution from neutrophils.

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G-CSF and Neutrophils are Non-Redundant Mediators of CNS Autoimmune Disease

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Granulocyte colony stimulating factor (G-CSF) plays a key role in the regulation of neutrophil production, function and survival. The G-CSF receptor (G-CSF-R) it most highly expressed by neutrophils and myeloid progenitors, and at lower levels by macrophages and monocytes. Here, we investigated the role of G-CSF and neutrophils in two extensively used models of central nervous system (CNS) autoimmunity both of which have long been described as T cell-mediated - experimental autoimmune encephalitis (EAE) and experimental autoimmune uveoretinitis (EAU).In $G\text{-}CSF^{-/-}$ mice and in WT mice treated with anti-G-CSF monoclonal antibody (mAb), disease severity in both models was dramatically reduced. A mixed population comprising macrophages ,T cells and neutrophils were identified by flow cytometric analysis of the ocular infiltrate in WT mice with EAU. The eyes of G-CSF-deficient and anti-G-CSF mAb-treated WT mice had reduced disease severity, with markedly reduced neutrophil infiltrate, but little or no change in other myeloid inflammatory cells. In the absence of G-CSF, antigen-specific T cell responses remained intact in both disease models and IL-17A production was maintained. We demonstrate that G-CSF controls ocular neutrophil infiltrate by modulating the expression of chemokine receptors - CXCR2 and CXCR4 - on peripheral blood neutrophils as well as CXCL2-mediated actin polymerization. These data establish an integral role for G-CSF-driven neutrophil responses in CNS autoimmunity, operating both within and outside the bone marrow and identify G-CSF as a potential therapeutic target in the treatment of human uveoretinitis and multiple sclerosis.

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The crystal structure of human interleukin-11: receptor binding site features and structural differences from interleukin-6

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Interleukin (IL)-11 is a member of the IL-6 family of pleiotropic cytokines that is characterised by shared use of the signal transducing receptor, GP130. Similar to IL-6, signalling by IL-11 is initiated by binding of soluble IL-11 to its membrane bound, specific receptor, IL-11Rα. This binary complex subsequently engages with GP130, inducing GP130 dimerisation and recruitment of JAK kinases, ultimately resulting in phosphorylation and activation of the transcription factor, Signal Transducer and Activator of Transcription (STAT)-3. As a result of the historical focus on the IL-6/STAT3 signalling axis, the IL-11 signalling machinery has remained relatively uncharacterised. No high resolution structural data have previously been reported for IL-11 or IL-11Rα and our understanding of the structure of the authentic signalling complex and the interactions between its components remains rudimentary. Here we report the crystal structure of human IL-11 and provide structural resolution of residues previously identified as important for IL-11 activity. Biophysical analysis indicates that IL-11 is a compact, stable, monomeric protein. While IL-11 is thought to signal via a complex analogous to that of IL-6, our comparisons show important differences between the two cytokines. Specifically, IL-11 shows a more elongated structure than IL-6 and lacks additional helical elements surrounding its central, 4-helix core. Detailed analysis of the GP130 binding regions suggest that IL-11 engages the first molecule of GP130 differently to IL-6. In addition to providing a structural platform for further study of IL-11, these data offer insight into the binding interactions of IL-11 with each of its receptors and the structural mechanisms underlying agonist and antagonist variants of the protein.

1. Putoczki T.L., Dobson R.C.J., Griffin M.D.W. (2014) The crystal structure of human interleukin-11 reveals receptor binding site features and structural differences from interleukin-6. Acta Crystallographica D. In Press doi:10.1107/S1399004714012267

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Strawberry notch homolog *2* gene expression is increased in the brain during endotoxin-induced systemic inflammation and is localized to numerous cells including astrocytes and microglia

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Aims: In an effort to better understand the function of the cytokine Interleukin (IL)-6 in the murine brain, our group identified the putative DExD/H-box helicase strawberry notch homolog 2 (*Sbno2*) as a novel IL-6/glycoprotein 130 regulated target gene in murine astrocytes and microglia *in vitro*. The aim of this study was to determine the regulation and cellular localization of *Sbno2* gene expression during neuroinflammatory conditions *in vivo*.

Methods: Upon endotoxin-induced systemic inflammation (2 mg/kg dual LPS injection, intra-peritoneal, 16 h plus 4 h or 24 h, respectively) in mice, total RNA was isolated from the brain and *Sbno2* mRNA levels were determined by RNase protection assay. From a second set of animals, paraformaldehyde-fixed, paraffin-embedded mouse brains were sagitally cut and analysed by in situ hybridization (ISH)/immuno-/histochemistry (IHC).

Results: *Sbno2* mRNA levels were highly increased at 4 h and decreased thereafter, but still had elevated levels up to 24 h after the second treatment. In ISH experiments, up-regulated *Sbno2* mRNA was mainly co-localized with GFAP-positive astrocytes while lesser numbers of selected tomato lectin-stained microglia and endothelial cells co-localized with *Sbno2* mRNA as well.

Conclusions: The results of the current study complement our previous *in vitro* data and strengthen the notion of a role for *Sbno2* in the innate inflammatory response during host-defence.
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Interferon gamma deficiency and Epstein-Barr virus reactivation in the pathogenesis of chronic lymphocytic leukemia

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Aims: Interferon (IFN)-gamma is a crucial regulatory cytokine in cellular immunity, and is important in immune surveillance of Epstein-Barr virus. Pathogenesis of chronic lymphocytic leukemia (CLL) is associated with an array of not completely understood disorders of cell- and humoral-mediated immunity. The aim of the study was an assessment of plasma concentration and intracellular expression of IFN-gamma, and its relationship with Epstein-Barr virus (EBV) DNA copy number, and selected clinical parameters in patients with CLL and healthy controls.

Methods: The study included samples of peripheral blood from 110 untreated patients with CLL. The control group comprised of 40 age- and sex-matched healthy individuals. The EBV-DNA copy number in peripheral blood mononuclear cells (PBMCs) was analyzed with EBV PCR kit (a single gene encoding EBV nuclear antigen 1 was amplified). The immunophenotype of PBMCs, including intracellular expression of IFN-gamma, was determined with flow cytometer. Concentration of INF-gamma in plasma was determined by ELISA.

Results: Presence of EBV DNA in PBMCs was found in 59 patients with CLL. There was a lack of detectable amounts of viral genetic material in healthy individuals. Decreased number of lymphocytes showing intracellular expression of IFN-gamma (CD3+/CD4+/IFN-gamma+ T cells, p=0.0027; CD3+/CD8+/IFN-gamma+ T cells, p=0.0044; CD19+/IFN-gamma+ B cells, p=0.0089), and low plasma concentration (p<0.0001) of this cytokine in CLL EBV(+) patients, as compared to EBV(-) subjects and healthy controls, reflect the suppression of cell-mediated immune response and non-reactivity of lymphocytes to the studied virus. The most important factors shortening the survival of CLL patients and time to progression were: EBV-DNA copy number (>17 copies/ug DNA) and low plasma concentration of IFN-gamma (<5.25 pg/ml).

Conclusion: These data show IFN-gamma deficiency in some patients with CLL and suggest that normal T- and B-cell response is crucial for favorable prognosis in this disease.

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Using Type III Interferon to Enhance Oncolytic Virus Function

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Introduction

Hepatocellular carcinoma (HCC) is responsible for more than 500,000 yearly deaths worldwide. The lack of effective therapies for this disease necessitates novel therapeutic approaches. Oncolytic viral vectors, such as vesicular stomatitis virus (VSV), are a promising new anticancer platform for HCC. In addition to direct cytopathology, VSV infection of cancer cells elicits specific and nonspecific immune responses, thus overcoming immunological tolerance to the tumor. The type III IFN family of cytokines (IFN-λ1, 2, and 3) have antiviral and immunomodulatory properties, and inhibit tumor progression in several different cancer models. We hypothesize that IFN-λ expression from VSV will improve oncolytic activity by both immunostimulatory effects and through increased selectivity of the virus for tumor cells.

Methods

We generated recombinant VSV vectors expressing mouse IFN-l2 either from the fifth position or the more highly expressing first position of the viral genome, and measured virus replication in cell culture and mice. Using a mouse model of HCC, we are examining the hypothesis that IFN-l expression from VSV will augment its antitumor activity.

Results

We found that IFN-l is expressed, secreted, and active when produced by VSV-infected cells. In cell culture, IFN-l expression from VSV attenuated virus replication in type III IFN-responsive cells, but not in type III IFN-nonresponsive cells, and after intranasal delivery to mice *in vivo* (Guayasamin et al., J. Virol., 2014). *In vitro* virus infection and spread assays demonstrate that VSV vectors expressing IFN-l are selectively cytopathic for type III IFN-nonresponsive liver cancer cells but not normal hepatocytes. Mouse models of HCC are being used to determine the antitumor potency of the VSV vectors and are ongoing.

Conclusion

These studies demonstrate the ability of the type III interferon cytokine family to enhance VSV oncolytic activity by increasing selectivity of the virus for type III IFN-resistant tumor cells.

Conclusions/Significance: We suggest that *B. pseudomallei*-infected $CD11b^{+}CD62L^{+}$ cells play an important role in inducing melioidosis with meningitis that could be due to the migration of infected leukocyte involving in a selectin-dependent manner.

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Functional Promiscuity in C-terminal Peptidic C3aR/C5a1 Ligands

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Background: The complement cascade is a highly sophisticated network of proteins that are well regulated and directed in response to invading pathogens or tissue injury. Complement C3a and C5a are key mediators produced by this cascade, and their dysregulation has been linked to a plethora of inflammatory and autoimmune diseases. Consequently, this has stimulated interest in the development of ligands for the receptors for these complement peptides, C3aR, and C5a1 (C5aR/CD88).

Aims: In this study we used computational methods to design novel C5a1 receptor ligands.

Results: Functional screening in HMDM using the xCELLigence label-free platform demonstrated promiscuity in the specificity of our ligands. No agonist/antagonist activity was observed at C5a1, but we instead saw that some ligands were able to agonize the closely related complement receptor C3aR. This was verified in the presence of C3aR antagonist SB 290157 and in a stable cell line expressing either C5a1 or C3aR alone.

Conclusions: C3a agonism has been suggested to be a potential treatment of acute neutrophil-driven traumatic pathologies, and may have great potential as a therapeutic avenue in this arena.

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Shifts in the microbiome during experimental chronic obstructive pulmonary disease (COPD)

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Chronic obstructive pulmonary disease is the 3rd commonest in the world. It is a heterogeneous disease characterised clinically by difficulties in breathing. It is induced by cigarette smoking that causes airway inflammation. Chronic inflammation results in tissue damage leading to airway remodelling and fibrosis, alveolar destruction and emphysema that combine to impair lung function. Current therapies alleviate some symptoms but do not reverse nor cure the disease. The microbiome encompasses all the microorganisms that inhabit the body. Recent studies show that a healthy microbiome is essential for maintaining homeostasis and health. Alterations in microbiomes in various tissues (eg gut) is associated with disease (eg colitis), potentially by inducing local and systemic inflammation. These changes may be reversed by microbiome transfer (eg colitis). Other recent studies have demonstrated that substantial immune and inflammatory cross-talk occurs between the lung and gut. We investigated the changes that occur in the gut microbiome in a mouse model of COPD that we developed. We used microbiome profiling to identify substantial shifts in microbial populations during the induction and progression of COPD. Transfer of fecal microbiomes from smoking groups to air groups and vice versa was performed, with transfer resulting from coprophagy. This suppressed the development of COPD in smoke-exposed mice exposed to the faeces of non-smoking mice. Profiling identified 2 species associated only with COPD and 2 species associated with protection. Analysis to identify genus-level shifts or key microorganisms that cluster with diseased or healthy states is ongoing.

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Autophagic Regulation of Pro-inflammatory Cytokines

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Autophagy is a conserved homeostatic mechanism for the lysosomal degradation of cytosolic constituents, including long-lived macromolecules, organelles and intracellular pathogens. Autophagosomes are formed in response to a number of environmental stimuli, including amino acid deprivation, but also by both host- and pathogen-derived molecules, including Toll-like receptor ligands and cytokines. In turn, autophagy regulates the production and secretion of a number of cytokines. In particular, inhibition of autophagy in LPS-treated macrophages and dendritic cells leads to increased secretion of IL-1α, IL-1β, IL-18 and IL-23. The mechanisms underlying these effects differ between cytokines; secretion of IL-1β is dependent on activation of inflammasomes and the TLR2/TLR4 adaptor molecule TRIF, while IL-1α secretion in response to autophagy inhibition is dependent on neither. Moreover, stimulation of autophagy leads to a decrease in intracellular levels of pro-IL-1β, while the secretion of IL-23 in response to inhibition of autophagy is dependent on IL-1. Moreover, supernatants from LPS-stimulated, autophagy-deficient, antigen presenting cells induce the secretion of IL-17, IL-22 and IFN-γ by $\gamma\delta^+$ T cells, suggesting that autophagy indirectly regulates Th17 responses through the modulation of IL-1 and IL-23. Thus, autophagy represents a potential target for the development of novel therapeutics for the treatment of inflammatory and autoimmune disorders, as well as in the development of treatment strategies for certain infectious diseases and vaccines.

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Antagonism of STAT3 signaling by lyssaviruses: a potent mechanism to inhibit cytokine responses, and potential target for antivirals

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Lyssaviruses, including rabies virus (RABV), are the etiological agents of rabies, an incurable disease with a case fatality rate of 100% that causes >60,000 human deaths/year. In common with other human-pathogenic viruses, RABV infection depends on evasion of host interferon (IFN)-mediated immunity, which is mediated through the interaction of the RABV IFN-antagonist P-protein with IFN-activated *signal transducers and activators of transcription* (STATs) 1 and 2, which has been shown to be critical to disease progression *in vivo.* Intriguingly, recent data indicate that RABV P-protein also targets STAT3, a major mediator of signaling by IFNs and by members of the IL-6 cytokine family. Similar reports for IFN-antagonists of several paramyxoviruses indicate that STAT3 targeting might play critical roles in infection by many viruses. However the molecular details underlying STAT3 targeting, and outcomes in terms of cytokine signaling and disease, remain largely unresolved.

Using quantitative cell imaging, protein interaction analysis and immune signaling assays, we have analysed STAT3 targeting by P-proteins from a panel of diverse lyssaviruses, finding that P-protein inhibits STAT3 responses to cytokines of **BOTH** the IFN and IL-6 families, and that STAT3-targeting is highly conserved across the genus, indicative of important roles. Nevertheless, we also identified subtle but significant differences in STAT3 targeting between different lyssaviruses, and identified specific mutations of P-protein able to prevent STAT3 interaction. Importantly, these mutations also reduce pathogenicity in mice. Using these tools, we are currently determining the precise molecular basis of P-protein's interaction with STAT3, and the roles of P-protein-STAT3 complexes in modulating antiviral immune signaling. This work should significantly enhance our understanding of the interaction of RABV with multiple cytokine signaling pathways, to reveal the extent of viral interference in immune signaling and its importance to disease, identifying new strategies to combat currently incurable rabies disease.

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G-CSF is a critical mediator of chronic inflammatory lung disease

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Chronic obstructive pulmonary disease (COPD) is a health problem of utmost global significance affecting tens of millions of people and it is resistant to current therapies. COPD is hallmarked by an irreversible and progressive deterioration in lung function, which is caused by destruction of lung parenchyma due to inflammation, and fibrosis in small airways. Most COPD is caused by irritants (smoke) and one near universal feature of COPD is a heightened chronic inflammatory response in lung involving macrophages, epithelial cells, granulocytes, dendritic cells and lymphocytes. Lung injury caused by cigarette smoke and irritants strongly induces the release of many inflammatory mediators including myeloid colony-stimulating factors (CSFs). CSFs link development of granulocytes and macrophages from the bone marrow with tissue inflammation, autoimmunity and host defence, and while GM-CSF is highly implicated in COPD pathogenesis, the role of G-CSF is much less well understood. The SH2 domain-containing inositol 5' phosphatase SHIP-1 is a negative regulator of myeloid cell proliferation and growth-factor mediated survival, exemplified in mice where deletion of SHIP-1 causes neutrophil and myeloid cell hyperplasia, myeloid cell accumulation in the spleen, lymph nodes and lungs, and the development of emphysema and spontaneous lung fibrosis that resembles human COPD. G-CSF levels are significantly elevated in the serum and bronchoalveolar lavage fluid of SHIP-1-deficient mice and the mice exhibit dramatically increased numbers of myeloid progenitors in spleen. We now show that deletion of G-CSF from SHIP-1-deficient mice leads to their increased survival, and the mice exhibit markedly reduced lung disease, loss of splenomegaly and lymphadenopathy phenotypes, and dramatic diminution of extramedullary hematopoiesis. Collectively, our findings show that G-CSF plays a crucial role in mobilizing hematopoietic stem cells from the bone marrow and inciting tissue inflammation in SHIP-deficient mice, which plays an important role in the development of chronic inflammatory lung disease.

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Human airway epithelial cells express ABCC4/MRP4, a transporter for uric acid and cAMP secretion, that contributes to innate and adaptive immune responses.

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HYPOTHESIS: Uric acid can be detected in airway lining fluids, although the mechanism regulating this process remains unclear. Uric acid is a potent anti-oxidant at physiological concentrations while becoming a danger associated molecular pattern triggering innate immune responses at pathological concentrations. Extracellular cAMP has also been shown to modulate immune responses. We tested the hypothesis that human airway epithelium expresses ABCC4/MRP4 which can regulate uric acid and cAMP secretion contributing to context dependent airway health and pathology.

METHODS: We used *in vitro* experiments with human airway epithelial cells and *in vivo* mouse models of airway disease. We performed candidate uric acid transporter gene expression, *in situ* immunohistochemistry, and *in vitro* pharmacological intervention experiments with human airway epithelial cells. We assessed the functional consequences of this system in a mouse model of allergic airways disease.

RESULTS: We demonstrate gene and protein expression of ABCC4/MRP4 in human airway epithelial cells *in vitro* and *in situ. In vitro* airway epithelial cells secrete basal levels of uric acid further induced with allergen treatment. ABCC4/MRP4 inhibition significantly blocked basal and induced uric acid secretion, without changes in cell death. This data suggests a uric acid transport system is active and does not require cell death. Inhibition of ABCC4/MRP4 also attenuated forskolin induced cAMP secretion. *In vivo* intervention studies targeting uric acid at the mucosal surface prevented allergic sensitization in a model of air pollution and allergen co-exposure.

CONCLUSIONS: Our results are the first to demonstrate the presence and function of a uric acid and cAMP transport system in human airway epithelial cells. Our results open the door to begin exploring how this transport system is regulated in health and disease, the downstream consequences of activation of the system, and the potential for the system to be active at other mucosal surfaces throughout the body.

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IFN-lambda production of CD8 cDCs in response to DNA-viruses, transfected dsDNA or cyclic-di-nucleotides depends on STING

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The $CD8^+$ conventional dendritic cells (CD8 cDCs) and the plasmacytoid dendritic cells (pDC) are major producers of IFN-lambda (IL-28/29). Previously we have shown that upon stimulation with dsRNA the murine CD8 cDCs were the exclusive source of IFN-lambda in vivo and in vitro via a TLR3/TRIF dependent recognition pathway. A finding confirmed for the human equivalent cDCs expressing CD141 (BDCA3). In contrast, pDCs ignored dsRNA but produced large amounts of IFN-lambda in response to TLR7 or TLR9 stimuli in a MyD88 dependent way. Here we demonstrate that both, the CD8 cDCs and pDCs, are able to produce IFN-lambda in response to DNA viruses such as HSV-1 or poxviruses. Our analysis of the pattern recognition receptors or adaptor molecules involved revealed that pDCs mainly use TLR9 and MyD88 for the detection of the DNA viruses, whereas CD8 cDCs depend on the adaptor molecule STING but not on MyD88. The transfection of CD8 cDCs with different forms of dsDNA or cyclic-di-nucleotides such as c-di-GMP or c-GAMP induced, similar to the infection with DNA viruses, large amounts of IFN-lambda in a STING dependent way. Thus, the immune system produces IFN-lambda in response to DNA via two different DC subsets (CD8 cDCs and pDCs), different pattern recognition receptors (cytoplasmic DNA receptors and TLR9) and adaptor molecules (STING and MyD88) as well as downstream signalling components (IRF3 and IRF7). We hypothesize; that this widespread redundancy counteracts viral or other pathogen encoded inhibitory mechanisms, possibly acting on the specific DC subsets, the pattern recognition receptors and adaptor molecules or other signalling components.

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Proteomic analysis of mitochondrial-associated ER membranes during RNA virus infection reveals dynamic changes in protein and organelle trafficking

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RIG-I pathway signaling of innate immunity to RNA virus infection is organized between the ER and mitochondria on a subdomain of the ER called the mitochondrial-associated ER membrane (MAM). The RIG-I adaptor protein MAVS transmits downstream signaling of antiviral immunity, with signaling complexes assembling on the MAM in association with mitochondria and peroxisomes. To identify components that regulate MAVS signalosome assembly on the MAM, we characterized the proteome of MAM, ER, and cytosol from cells infected with either chronic (hepatitis C) or acute (Sendai) RNA virus infections, as well as mock-infected cells. Comparative analysis of protein trafficking dynamics during both chronic and acute viral infection reveals differential protein profiles in the MAM during RIG-I pathway activation. We identified proteins and biochemical pathways recruited into and out of the MAM in both chronic and acute RNA viral infections, representing proteins that drive immunity and/or regulate viral replication. In addition, by using this proteomics approach, we identified 3 new MAVS-interacting proteins, RAB1B, VTN, and LONP1. Our proteomic analysis also reveals a dynamic cross-talk between subcellular compartments during both acute and chronic RNA virus infection, and demonstrates the importance of the MAM as a central platform that coordinates innate immune signaling to initiate immunity against RNA virus infection.

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Identification and functional characterization of K+ transporters encoded by Legionella pneumophila kup genes.

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Legionnaires' disease is an emerging, severe, pneumonia-like illness caused by the Gram-negative intracellular bacteria *Legionella pneumophila*, which are able to infect and replicate intracellularly in macrophages. Little is known regarding the mechanisms used by intracellular *L. pneumophila* for the acquisition of specific nutrients that are essential for bacterial replication. Here, we investigate three *L. pneumophila* genes with high similarity to the *Escherichia coli* K+ transporters. These three genes were expressed by *L. pneumophila* and have been designated *kupA*, *kupB* and *kupC*. Investigation using the *L. pneumophila kup* mutants revealed that *kupA* is involved in K+ acquisition during axenic growth. The *kupA* mutants replicated efficiently in rich axenic media, but poorly in a chemically defined medium. The *kupA* mutants were defective in the recruitment of polyubiquitinated proteins to the Legionella-containing vacuole that is formed in macrophages and displayed an intracellular multiplication defect during the replication in *Acanthamoeba castellanii* and in mouse macrophages. We found that bafilomycin treatment of macrophages was able to rescue the growth defects of *kupA* mutants, but it did not influence the replication of wild-type bacteria. These defects identified in *kupA* mutants of *L. pneumophila* were complemented by the expression *E. coli trkD/kup* gene in trans, a bona fide K+ transporter encoded by *E. coli.* Finally, we observed that the *kupA* mutant failed to activate the caspase-1 and also showed a reduction in the secretion of the IL-1β cytokine. Collectively, our data indicate that KupA is a functional K+ transporter expressed by *L. pneumophila* that facilitates the bacterial replication intracellularly and in nutrient-limited conditions. However, KupA can also be involved with the activation of the inflammasomes.

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Examination of inflammatory pathway induction in the fruit bat.

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Bats are known to be the natural reservoir hosts of multiple pathogenic viruses with frequent zoonotic spillover events into human populations. However, most virus infections are completely asymptomatic in bats. These same virus infections cause mass-inflammation in animals and humans. We are investigating how pathogen detection and activation of the Interferon signaling pathways, and cross talk to the inflammasome pathway may be altered in the Australian black flying fox. Our data so far suggests that while bats can mount a sufficient, yet altered, induction of the IFN pathway with production of IFN α, β and λ and various ISGs, and induction of NFκB signaling, most pathogen detection does not result in activation of the inflammasome signaling complexes. Interrogation of the inflammasome pathways reveals poor Asc-recruitment and low caspase activation in response to natural and synthetic ligands. Whether this is due to altered kinetics, a decrease in sensitivity or redundancy of signaling pathways is currently under investigation.

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Regulation of cell survival signals by Interleukin-3 in myeloid cells

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Survival and proliferation of haematopoietic cells is tightly regulated by cytokines, such as Interleukin (IL)-3. When IL-3 is not available, dependent cells arrest in G1 and activate intrinsic apoptosis pathways. The Bcl-2 family of proteins regulates intrinsic cell death in response to IL-3 loss. We have previously shown that deletion of the Bcl-2 family member, a BH3-only protein Puma, in myeloid cells affords protection from Interleukin-3 (IL-3) withdrawal mediated apoptosis. In the absence of IL-3, Puma protein levels increase in a p53-dependent manner. If IL-3 is restored before cells commit to apoptosis, Puma levels decrease in a manner dependent on post-translational phosphorylation of Puma at serine 10 by IKK1. These results indicate a role for the IKK complex downstream of IL-3 signalling. Using myeloid cells lacking *IKK1* or *IKK2* we show the relevance of IKK dependent survival signals downstream of the IL-3 receptor.

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An increased level of IL-6 suppresses NK cell activity in peritoneal fluid of patients with endometriosis via regulation of SHP-2 expression

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Endometriosis is known to be related to a defect in NK cell cytolytic activity. Additionally, the levels of inflammatory cytokines are elevated in the peritoneal fluid (PF) of women with endometriosis. Here, we show the decrease of natural killer (NK) cell cytolytic activity in the PF of endometriosis patients by primary cytokine activity. An increased level of interleukin-6 (IL-6) in the PF of patients with endometriosis suppresses NK cell cytolytic activity by down-regulating cytolytic granule components, such as granzyme B and perforin, through the modulation of Src homology region 2-containing protein tyrosine phosphatase-2 (SHP-2) expression, suggesting that IL-6 plays a crucial role in the reduction of NK cell activity in the PF of patients with endometriosis.

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Functional expression of umami taste receptor T1R1/T1R3 in mouse neutrophils

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Although many different types of G-protein coupled receptors (GPCRs) are functionally expressed in neutrophils, no reports have demonstrated functional expression of umami taste receptor in these cells. In this study, we observed that mouse neutrophils express the umami taste receptor heterodimeric *T1R1/T1R3* through quantitative RT-PCR analysis. We also found that stimulation of mouse neutrophils with L-alanine or L-serine, which is a ligand for the umami taste receptor, elicited not only ERK or p38 MAPK phosphorylation but also neutrophil chemotactic migration. Moreover, addition of L-alanine or L-serine markedly blocked the production of several cytokines including TNF-α, CCL2, and IL-10 induced by lipopolysaccharide through inhibition of NF-κB activity or STAT3 phosphorylation in neutrophils. Our findings demonstrate that neutrophils express the umami taste receptor, and tastants stimulate neutrophils, resulting in chemotactic migration, and attenuation of LPS-induced inflammatory response.

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Interferon-induced autophagy and antibacterial defenses occur through the Apoptosis stimulating kinase-1

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IFN-g induces multiple gene regulatory pathways to combat pathogens and tumors. The death-associated protein kinase 1 (DAPK1) is an important IFN-induced regulator of cell death and autophagy. Recently we have identified that ATF6, an ER resident transcription factor, in association with the transcription factor CEBP-b regulates the IFN-g-induced expression of *Dapk1* though a novel enhancer element. ATF6 migrates to Golgi in response to IFN-g, where it undergoes proteolysis to yield the transcriptionally active form that enters nucleus and induces gene expression. IFN-g-induced proteolytic processing of ATF6 and phosphorylation of C/EBP-b were essential for the formation of a novel transcriptional complex that regulates *Dapk1*. ASK1 (MAP3K5) is activated by various stress stimuli. Although originally identified as a kinase that stimulates apoptosis, ASK1 also contributes to cytokine responses, cell differentiation and immunity. However, the exact targets of this kinase are unclear. Here, we report that IFN-γ activates ASK1-MKK3/MKK6-p38MAP kinase pathway for controlling the activity of ATF6. The terminal enzyme in this pathway, p38MAP kinase, phosphorylates a critical threonine residue in ATF6, upstream of its DNA binding domain, which is required for its proteolytic processing. ATF6 mutants lacking the p38 MAPK phosphorylation site fail to undergo proteolytic processing in the Golgi, drive IFN-g induced gene expression and autophagy. We also show that mice lacking *Ask1* are highly susceptible to lethal bacterial infection owing to defective autophagy. Together, these results identify a novel host-defense pathway controlled by IFN-g.

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Cytokine-induced tumor suppression: a GRIM lesson

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GRIM-19, a STAT3 inhibitory protein, was isolated as a growth suppressive gene product using a genome-wide expression knockdown screen as an Interferon/retinoid induced growth suppressors. Loss of expression and occurrence of mutations in the *GRIM-19* gene in a variety of primary human cancers, suggesting its importance as a novel tumor suppressor. A number of DNA viral and cellular oncogenes are blocked by GRIM-19. We generated a *Grim-19* conditional knockout mouse for understanding its tumor suppressor function *in vivo.* Deletion of *Grim-19* significantly increased susceptibility of mice to chemical carcinogenesis resulting in development of squamous cell carcinomas. These tumors had high STAT3 activity and an increased expression of STAT3 responsive genes. Surprisingly, mono-allelic loss of *Grim-19* gene was sufficient to promote carcinogen-induced formation of invasive squamous cell carcinomas. Loss of *Grim-19* also caused mitochondrial electron transport dysfunction and assembly of the ETC complexes, and altered the expression of several cellular genes involved in glycolysis and promoted Warburg effect. These observations highlight the critical role of GRIM-19 as a cytokine-induced tumor suppressor.

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Dectin-1 modifies colonic microflora by inducing cytokine-dependent antimicrobial peptide secretion from intestinal epithelial cells.

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The mucosal immune system regulates the balance of intestinal microflora by innate immunological molecules such as antimicrobial peptides (AMPs). Dectin-1 is the receptor for β-glucans which are a component of fungal cell walls and are contained in various foods, suggesting that Dectin-1 signaling may regulate intestinal mucosal immunity. Here, we found that calprotectin, one of AMPs, was exclusively produced by mouse colonic epithelial cell (cEC), and Dectin-1 deficient mice showed significantly impaired calprotectin expression in cEC. Calprotectin was induced by a special IL-17 family cytokine that was mainly produced by macrophages in colonic lamina propria. Calprotectin specifically suppressed the growth of a species of intestinal commensal bacteria belonging to *Firmicutes*, and the population of this species was increased in both Dectin-1 and this IL-17 family cytokine deficient mice. Furthermore, the ligand of Dectin-1 in mouse intestine was identified as food-derived β-glucans. These findings suggest that oral administration of β-glucans regulates the balance of commensal bacteria population via the Dectin-1-induced cytokine-dependent AMP secretion to control the homeostasis of intestinal immunity.

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The IL-3 receptor α subunit N-terminal domain: role in receptor assembly and signalling

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Interleukin (IL)-3, granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-5 are responsible for the survival, proliferation, differentiation and activation of hematopoietic cells. IL-3 signals through a receptor consisting of a ligand-specific α subunit (IL3Rα) and a β subunit (βc), which is shared with the GM-CSF and IL-5 receptors. IL-3Rα is overexpressed on the surface of leukaemic stem cells in patients with acute myelogenous leukaemia, allowing the targeting of leukaemic stem cells with specific antibodies[1]. Our previous studies demonstrated that the GM-CSF receptor assembles into a hexamer consisting of 2 GM-CSF molecules, 2 α subunits and a βc homodimer, which subsequently forms a higher-order dodecamer complex upon cytokine binding that is required for Jak2 transphosphorylation and full activation[2]. We have recently solved the structure of IL3Rα, which contains the N-terminal domain (NTD) and domains 2 and 3 and demonstrated that IL-3 can bind IL-3Rα through “open” and “closed” conformations of the receptor[3]. What remains unknown is how the NTD contributes to receptor signalling and its functional significance.

The aim of the present study is to determine the role of the NTD of IL-3Rα in IL-3 binding, higher-order receptor assembly and cell signalling. We generated several mutants that disrupt the conformation of the NTD, which were tested in signalling and functional studies. The results showed that the binding affinity of IL-3 to the mutant receptors and proliferative activity in immortalized mouse fetal liver cells expressing the mutants were reduced and similar to that observed for IL-3Rα that lacks the NTD. We will present further biochemical data that suggests that the mutants follow different pathways to the formation of the receptor dodecameric complex. Although the IL-3Rα NTD is not essential for ligand binding, its conformation once ligand-bound plays a crucial role in initiating and directing the assembly of a functional higher order complex.

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Re-Zincing the macrophage antimicrobial response to Salmonella typhimurium.

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Zinc deficiency predisposes to infectious diseases and zinc supplementation is associated with improved outcomes in severe diarrheal disease. Cytokines, including TNF and IL-6, released during inflammatory events induce an acute redistribution of the zinc, leading to plasma zinc depletion. Here we investigated potential antimicrobial effects of zinc in human macrophages.

We show Toll-like Receptor (TLR) signalling promotes the delayed accumulation of vesicular zinc within primary human macrophages. Treatment of macrophages with exogenous zinc does not alter LPS-dependent TNF and IL-6 production but increased the intracellular zinc pool. After infection, the vesicular zinc is delivered to non-pathogenic *Escherichia coli* (*E. coli*) strain MG1655 for microbial clearance. In contrast, *Salmonella enterica* serovar Typhimurium (S. Typhimurium) evades this response via a mechanism dependent upon Salmonella pathogenicity island-1 (SPI-1); a SPI-1 mutant, but not wild type S. Typhimurium, co-localized with zinc-containing vesicles. The antimicrobial effect of exogenous zinc was more pronounced with E. coli than with S. Typhimurium, suggesting that S. Typhimurium may employ additional mechanisms to evade zinc-mediated antimicrobial responses. Indeed, intramacrophage S. Typhimurium upregulated expression of the zinc exporter zntA, which effluxes zinc when concentrations reach cytotoxic levels. The SPI1 mutant displayed prolonged up-regulation of zntA within macrophages, consistent with its sustained exposure to zinc in this environment. Interestingly, Salmonella zntA mRNA is upregulated by both zinc and copper, and both metals ions colocalise in vesicles in TLR-activated macrophages. Furthermore, copper and zinc synergized to reduce bacterial growth of the Salmonella zntA mutant.

Our data thus suggest that S. Typhimurium employs multiple mechanisms of zinc evasion including SPI-1-dependent subversion of zinc delivery, upregulation of zntA and an additional yet to be characterized mechanism. In total, our findings identify roles for zinc and zinc trafficking in human macrophage antimicrobial pathways against Gram-negative bacteria, and provide insights into host subversion.

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Suppressor of cytokine signalling (SOCS) 5 regulates the innate anti-viral responses to influenza A infection.

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The suppressors of cytokine signalling (SOCS) proteins are negative regulators of cytokine signalling, immune cell development and function, and the inflammatory response. SOCS1-3 and CIS have defined roles in controlling the magnitude of response to various cytokines including the interferons, gp130 cytokines, IL-12, IL-10, G-CSF, prolactin, growth hormone and IL-2 family cytokines. We have recently shown SOCS4 to be a critical regulator of the anti-viral response, with loss of functional SOCS4 leading to increased susceptibility to influenza infection (1). Given the similarity between the SOCS4 and 5 SH2 domains, and the lack of an obvious phenotype in mice lacking SOCS5 protein (Socs5-/-), we have investigated its role during influenza infection. Socs5-/- mice were also highly susceptible to infection with H1N1 Puerto Rico/8/34 (PR8) influenza A virus, displaying an increased morbidity and mortality, which is associated with increased weight loss, elevated proinflammatory cytokines and delayed viral clearance. In contrast to Socs4-deficient mice, these differences are evident at day 2 post-infection, suggesting that the innate immune response is perturbed. In addition, Socs5 mRNA is expressed at significantly higher levels than Socs4 in lung epithelium, the primary site of viral replication, and is upregulated in response to infection. An intrinsic defect in epithelial cells was confirmed by infection of cultured primary lung epithelial cells from Socs5-/- mice. Moreover, wild-type bone marrow transfer into irradiated Socs5-/- mice did not rescue the phenotype, confirming the non-hematopoietic nature of the defect. We are currently using proteomics and siRNA knock-down studies to identify the innate signalling pathways that are regulated by SOCS5.

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Differential effects of pro-inflammatory cytokines IL-1β and TNFα on cell adhesion molecules in an *in vitro* model of the human blood-brain barrier.

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The central nervous system (CNS) is an immune privileged site where highly specialised endothelial cells, which comprise the blood-brain barrier (BBB), acts as a selectively permeable interface to control the passage of nutrients and inflammatory cells into the brain. Disruption of the BBB and leukocyte infiltration are among the abnormalities seen in neuroinflammatory diseases such as multiple sclerosis. Using a recently developed human cerebral microvascular endothelial cell line (hCMVEC), we investigated the effects of two pro-inflammatory cytokines IL-1β and TNFα on the inflammatory response of these cells. Specifically, temporal expression of cell adhesion molecules ICAM-1 (CD54) and VCAM-1 (CD106), and cytokine secretion were investigated using flow cytometry and multiplex cytokine arrays. The endothelial cells demonstrated differential expression of cell adhesion molecules, where TNF-α induced higher expression of ICAM-1 and VCAM-1 over the time course of 6 days. Quantification of soluble ICAM-1 and soluble VCAM-1 in endothelial conditioned media revealed that more adhesion molecules were cleaved from the cell surface when cells were stimulated by IL-1β as opposed to TNFα over a period of 72 hours. IL-1β induced a substantially different cytokine secretion profile in comparison to TNFα. Our study shows that these two key pro-inflammatory cytokines differentially regulate the inflammatory response of brain endothelial cells.

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Effects of caffeic acid phenethyl ester on *Prevotella intermedia* lipopolysaccharide-induced production of proinflammatory mediators in murine macrophages

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Caffeic acid phenethyl ester (CAPE) has numerous potentially beneficial properties, including anti-oxidant, immunomodulatory and anti-inflammatory activities. However, the effect of CAPE on periodontal disease has not been studied before. This study was designed to investigate the efficacy of CAPE in ameliorating the production of proinflammatory mediators in macrophages activated by LPS from *Prevotella intermedia*, a pathogen implicated in periodontal disease. CAPE exerted significant inhibitory effects on *P. intermedia* LPS-induced production of NO, IL-1β and IL-6 as well as their mRNA expression in RAW264.7 cells. CAPE induced HO-1 expression in cells activated with *P. intermedia* LPS, and selective inhibition of HO-1 activity by tin protoporphyrin IX significantly attenuated the inhibitory effect of CAPE on LPS-induced NO production. CAPE did not interfere with IκB-α degradation induced by *P. intermedia* LPS. Instead, CAPE decreased nuclear translocation and DNA binding activity of NF-κB p50 subunit induced with LPS. In addition, CAPE showed strong inhibitory effects on LPS-induced STAT1 and STAT3 phosphorylation. Further, CAPE significantly elevated the expression of SOCS1 mRNA in *P. intermedia* LPS-stimulated cells. Overall, this study suggests that CAPE inhibits *P. intermedia* LPS-induced production of NO, IL-1β and IL-6 in murine macrophages through anti-inflammatory HO-1 induction and inhibition of NF-κB, STAT1 and STAT3 activation, which is possibly related to the activation of SOCS1 signaling. Modulation of host response by CAPE may represent an attractive strategy towards the treatment of periodontal disease. In vivo studies are underway to further appraise the potential of CAPE as an immunomodulator in the treatment of periodontal disease.

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Suppressors of Cytokine Signalling (SOCS) 1 and SOCS3 coordinate regulation of macrophage polarization

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Macrophages are multi-functional innate immune cells, which play a critical role in the host defence against various pathogens such as bacteria, viruses and parasites. Once activated, they produce cytokines and chemokines to promote cell recruitment and proliferation, tissue repair and wound healing. They display a high degree of heterogeneity and phenotypic plasticity depending on their origin, the detection of pathogen-associated molecular pattern molecules (PAMPs) and the surrounding cytokine milieu. At present macrophages are broadly classified into M1 (classically activated) and M2 (alternatively activated), based on the activating stimuli and corresponding expression of signature markers. The SOCS family proteins (CIS and SOCS1-7) play an important role in the negative regulation of JAK-STAT signalling in various cytokine pathways. Genetic deletion of either *Socs1* or *Socs3* has a dramatic effect, resulting in excessive cytokine-driven inflammation. In order to address the possible redundancy between SOCS1 and SOCS3 in regulating macrophage polarization, we have generated CSF-1-cultured bone marrow-derived macrophages deficient in SOCS1, SOCS3 or both SOCS1 and SOCS3, and polarized them in the presence of interferon (IFN)γ (M1), interleukin (IL)-4 (M2) or lipopolysaccharide (LPS). Analysis of M1/M2 markers confirmed that SOCS1 is a negative regulator of IFNγ and IL-4 signalling, and that SOCS3 regulates cytokine-driven STAT3 phosphorylation following LPS stimulation. Interestingly, compound deletion of SOCS1 and SOCS3 synergistically up-regulated both inducible nitric oxide synthase (iNOS) and arginase I (ArgI) in response to LPS, and ArgI in response to IL-4 stimulation. In conclusion, we find no evidence for redundancy between SOCS1 and SOCS3 as each regulate specific cytokine signalling cascades, however those signals converge on iNOS and ArgI. These two enzymes compete for a common substrate to

regulate production of nitric oxide, and thus SOCS1 and SOCS3 coordinate regulation of the iNOS/ArgI axis and an important anti-pathogen effector mechanism.

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Cell crowding induces interferon regulatory factor 9 which confers resistance to chemotherapeutic drugs

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Background: Intrinsic tumor resistance to therapy remains a major problem in the management of cancer patients. Multiple mechanisms are believed to contribute to this phenomenon. Recently, a subset of interferon-stimulated genes (referred to as interferon-related DNA damage signature or IRDS) has been associated with poor therapy response and short overall survival for several cancer types. In this study we aimed to investigate the mechanism of IRDS regulation in different cancer types.

Materials and methods: Using both 2D and 3D cancer cell line models, RNAi and over expression approaches, we investigated the expression of IFN-stimulated genes using qRT-PCR and Western blotting approaches.

Results: a microarray analysis of a 2D and a 3D cultures(multicellular spheroids, MCS) of a human carcinoma cell line HCT116 revealed that IRDS genes are highly enriched in MCSs what reflects their relative resistance to chemotherapeutics. Analysis of a panel of cancer cell lines (MCF7, SKOV3, DLD1, HCT116) demonstrated that IRF9 has a leading role in the regulation of interferon-stimulated genes (e.g. *STAT1*, *STAT2*, *IFITM1*, *IFI27*, *OAS1*) and promoting therapy resistance. We have also demonstrated that this phenomenon occurs in the absence of interferons and does not depend on STAT1, the main regulator of IFN-stimulated genes, but rather depends on STAT2. Furthermore, we showed that a subset of IRDS is regulated by crowding and might depend on cell-to-cell contacts and/or adhesion molecules.

Conclusion: Our findings demonstrate that IRF9 can act as a main regulator of interferon-stimulated genes rendering tumors resistant to therapy. Therefore, it can potentially be developed as a marker of clinical response in oncology.

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IL-1-Induced Production of Chemokines CCL5 and CXCL10 requires K63-linked Polyubiquitination of IRF1

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IL-1 regulates a broad range of both immune and inflammatory responses and plays a critical role in autoinflammatory diseases. Although IL-1 activates NF-kB that regulates many IL-1 controlled processes, IL-1 also induces expression of transcription factor IRF1, yet the roles and mechanisms of its activation remain elusive. In contrast to TLRs that effectively activate IRF3 and IRF7 and induce chemokine production and efficient recruitment of mononuclear cells to sites of infection, we show that the recruitment of mononuclear cells to sites of sterile inflammation is coordinated by IL-1R-mediated activation of IRF1. We found that IRF1 is essential for IL-1-induced expression of the chemokines CXCL10 and CCL5 that in turn recruit mononuclear cells into sites of sterile inflammation. Newly synthesized IRF1 acquires K63-linked polyubiquitination mediated by cellular inhibitor of apoptosis 2 (cIAP2), whose E3 ligase activity is enhanced by the bioactive lipid sphingosine-1 phosphate (S1P). In response to IL-1, cIAP2 and sphingosine kinase 1, the enzyme that generates S1P, form a complex with IRF1, which leads to its activation. Thus, IL-1 triggers a novel signaling cascade that controls induction of IRF1-dependent genes important for sterile inflammation. Targeting of this newly discovered IL-1-induced cascade may be clinically important in the future.

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Identification of a novel low molecular weight inhibitor targeting macrophage migration inhibitory factor (MIF).

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Aim: Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine involved in the regulation of both innate and adaptive immune responses. As elevated levels of MIF are implicated in the pathogenesis of autoimmune disorders, it is an attractive target for therapeutic intervention. Several classes of small-molecule inhibitors of MIF, including ISO-1, attenuate MIF's pro-inflammatory activities. We have identified a novel and potent MIF antagonist, COR123625, which is effective *in vivo* and *in vitro*.

Methods and Materials: To assess the effects of COR123625 in endotoxic shock *in vivo*, mice were treated with COR123625 (0.1-10mg/kg) by intraperitoneal injection 24 and 1 hour prior to administration of LPS (1.0mg/kg). Serum was collected after 90 min and levels of TNF-α measured by ELISA. In vitro, murine immortalised bone marrow-derived macrophages (iBMMs) were pre-treated with COR123625 or ISO-1 (0-100μM) prior to the addition of LPS (100ng/mL). ELISA was then used to measure levels of secreted cytokines in cultured supernatants and qPCR to quantitate pro-inflammatory mRNA expression.

Results: At a concentration of 1 mg/kg, COR123625 significantly reduced LPS-induced serum TNF-α. This effect was not observed with ISO-1 at doses between 0-20 mg/kg. Both COR123625 and ISO-1 significantly reduced LPS-induced expression of TNF-α and MKP-1 mRNA, while MCP-1 levels were unaffected. No inhibition of secreted TNF-α and MCP-1 were observed when compared to LPS only cultured supernatants.

Conclusions: We show that COR123625 is a potent inhibitory compound against the promotion of TNF-α by MIF *in vivo*. In contrast to *in vivo* findings, both COR123625 and ISO-1 were not as effective in reducing cytokine expression at the transcriptional and translational level *in vitro*, which may be accounted for by cell-specific properties and suggests the effects observed *in vivo* may be dependent on interactions with other cells.

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Shedding light on preterm immunity

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Background: There is little knowledge on the immune system of extremely premature infants. This paucity of evidence impedes advances in the fight against bronchopulmonary dysplasia (BPD), a common, severe chronic lung disease that entails significant morbidity and mortality. No safe and effective treatment exists.

Method: Blood was collected from extremely preterm infants at 5 timepoints [birth, days 1, 7 and 14 and 36 weeks corrected gestational age (WCGA)]. Following overnight stimulation with PMA/ionomycin, LPS or vehicle in whole blood assays, flow cytometry was used to explore T cells and their polarisation, macrophages and dendritic cells (DC) and their activation status and endothelial progenitor cells (EPC).

Results: In preterm babies, expression of the activation marker MHC II progressively increased over time on macrophages (d1, 48% vs 36-WCGA, 86%) and DC (d1, 24% vs 36-WCGA, 56%). Comparing the one infant that developed BPD with the two that did not at 36-WCGA, we observed a marked increase in macrophage (6-fold) and DC activation (2-fold), but fewer circulating EPC (0.3% vs 1.4% of viable cells). Unexpectedly, we also found that preterm infants are capable of producing the cytokines IFN-gamma and IL-17A.

Conclusions: It only took the results from three preterm infants to reveal that this first-of-its-kind study will revolutionise the understanding of preterm immunity; for example, these data suggest that, contrary to current belief, preterm babies are capable of producing IFN-gamma and IL-17. BPD appears associated with markedly increased cellular activation - a promising finding that may provide a basis for therapeutic innovations.

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Inhibitor of apoptosis (IAP) proteins repress spontaneous inflammatory joint disease

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Inhibitor of apoptosis proteins (IAPs), including x-linked (XIAP) and cellular IAP 1/2 (cIAP1/2), are important regulators of TNF receptor induced cell death and cytokine production. We have previously demonstrated that, *in vitro,* IAP loss sensitises macrophages to TNF induced caspase-8 activation and apoptosis, and limits RIPK3 mediated TNF and IL-1β secretion. Here we report that mice with genetic deletion of XIAP and cIAP2 ubiquitously and cIAP1 in myeloid cells ($c1^{LysMcre}x^{-/-}$ $c2^{-/-}$) develop a mild spontaneous inflammatory arthritis and dermal inflammation, which is associated with increased myelopoiesis and elevated cytokines, particularly IL-1β. In contrast, mice doubly deficient in cIAPs ($c1^{LysMcre}c2^{-/-}$) but retaining XIAP, exhibit severe arthritis accompanied by elevated TNF levels. Predisposition to inflammation upon IAP deletion in myeloid cells was further highlighted by increased disease severity in IL-1β dependent K/BxN serum induced arthritis. Consistent with the role of IAPs as important negative regulators of RIPK3 and caspase-8 activity, we further show that K/BxN arthritis is reduced in mice lacking RIPK3 alone, or in combination with apoptotic caspase-8. In contrast, deficiency in the necroptotic effector MLKL did not alter disease pathogenesis. These results show that deletion of cIAP1 concurrently with either cIAP2 or XIAP is sufficient to cause spontaneous inflammation, albeit with distinct cytokine profiles. Furthermore, our findings overturn the common assumption that reduced inflammatory disease in RIPK3-deficient mice is due to a block in necroptosis.

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Role of STAT3 in regulation of cancer metabolism

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STAT3 is a latent cytoplasmic transcription factor that responds to cytokine signaling and tyrosine kinase oncoproteins by nuclear translocation when tyrosine phosphorylated and has been implicated in a wide variety of human cancers. We have found that malignant transformation by activated Ras is impaired without STAT3, in spite of the inability of Ras to drive STAT3 tyrosine phosphorylation or nuclear translocation. STAT3 mutants that cannot be tyrosine phosphorylated, are retained in the cytoplasm, or cannot bind DNA are nonetheless capable of supporting Ras-mediated transformation. In addition to being a cytoplasmic protein that translocates to the nucleus in response to tyrosine phosphorylation, STAT3 accumulates in mitochondria, and its presence in mitochondria is sufficient to support Ras-mediated transformation. Mitochondrial STAT3 modulates metabolic output, influencing both glycolytic and oxidative phosphorylation activities characteristic of cancer cells. These actions of mitochondrial STAT3 depend on its phosphorylation on serine 727, which is mediated by the MEK-ERK pathway in Ras-transformed cells.

K-Ras-dependent myeloid proliferative neoplasm in mice displays serine but not tyrosine phosphorylated STAT3. A point mutation abrogating STAT3 S727 phosphorylation delayed onset and decreased disease severity in mice with oncogenic K-Ras expressed in hematopoietic progenitors and significantly extended their survival. Activated K-Ras also required STAT3 for cytokine-independent growth of myeloid progenitors *in vitro*, and mitochondrially restricted STAT3 and STAT3-Y705F, both transcriptionally inert mutants, supported factor-independent cell growth. Although STAT3 was dispensable for growth of myeloid progenitors in response to cytokines, abrogation of STAT3-S727 phosphorylation blocked K-Ras-driven, growth factor-independent malignant growth. These data document that serine phosphorylated, mitochondrially-restricted STAT3 supports hematopoietic neoplastic cell growth induced by K-Ras. A series of small molecules that impair growth of malignant cells appear to depend on the metabolic function of STAT3, suggesting that mitochondrial STAT3 is a viable cancer target.

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Inhibition of JAK signalling by SOCS1

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Hypothesis

The Janus Kinase family of proteins (JAKs) are constitutively associated with the intracellular portion of cytokine receptors. Upon cytokine stimulation of the receptor, JAKs provide the kinase activity necessary for the phosphorylation of substrate molecules, resulting in a subsequent biological response. The Suppressors of Cytokine Signalling (SOCS) proteins inhibit JAK activity, regulating cytokine signalling. SOCS1 and SOCS3 have a distinct method of inhibiting JAK1, JAK2 and TYK2 by competing with protein substrates, directly inhibiting JAK catalytic activity.

We have solved the structure of SOCS3 in complex with JAK2 and a fragment of the gp130 receptor. This structure shows that SOCS3 binds JAK and receptor simultaneously. Importantly, SOCS3 is able to obtain specificity for the downregulation of certain cytokine pathways by binding preferentially to some receptors.

In light of this information, we now aim to elucidate the mechanism through which the similar SOCS1 inhibits JAK catalytic activity.

Aims and methods

We have recently been able to recombinantly produce SOCS1, allowing structural and biophysical studies to be undertaken for the first time.

Our preliminary data indicate that SOCS1 is a more potent inhibitor of JAK catalytic activity than SOCS3. We are currently investigating the structural features of SOCS1 which contribute to such inhibitory activity. We are also investigating the features allowing SOCS1 to specifically inhibit different cytokine pathways to those regulated by SOCS3.

Specifically, we aim to obtain the structure of SOCS1 bound to a JAK family member to understand its mode of inhibition. Further, studies of engineered SOCS1 and JAK mutants can now reveal the direct contributions of particular regions of each protein to binding and inhibition kinetics, as well as those regions which provide specificity of inhibition of certain cytokine pathways.

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Understanding emergency haematopoiesis using cellular barcoding

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Haematopoiesis in the steady-state is reasonably well understood. However, upon emergency situations such as infection or inflammation, homeostasis is broken and cell numbers can rise substantially e.g. neutrophil numbers after infection with Listeria monocytogenes. What is not currently clear is whether this is due to the same progenitors producing more of a given cell type, or instead if there is recruitment of 'emergency' progenitors for this purpose. Cellular barcoding is a novel technology that can track the output of single stem and progenitor cells. Here, cells are transduced with lentivirus where each particle has a different DNA 'barcode' that is integrated into the genome, and thus inherited by daughter cells. By establishing the barcode signatures in progeny cell types in steady-state vs inflammatory conditions I aim to assess progenitor fate on the single cell level that gives rise to higher cell numbers. In my model I will be assessing the affect of G-CSF and flt3 ligand on the output of several immune cell types.

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Oncostatin M and IL-6 induce pro-inflammatory gene signatures in articular chondrocytes

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Aims. This study describes gene expression in chondrocytes stimulated with two gp130 family cytokines - Oncostatin M (OSM) or IL-6 in conjunction with soluble IL-6 receptor (IL-6/sIL-6R, i.e. IL-6 trans-signaling) and examines the impact of deleting Suppressor of Cytokine Signaling-3 (SOCS3) in this cell type.

Methods. Wild type (WT) and SOCS3-deficient (*Socs3*$^{\Delta/\Delta col2}$) murine primary chondrocytes were stimulated *in vitro* with OSM or IL-6/sIL-6R, for 4 hours. Total RNA was extracted and expressed genes were identified by microarray analysis (*Illumina MouseWG-6 v2.0 Expression BeadChip*). Validation of microarray results was performed using Taqman probes on RNA derived from chondrocytes stimulated with OSM or IL-6/sIL-6R for 1, 2, 4 or 8 hours. Gene set testing was undertaken using ROAST (rotation gene set testing). Gene ontology was performed using DAVID v6.7.

Results. Preliminary data showed that chondrocytes rely on IL-6 trans-signaling. The gene transcription profiles between OSM and IL-6/sIL-6R were highly correlated. Using pathway analysis, OSM was found to have more profound effects on chondrocyte gene expression compared to IL-6/sIL-6R, and induced greater changes in biological processes, including “Cytokine activity”, “Metallopeptidase activity” and “Proteolysis”. Further analysis distinguished between acute-phase (e.g. *Ccl7*) and late-stage (e.g. *Il19* and *Saa1*) changes in gene expression for both OSM and IL-6. In the absence of SOCS3, OSM and IL-6/sIL-6R stimulation induced an IFN-like gene signature.

Conclusion. We found similarities and differences between OSM- and IL-6/sIL-6R-induced inflammatory gene signatures in chondrocytes. SOCS3 plays an important regulatory role in this cell type, as it does in hematopoietic cells. Our results suggest OSM may be a target for therapeutic intervention in inflammatory arthritis.

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Targeting interleukin 6 in endothelial cells to prevent undesired formation of new blood vessels

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Interleukin 6 (IL-6) is a multifunctional cytokine that plays a central role in inflammation by controlling differentiation, proliferation, migration and apoptosis of targeted cells. In recent years it has been shown that IL-6 induces release of vascular endothelial growth factor, and thus might play an important role in angiogenesis.

Aim: The present study aimed at investigating the involvement of IL-6 in angiogenesis by studying migration and tube formation of vascular endothelial cells.

Methods: Migration and tube formation of human umbilical vein endothelial cells (HUVEC) were studied after treatment with IL-6, soluble IL-6 receptor or the combination of IL-6 and the soluble IL-6 receptor. Migration and tube formation was also studied in HUVEC after knock-down of IL-6 using siRNA. Knock down of IL-6 was confirmed using ELISA.

Results: Treatment with IL6 (50ng/ml), soluble IL-6 receptor (100 ng/ml) or the combination of IL6 and soluble IL6 receptor did not affect tube formation or migration. Knock down of IL-6, on the other hand, resulted in reduced tube formation (number of tubes and total tube length) as well as slightly reduced migration of HUVEC. This inhibition could be restored after addition of exogenous IL-6 (50ng/ml).

Conclusion: Our data suggests that IL-6 is involved in angiogenesis and that IL-6 produced by the endothelial cells themselves is enough in to maintain normal tube formation and migration. Targeting IL-6 may be a novel strategy to prevent undesired formation of new blood vessels.

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Detection, localisation and quantification of the interaction between IL-37 and its cell surface receptor in human peripheral blood mononuclear cells via dSTORM super-resolution imaging

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Aims

The elucidation of protein complexing and their cellular localisation are foundation to cytokine and signalling research. Traditional methodologies such as immunoprecipitation and immunofluorescence cannot show complexing, localisation and abundance of molecules simultaneously. We aim to co-opt super-resolution imaging techniques to study the interactions between the powerful anti-inflammatory cytokine IL-37 and the receptor chains IL-18 receptor alpha (IL-18Ralpha) and SIGIRR. We describe cytokine-receptor binding/complexing, subcellular localisation and molecular numbers of these three proteins in their endogenous, native context, i.e. without exogenous additions, transfections or lysis.

Methods

The basis for our technique is direct stochastic optical reconstruction microscopy (dSTORM, 1). AlexaFluor488-, AlexaFluor568- and AlexaFluor647-tagged secondary antibodies were used to label the ligand (IL-37) and the two subunits of its heterodimeric receptor complex (SIGIRR and IL-18Ralpha) in PBMC from healthy human donors. The fluorophores were induced into a dark state by high intensity laser illumination and reducing buffer conditions. The stochastic return of the fluorophores to an active state is imaged and statistically analysed, allowing detection and localisation at 20-50nm precision. We then derive absolute localisation (positioning) and proximity of all three proteins relative to each other on the cell surface, and infer complexing and interaction based on clustering and co-location at molecular binding distances. As the technique is single molecule sensitive, we further measure and sample the each protein at the cell surface and derive the molecular proportion that is involved in complexing.

Results and conclusions

IL-37 forms a ligand:receptor complex with SIGIRR and IL-18Ralpha (sub 50nm proximity with each other) on the cell surface of primary, untransfected human PBMC. Thirty min after LPS, approximately 15% of SIGIRR and 6% of IL-18Ralpha molecules are involved in complexing with IL-37 on the cell surface. This is the first demonstration of any super-resolution technique to study three-way protein interactions in primary human samples.

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Regulation of myeloid cell population kinetics and phenotype by CSF-1 and GM-CSF

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GM-CSF (CSF-2) and CSF-1 are key cytokines for the mononuclear phagocytes, including monocytes, macrophages and dendritic cells (DCs). GM-CSF is critical for in vitro monocyte-derived DCs (Mo-DCs) generation and the development of resident $CD103^+$ DCs in some tissues. In contrast, CSF-1 is essential for the homeostasis of monocyte/macrophage lineage cells. However, clear delineation of DC subsets, Mo-DCs and classical DCs (cDCs), has remained relatively unclear because of many overlapping features of these cells such as surface markers, making it difficult to discern the differential roles of GM-CSF or CSF-1 in controlling such cells.

In this study, we assess the effects of GM-CSF and CSF-1 on myeloid-lineage populations during inflammation utilizing a number of animal models. Peritoneal exudate cells (PECs) were taken from antigen (mBSA)-induced peritonitis (AIP) or thioglycolate-induced peritonitis. Spleen cells from intravenous LPS-challenged mice were examined. Mice also received anti-GM-CSF (22E9), anti-M-CSFR (AFS98), or control mAbs. Monocytes, macrophages, and DC subsets (Mo-DCs and cDCs) were monitored by flow cytometry.

We classified all monocyte descendants, including monocytes, monocyte-derived macrophages and Mo-DCs, by their CSF-1 receptor/CD115 expression, setting them apart from cDC lineage. Inflammatory Mo-DCs were found in the AIP PECs, but not in thioglycolate-PECs or LPS-challenged spleens, and were depleted specifically in $GM\text{-}CSF^{-/-}$ or anti-GM-CSF-treated mice. AIP Mo-DCs, macrophages, and monocytes were all depleted following CSF-1R neutralization. In contrast, cDCs subsets in all tissues examined were relatively unaffected by the GM-CSF or CSF-1R neutralization. In summary, GM-CSF deletion or anti-GM-CSF treatment abrogated the accumulation of inflammatory Mo-DCs, with no effect on nonlymphoid tissue cDCs, in T cell-dependent inflammation; in contrast, the CSF-1 receptor was important for all inflammatory monocyte descendants, but not the cDC lineage populations.

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Understanding the Role of Pro-inflammatory Cytokines in the Development of Gastric Cancer

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NF-κB, in particular the RelA subunit, is an important transcriptional regulator of many genes involved in tumour-promoting inflammation, cell proliferation and survival, and has frequently been found to be aberrantly over-activated in infiltrating immune cells in gastritis and in gastric cancer (GC). Our laboratory has discovered that ageing NF-κB1-deficient (*nfκb1*$^{-/-}$) mice develop GC in a manner that mimics the progression of human invasive intestinal-type GC. These mice develop chronic gastritis, gastric mucosal atrophy or dysplasia, with a high incidence of invasive gastric adenocarcinomas. We observed abnormally increased infiltration of F4/80-positive macrophages and CD45-positive leukocytes in the stomachs of young *nfκb1*$^{-/-}$ mice. Concurrent with the recruitment of inflammatory cells into the gastric mucosa, elevated levels of pro-inflammatory cytokines and chemokines were found in the serum of young *nfκb1*$^{-/-}$ mice, and also in established tumours of these mice. This implicates deregulated expression of pro-inflammatory cytokines in GC disease progression.

Inflammatory cells recruited into the gastric mucosa during chronic gastritis secrete pro-inflammatory cytokines and chemokines. Interestingly, loss of NF-κB1 has been found to exacerbate macrophage M1-driven inflammation, augmenting pro-inflammatory cytokine production. In order to characterise the role of abnormally elevated cytokines in the onset and progression of GC in *nfκb1*$^{-/-}$ mice, we generated compound mutant mice lacking both NF-κB1 and individual pro-inflammatory cytokines that have been linked to cancer development. The survival, histopathology, immune cell infiltration, cytokine and chemokine levels of these mice is being examined at multiple time points. We have also characterised the cell populations in the gastric mucosa and have quantified the levels of phosphorylated-Stat3 and phosphorylated-p65/NF-κB levels in gastric epithelial cells and infiltrating leukocytes of these compound mutant mice. Our results determine the roles these cytokines have in driving GC in the *nfκb1*$^{-/-}$ mice and highlight the therapeutic potential in targeting pro-inflammatory cytokines for the treatment of GC.

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Therapeutic deletion of IL-17 producing CD8+ T cells (Tc17) attenuates GVHD without impairing GVL.

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Donor T cell polarization is a critical factor influencing the severity and tissue distribution of graft-versus-host disease (GVHD) and the potency of graft-versus-leukemia (GVL) effects after bone marrow transplantation. We have recently reported that G-CSF treatment promotes type-17 differentiation in both CD4 and CD8 T cells and that donor IL-17A, predominantly from CD8 T cells (Tc17), mediates fibrotic skin pathology manifesting late after transplant as scleroderma. To study Tc17 development and function we utilized the IL-17ACreRosa26ReYFP 'fate-mapping' reporter mouse and observed that donor Tc17 cells differentiate early post allogeneic transplant and transition rapidly towards a Tc1-like phenotype. Tc17 differentiation is dependent upon IL-6, host-DC and is regulated by the presence of IFNγ. Tc17 cells appear highly inflammatory, displaying considerable promiscuity in their transcriptional profile and inflammatory cytokine production. Furthermore, targeted deletion of Tc17 early post transplant was protective in a lethal model of acute GVHD. In contrast, Tc17 express only low levels of the CTL effector molecule Granzyme B and display strikingly poor GVL activity in vivo. These data demonstrate that Tc17 differentiation is an early and highly plastic differentiation program, culminating in a poorly-cytolytic, inflammatory population that mediates GVHD without contributing to GVL. Thus, early therapeutic targeting of Tc17 development via IL-6 inhibition represents a highly attractive avenue for GVHD prevention.

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PB1-F2 from H7N9 Influenza A virus activates the NLRP3 inflammasome to induce inflammation

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Emerging influenza A virus (IAV) strains that cross over into humans present a global health danger due to their potential high mortality. These pandemic infections are characterized by the hyper-inflammatory response, or 'cytokine storm' which can lead to severe clinical symptoms and death.

We recently identified the virulence factor PB1-F2 in the highly pathogenic mouse-adapted A/PR8 IAV strain as a potent activator of the NLRP3 inflammasome, inducing secretion of mature pyrogenic IL-1b. Activation of the inflammasome was directly linked to pathogenic IAV aetiology. We have now identified that PB1-F2 peptides derived from highly pathogenic strains of the 20th century are also able to induce IL-1b secretion.

Recent sporadic infections of humans in China with avian H7N9 IAV have caused concern due to high mortality, yet virulence factors associated with severe pathology are unclear. H7N9 viruses isolated from human express full length PB1-F2 which may act as a virulence factor. We have found that PB1-F2 peptide derived from H7N9 is a potent inducer of IL-1b secretion, comparable to A/PR8. Importantly, IL-1b secretion is reduced by inhibiting phagocytosis, lysosomal acidification and Caspase-1 activation while macrophages deficient in ASC, Caspase-1 and NLRP3 display ablated IL-1b secretion. Our data therefore identify H7N9 PB1-F2 as a novel inflammasome activator. Critically treatment of mice with H7N9 PB1-F2 peptide induced leukocyte infiltration and IL-1b secretion into the lung airspaces.

Given the high mortality associated with pathogenic IAV outbreaks, we further investigated if suppression of the inflammasome with the inhibitor MCC950 could reduce inflammation. We have subsequently found that MCC950 is able to potently inhibit PB1-F2-induced IL-1b secretion and inflammasome activation. Importantly within the context of pathogenic IAV outbreaks, treatment of mice with MMC950 three days post-intranasal infection with pathogenic PR8 IAV delays the onset of disease.

These findings suggest that H7N9 IAV pathogenicity may be associated with PB1-F2-induced inflammasome activation and that targeted inhibition of the inflammasome may provide a therapeutic intervention to alleviate the excessive inflammation associated with pathogenic IAV infections.

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Positive and Negative Epigenetic Regulatory Steps During IFN-Stimulated Transcriptional Initiation and Elongation

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IFN-stimulated gene (ISG) expression is mediated by the ISGF3 transcription factor complex, composed of tyrosine phosphorylated STAT1 and STAT2 and the DNA binding partner, IRF9. The transactivation domain of STAT2 recruits transcriptional activators and chromatin modifiers that regulate polymerase recruitment and elongation. Interestingly, both histone acetyltransferase (HAT) and histone deacetylase (HDAC) activities are required for transcription of ISGs and establishment of an antiviral state. Inhibition of HDAC activity or reduction of HDAC1, 2, and 3 abrogates ISG transcription without altering the activation or chromatin recruitment of ISGF3. To pinpoint this HDAC requirement, we examined ISG transcription in vitro and ISG epigenetic regulation in vivo. While transcription of ISGs on nucleosome-free DNA in vitro was unaffected by HDAC activity, chromosomal remodeling occurred at ISG promoters in vivo in response to IFN stimulation and this remodeling required HDAC activity. To discover factors required for ISG transcription, we purified native ISGF3 complexes and identified STAT2-interacting proteins by mass spectrometry. The DNA helicases Rvb1 and Rvb2 associated with the transactivation domain of STAT2 and reducing their expression by RNA interference impaired ISG transcription. Neither Rvb1 nor Rvb2 were required for induction of IFN-gamma or TNF-alpha induced transcription. Interestingly, IFN-alpha stimulation recruited RNA Pol II to ISG promoters even in the absence of HDAC activity, and the recruited Pol II became phosphorylated on Ser-5, a hallmark of transcriptional initiation. However, RNA Pol II did not become phosphorylated on Ser-2 and failed to transcribe IFN target genes in the absence of HDAC activity. Moreover, BRD proteins, PAF1, and SAGA complexes were found to regulate ISG transcription, including requirements for regulated and possibly sequential histone acetylation, deacetylation, and ubiquitinylation. Individual chromatin modification steps were impaired when HAT or HDAC enzymes were inhibited. These data define an ordered set of chromatin modifications that coordinate individual regulatory events necessary for recruitment of positive factors and dismissal of negative factors during the ISG transcriptional initiation and elongation cycle.

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Investigating type I interferon gene signatures in breast cancer patients

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Bone metastasis is a key characteristic of end-stage breast cancer and severely reduces patient survival. While staging and grading of primary tumour cells help to characterise the invasive nature of the tumour, currently there is no way of definitely predicting prognosis in breast cancer patients. Interferon regulatory factor 7 (IRF7) is a transcription factor known to be a key regulator of type I IFN. We recently showed that the loss of IRF7 expression in bone metastases compared to primary breast tumour cells results in reduced type I IFN activation of peripheral anti-tumour immunity; thus enabling metastasis[1]. The aim of this project is to investigate the type I IFN-regulated gene expression in human peripheral blood and whether a signature exists in breast cancer patients as a potential marker for prognosis. Transcription profiling microarray experiments will be performed using peripheral blood mononuclear cells (PBMC) from healthy donors; breast cancer patients who responded to chemotherapy; and non-responders. The gene signature derived in the previous aim will be refined using Interferome v2.0[2] and 'Enrich' and used to examine whether the type I IFN-regulated gene signature is expressed differentially as a reflection of the presence or absence of an intact IFN/IRF7 pathway in primary breast cancer cells and further, whether differential expression indicates prognosis.

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Arid5a enhances the development of Th17 cells by stabilizing Stat3 mRNA

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Th17 cells play a critical role not only in host defense and mucosal defense but also in tissue inflammation and autoimmunity. Although the mechanism of regulation of Th17 cell differentiation at transcriptional level is extensively studied, little is known about the post-transcriptional gene regulation in Th17 cells. Here we report for the first time that AT-rich interactive domain 5a (Arid5a) controls the differentiation of Th17 cells through stabilization of Stat3 mRNA. Arid5a is specifically induced under Th17 cell polarizing condition but not Th1, Th2, and Treg cell condition. Consequently, enhanced Arid5a protein binds to the stem-loop sequence of the 3' untranslated region (UTR) of the Stat3 mRNA, in turn, stabilizes Stat3 mRNA by competing with Regnase-1 on the same portion. Conversely, Arid5a deficiency led to reduction of the frequency of Th17 cell population, in which the expressions of the Stat3-regulating gene, *Rorc*, *IL-21*, *IL-23R* was significantly lower than those of WT T cells. Moreover, we have shown that such intrinsic role of Arid5a in T cells is critical for induction of experimental autoimmune encephalomyelitis (EAE) in Rag2 deficient mice by adoptively transferred T cells. Thus, we demonstrated that Arid5a plays an important role in the differentiation of Th17 cells through control of stabilization of Stat3 mRNA, which results in exacerbation of autoimmune inflammation.

ILK inhibition. Instead, ILK is involved in an alternative activation of NF-kB signaling by modulating the phosphorylation of p65 at Ser536. Furthermore, ILK-mediated alternative NF-kB activation through p65 Ser536 phosphorylation also occurs during *Helicobacter pylori* infection in macrophages and gastric cancer cells. Moreover, ILK is required for *H. pylori*-induced TNF-α secretion in macrophages. While ILK-mediated phosphorylation of p65 at Ser536 is independent of the phosphotidylinositol 3-kinase (PI3K)/Akt pathway during LPS stimulation, upon *H. pylori*infection this event is dependent on the PI3K/Akt pathway. Moreover, in mouse model of DSS-induced colitis, DSS-induced colon tissue damages, macrophages infiltration and colitis disease activity (measured by colon length, body weight loss and disease activity index) were significantly reduced in ILKfl/fl;LysMCre mice compared to the control mice, indicating a critical role of ILK in inflammatory diseases.

Conclusions:
Our findings implicate ILK as a critical regulatory molecule for pro-inflammatory signaling and a potential target for therapeutic intervention in inflammatory diseases.

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Decidual-trophoblast interactions are critical for maternal tolerance of pregnancy.

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Invasion of specialized fetal cells, the 'extravillous trophoblast' (EVT) into the uterine decidua is critical for the establishment of pregnancy. This presents an immunological paradox – why do invasive EVTs escape rejection?

One hypothesis is that decidual leukocytes are 'specialized' compared to peripheral leukocytes with pregnancy-specific functions. However, the mechanisms leading to the differentiation of decidual-specific leukocyte populations are not well understood. The local endometrial environment likely regulates leukocyte differentiation and activation. Prior to implantation endometrial stromal cells (ESC) differentiate to become 'decidualized' and decidual leukocyte numbers increase such that during the 1st trimester, the period of maximal EVT invasion, around 30-40% of cells in the endometrium are leukocytes, predominantly uterine Natural Killer cells and macrophages. Abberant leukocyte activation and impaired decidualization are features of recurrent miscarriage and preeclampsia. We aimed to investigate how interactions between decidual cells and the invading EVTs might influence maternal tolerance.

Cytokine secretion by primary human decidual biopsies (n=3) was identified by Luminex. Primary human ESC were decidualized in vitro and conditioned media (CM) collected from non-decidualized and decidualized ESC. Primary human EVT (n=6) were treated with pooled (n=14) ESC CM and secreted proteins <30kD identified by mass spectrometry and validated by immunohistochemistry and Western blot.

Decidual biopsies secreted high levels of G-CSF, GM-CSF, IL6, IL8 and MCP1. The presence of EVTs within this tissue elevated IL1RA secretion and reduced IL6, IL15 and MCP1 secretion. EVTs exposed to decidualized CM expressed cell surface factor CD59, which inhibits formation of the complement membrane attack complex. In contrast, EVTs exposed to non-decidualized CM secreted pro-inflammatory proteases including Annexin A2, which can activate macrophages via TLR4 and DPP1, which activates granzymes.

Overall, our data suggests decidualization and appropriate interactions between ESC and EVTs are critical for maternal tolerance. Impaired decidualization may lead to EVT expression of factors which aberrantly activate maternal leukocytes.

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Mitochondrial apoptosis is dispensable for NLRP3 activation but non-apoptotic caspase-8 is required for inflammasome priming

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The NOD-like receptor protein 3 (NLRP3) inflammasome senses a variety of pathogen, host and environmental molecular patterns to mediate caspase-1 activation, thereby promoting caspase-1 processing and secretion of the pro-inflammatory cytokines IL-1b and IL-18. The current paradigm states that mitochondrial damage is a critical determinant of NLRP3 inflammasome activation. Here, we genetically assess whether mitochondrial signalling represents a unified mechanism to explain how NLRP3 is activated by divergent stimuli. Neither co-deletion of the essential executioners of mitochondrial apoptosis BAK and BAX, nor removal of the mitochondrial permeability transition pore component cyclophilin D, nor loss of the mitophagy regulator Parkin, nor deficiency in MAVS affects NLRP3 inflammasome function. In contrast, caspase-8, a caspase essential for death-receptor-mediated apoptosis, is required for efficient Toll-like-receptor-induced inflammasome priming and cytokine production. Collectively, these results demonstrate that mitochondrial apoptosis is not required for NLRP3 activation, and highlight an important non-apoptotic role for caspase-8 in regulating inflammasome activation and pro-inflammatory cytokine levels.

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Clostridium difficile toxin B induces an inflammatory cytokine response in a mouse model of infection

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Clostridium difficile is an important nosocomial pathogen of humans. Currently the role of toxin A and toxin B in disease pathogenesis and in the development of severe disease resulting from *C. difficile* infection (CDI) is poorly characterised. Furthermore, the factors that predispose some patientsto develop mild disease while others develop life-threatening disease are unknown. Understanding how *C. difficile* modulates the host immune response during infection may provide insight into this phenomenon and may provide a better understanding of the role that each toxin plays in disease. In this study, we examined the role of both toxins in pathogenesis and the induction of the host immune response with disease progression. The analysis of an isogenic panel of independently derived toxin gene mutants in a BI/NAP1/027 *C. difficile* strain using the mouse model of CDI showed that toxin B plays a critical role in the development of severe disease and in the induction of the host immune response. Increased levels of pro-inflammatory cytokines and chemokines such as C5a, TNF-α, MCP-1 and CXCL1, 9 and 10 were found in colonic tissue isolated from mice infected with a toxin A mutant, at levels similar to those detected in tissues from mice infected with the wild-type strain. Cytokine induction coincided with the development of severe colonic damage and fulminant disease. By contrast, infection with toxin B mutants resulted in only self-limiting diarrhoea and mild disease and led to the induction of significantly lower levels of pro-inflammatory cytokines than observed in mice infected with the wild-type strain. Toxin B therefore appears to be the major virulence factor of *C. difficile* with toxin A playing a more minor role. This study provides valuable insights into the role of toxin A and toxin B in *C. difficile* disease and the host immune response

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Cytokines differentially regulate complement receptor immunoglobulin (CRIg) in human macrophages: a control point in inflammation

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Aim(s) and Introduction

CRIg, a recently characterized complement receptor, is structurally and functionally distinct from the classical complement receptors, CR3/CR4. CRIg expression is restricted to macrophages. While these receptors promote phagocytosis, CRIg also expresses anti-inflammatory/immunosuppressive activity. Reduced ratio of CRIg to CR3 expression in macrophages skews the response towards inflammation. We propose that CRIg is a control point in inflammation and its expression is regulated by cytokines in a manner that is commensurate with the action of the cytokines (that cytokine actions occur via regulating CRIg expression).

Methods

Cytokines were examined for their effects on (i) the differentiation of human monocytes to CRIg+ macrophages in culture, (ii) CRIg expression in monocyte-derived macrophages (MDM) and (iii) CRIg expression in dendritic cells (DC) generated from GM-CSF/IL-4 treated monocytes. The range of cytokines investigated included Th1, Th2, pyrogenic, regulatory and haematopoietic growth factors types (LT, IFN-γ, IL-4, IL-13, TNF-α, IL-1β, IL-6, IL-10, TGF-β1, M-CSF and GM-CSF). CRIg mRNA was measured by qRT-PCR and protein by Western blot and/or flow cytometry.

Results

Development of CRIg+ macrophages and CRIg expression in MDM and DC was positively or negatively regulated by cytokines. The changes caused by these cytokines were dependent on the macrophage type or process and conducive with their role in promoting or protecting against inflammation associated with rheumatoid arthritis and atherosclerosis. The cytokines similarly regulated both spliced forms of CRIg. The results also showed that the cytokines differentially regulated CR3/CR4 compared with CRIg expression. Furthermore, using PKCα-deficient MDM generated by RNAi, our data demonstrated that PKCα plays an important role in regulating CRIg expression in macrophages and that cytokines such as TNF-α regulated CRIg expression via PKCα.

Conclusions

Cytokines regulate the development of CRIg+ macrophages and CRIg expression on macrophages and DC, in a manner conducive with their role in inflammation.

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Candida induces pyroptosis during escape from macrophages

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The human fungal pathogen *Candida albicans* exists as commensal yeast cell but becomes invasive as hyphae. While several pattern recognition receptors recognize the carbohydrate rich cell wall of *Candida*, yeast and hyphae induce different cytokine and immune responses. This is largely because intracellular hyphae, but not yeast cells, are detected by the NLRP3 inflammasome leading to caspase-1 activation and Il-1β secretion. Here we show activation of the NLRP3 inflammasome by intracellular *Candida* also induces pyroptosis. In the absence of NLRP3, ASC or caspase-1, *Candida* is still able to form intracellular hyphae but fails to kill and escape from macrophages soon after phagocytosis. Conversely, we have generated *Candida* mutants that still form hyphae but display reduced levels of caspase-1 activation, pyroptosis and Il-1β secretion. These mutants show a breakdown of the cell wall architecture, in particular reduced expression of glucans, which are known targets of macrophage receptors. Importantly, despite forming hyphae in susceptible mice these *Candida* mutants are heavily attenuated in virulence, suggesting that pyroptosis is a critical escape mechanism of *Candida*. This is surprising, as we have recently shown that intracellular hyphae can also induce delayed macrophage death to escape, even in the absence of pyroptosis and other programmed cell death pathways. Thus, we provide the first report of a fungal pathogen to induce pyroptosis in macrophages and provide evidence that early macrophage escape by hijacking pyroptosis contributes to*Candida* virulence.

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Inhibition of IFN-regulated mediators of the IRF3 signaling pathway using small molecule inhibitors targeting TBK1

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Aims

Interferon-regulatory factor 3 (IRF3) is a key transcription factor that mediates TLR3 and TLR4 activation of IFN-regulated gene expression in inflammatory diseases such as rheumatoid arthritis (RA). The IKK-related kinase, TANK-binding kinase 1 (TBK1), is essential for IRF3 activation. Here, we investigate the *in vitro* effects of a novel small molecule inhibitor that selectively inhibits TBK1 and inflammatory mediators downstream of IRF3 signaling.

Methods

TBK1 kinase assays were performed using recombinant TBK1 protein in an ADP luminescent kinase assay. The ability of TBK1 inhibitors (WEHI-112 and MRT67307) to inhibit LPS induced phosphorylation of IRF-3 was assessed in primary mouse synoviocytes and human THP-1 macrophages by Western Blot. The effect of TBK1 inhibitors on LPS or Poly I:C induced cytokine production (IP10, RANTES and TNF) by RAW264.7 cells was measured in cell culture supernatant by ELISA. IFNα/β gene expression and protein levels in culture supernatants were detected using QPCR and the B16-reporter cell line, respectively.

Results

The TBK1 inhibitor WEHI-112 suppressed TBK1 activity with similar potency to the previously described compound MRT67307 (IC_{50} 14nM and 43nM respectively). Western blot analysis demonstrated that WEHI-112 inhibited LPS and Poly I:C-induced phosphorylation of IRF3 in mouse synoviocytes and THP-1 macrophages, comparable to MRT67307. In addition, both WEHI-112 and MRT67307 potently inhibited LPS- and poly I:C-induced IP10, RANTES and type I IFN production by RAW macrophages. TBK1 inhibitors failed to modulate TNF production, demonstrating selectivity for the IRF3 signaling pathway.

Conclusion

We have developed a novel, small molecule inhibitor of TBK1 that potently suppresses TBK1 activated IRF3 phosphorylation and the production of IP10, RANTES and type I IFN. This study demonstrates a promising avenue for the development of small molecule inhibitors targeting TBK1 as a potential therapeutic in inflammatory diseases, including RA.

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Understanding the Role of Cytokines in the Onset and Progression of Intestinal-Type Gastric Cancer

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Gastric cancer (GC) is the fourth most prevalent cancer worldwide, and the third most common cause of cancer-related death. The disease is generally asymptomatic and as a result is often diagnosed at an advanced stage, at which point metastasis is present in greater than 80% of patients. Treatment options for GC are limited; however, current therapeutic strategies have embraced the concept of targeting components of the inflammatory microenvironment. In light of this, we aimed to characterise the role of cytokines produced by mucosal immune cell populations during onset and progression of intestinal-type gastric cancer (IGC). We compared microarray data from a panel of >40 human IGC biopsies and adjacent non-tumour tissue to characterise the expression of cytokines and transcription factors classically associated with chronic inflammation. Since we could detect numerous pro-inflammatory cytokines in human IGC tumour tissue, we characterised the role of individual cytokines in disease progression using a validated mouse model of IGC, referred to as *gp130*^{Y757F} (1). We found that genetic restriction of IL-10 and IL-12 (p40) in did not alter disease progression in *gp130*^{Y757F} mice. Meanwhile, loss of IL-6, IL-17A and IL-23 (p19) expression delayed gastritis, but did not alter the progression of established gastric adenomas. In contrast, genetic inhibition of IL-18, or therapeutic inhibition of IL-22 significantly reduced gastric tumour burden. Our results suggest stage-specific roles for various cytokines during IGC progression, and identify IL-18 and IL-22 as potential therapeutic targets for this disease.

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A Two-Site Interaction Underpins TRIM25 Activation of the RIG-I Anti-Viral Response

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The **r**etinoic acid-**i**nducible **g**ene-**I** (**RIG-I**)-like receptors are an important family of cytosolic viral RNA sensors. RIG-I recognizes short 5'-triphosphate base-paired viral RNA and is a critical mediator of the innate immune response against viruses such as influenza A, HIV and hepatitis C. This response requires a carefully orchestrated interaction with **tri**partite **m**otif 25 (**TRIM25)**. The binding of viral RNA and the hydrolysis of ATP induce a conformational change in RIG-I, which releases its tandem CARD domains for interaction with the TRIM25 B30.2 domain. TRIM25 then functions as an E3 ubiquitin ligase to stabilize the formation of a RIG-I tetramer and facilitate RIG-I interaction with MAVS (mitochondrial anti-viral signalling) at the mitochondrial membrane. The net result is expression of the type I and III interferon (IFN)s and the establishment of an anti-viral state. We have previously solved the crystal structure of the mouse TRIM25 B30.2 domain and identified key residues that are critical for the interaction with the RIG-I CARDs (Site 1) (1). We have now identified a second CARD-binding site on the TRIM25 B30.2 domain that is revealed by removal of an N-terminal alpha-helix (mimicking TRIM25 dimerization) (Site 2). We provide biochemical evidence to suggest that both CARDs participate in this interaction and that a conformational change is required to expose a structurally-similar helix in CARD2. This suggests a model whereby the RIG-I CARDs interact with opposing sides of the TRIM25 B30.2 domain to form a higher order TRIM25/RIG-I complex that facilitates RIG-I tetramerisation. The characterization of a dual binding mode for the TRIM25 B30.2 domain is a first for the SPRY/B30.2 family and has broader implications. For instance, disease-causing mutations in the MEVF gene encoding Pyrin (TRIM20) map to its B30.2 domain in a region analogous to "Site 2".

(1) D'Cruz et al., Biochem J. 2013.

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IL-37 requires IL-18Rα and SIGIRR to carry out its multi-faceted anti-inflammatory program on innate signal transduction

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Aims. Both IL-37 and SIGIRR (TIR8, IL-1R8) are anti-inflammatory orphan IL-1 ligand and receptor family members. Association of recombinant IL-37 with IL-18 receptor alpha (IL-18Rα) was reported, but no biological activity was observed. Therefore, we investigated the interactions between IL-37, SIGIRR and IL-18Rα, and their effects on intracellular signaling.

Methods and results. In IL-37-transfected THP-1 macrophages, we observed an 83% reduction in IL-1β, but only 34% when endogenous SIGIRR was silenced. A similar attenuation of IL-37-induced anti-inflammation was demonstrated in LPS-stimulated human PBMC, and by IL-18Rα silencing. By immunoprecipitation and immunofluorescence, IL-37 associates with SIGIRR and IL-18Rα in A549 epithelial cells and RAW macrophages, both transfected with IL-37. Using proximity-ligation assays and FRET in PBMC, thus exploring interactions of the naturally occurring pairs IL-37:SIGIRR, IL-37:IL-18Rα and SIGIRR:IL-18Rα, we demonstrated each pair's sub-40nm co-localization. These cell surface-interactions were maximal 30min after LPS. Formation of the three-component ligand-receptor complex was confirmed by BRET and super-resolution microscopy. We next generated a strain of SIGIRR-KO mice transgenic for IL-37 (IL-37tg-SIGIRR-KO). As expected, IL-37tg mice were protected from endotoxic shock; however, IL-37tg-SIGIRR-KO mice were not: LPS induced severe hypothermia (trough 25°C) and acidosis (pH7.16) in wild-type, but not in IL-37tg mice (29°C, pH7.32). In IL-37tg-SIGIRR-KO mice, these protective effects were considerably weaker. Mechanistical exploration of the effects of the IL-37 receptor complex on intracellular signaling by proteomic and transcriptomic methods revealed that, via SIGIRR, IL-37 exploits the activities of anti-inflammatory mediators, e.g. Dok1, and inhibits not only NF-κB and MAPKs, but also mTor and Tak1.

Conclusions. IL-37 engages in a cell surface-complex with IL-18Rα and SIGIRR to limit the severity of inflammation by activating an intracellular anti-inflammatory program. As it blocks mTor, IL-37 may function as an endogenous rapamycin. SIGIRR utilizes an unexpected second mechanism of action in addition to the known decoy functions.

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IL-1 receptor antagonist (IL-1Ra) prevents murine bronchopulmonary dysplasia (BPD) induced by perinatal inflammation and hyperoxia.

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BPD is a common lung disease of premature infants, with devastating short- and long-term consequences. The pathogenesis of BPD is multi-factorial, but all triggers cause pulmonary inflammation. No therapy exists; thus, we investigated whether the anti-inflammatory IL-1Ra prevents murine BPD.

We precipitated BPD by perinatal inflammation (LPS injection to pregnant dams) and rearing pups in hyperoxia (65% or 85% O_2). Pups were treated daily with IL-1Ra or vehicle.

Vehicle-injected animals in both levels of hyperoxia developed a severe BPD-like lung disease (alveolar number and gas exchange area decreased by 60%, alveolar size increased 4-fold). IL-1Ra prevented this structural disintegration at 65%, but not 85% O_2. Hyperoxia depleted pulmonary immune cells by 67%; however, extant macrophages and dendritic cells were hyper-activated, with CD11b and GR1 highly expressed. IL-1Ra partially rescued the immune cell population in hyperoxia (doubling viable cells), reduced the percentage that were activated by 63%, and abolished the unexpected persistence of IL-1alpha and IL-1beta on d28 in hyperoxia/vehicle-treated lungs. On d3, perinatal inflammation and hyperoxia each triggered a distinct pulmonary immune response, with some pro-inflammatory mediators increasing 20-fold and some amenable to partial or complete reversal with IL-1Ra.

In summary, our analysis reveals a pivotal role for IL-1 in murine BPD and an involvement for MIP-1 and TREM-1. Because it effectively shields newborn mice from BPD, IL-1Ra emerges as a promising treatment for a currently irremediable disease that may potentially brighten the prognosis of the tiny preterm patients.

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Regulation of Th2 cell responses by Grail

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Aims: T helper (Th)-2 cells are the major players in allergic asthma; however, the mechanisms that control Th2-mediated inflammation are poorly understood. Our previous studies showed that E3 ubiquitin ligase, Grail, is associated with T cell tolerance. In addition to tolerant T cells, Grail mRNA is upregulated during normal T cell activation, suggesting that Grail function might not be restricted to T cell tolerance. In fact, we determined a significant expression of Grail in Th2 cells compared to other T helper subsets, suggesting the role of Grail in controlling Th2 programming.

Methods: The regulation of Grail expression in Th2 cells was assessed by quantitative Real-Time PCR, Chromatin Immunoprecipitation (ChIP) assay, luciferase reporter assay and retroviral transduction. Wild-type and Grail deficient T cells were utilized for T helper cell differentiation assay to evaluate the role of Grail in Th2 responses *in vitro.* For *in vivo* studies wild-type and Grail deficient mice were subjected to Ova immunization and asthma model. Immunoblot analysis and ubiquitination assay were performed to explore the mechanism whereby Grail controls Stat6 expression in Th2 cells.

Results: In the current study, we found that Grail is selectively induced upon IL-4 stimulation in a time dependent manner and depends on Th2-specific factors Stat6 and Gata3 that bind to and transactivate the Grail promoter. Grail deficiency in T cells leads to enhanced Th2 development *in vitro* and *in vivo;* Grail deficient mice are more susceptible to allergic asthma. Mechanistically, the enhanced effector function of Grail-deficient Th2 cells is mediated by increased expression of Stat6 and IL-4 receptor α-chain. Grail interacts with Stat6 and targets it for ubiquitination and degradation.

Conclusions: Our results suggest an important link between the Th2 specific expression of Grail and its role in control of Th2 developmentand Th2-mediated pathogenesis and immunity through a negative feedback loop.

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$CD141^+$ DC and IFN-λ production in the peripheral blood of a young cohort of injecting drug users (IDUs) with chronic HCV infection

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Polymorphisms within and upstream of Interferon-lambda (IFN-λ) gene 3 (IFN-λ3, *IL-28B gene*) are strongly associated with the clearance of Hepatitis C virus (HCV) in chronically infected patients treated with interferon-alpha (IFN-α). Human blood and liver dendritic cells (DC) expressing CD141 and Clec9A are known to produce IFN-λ in response to dsRNA and in particular, to HCV. In this study we enumerated the $CD141^+$ DC in the peripheral blood of a young cohort of injecting drug users (IDUs), with chronic HCV infection, not undergoing treatment, and assayed their ability to produce IFN-λ. DCs from chronic HCV-infected IDUs expressing the protective IL-28B genotype (rs8099917, TT and rs12979860, CC) were compared to those expressing IL-28B rs8099917, GT or GG and rs12979860 CT or TT. Regardless of the IFN-λ genotype, IFN-λ production was reduced from the total PBMCs of chronically infected HCV donors. This reduction was commensurate with a common loss of $CD141^+$ DC from the blood of all chronically infected individuals. However, the production of IFN-λ was increased at least 2-fold by the pre-treatment of PBMC with IFN-α in healthy subjects and in

chronically infected patients, regardless of genotype. Thus those cells most efficient at IFN-λ production, $CD141^+$ DC, although substantially reduced in blood in the cohort we have examined, respond to IFN-α with increased IFN-λ production, regardless of the IL-28B genotype.

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Loss of NF-κB1 Promotes Gastric Cancer through Cytokine Driven Chronic Inflammation

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Gastric cancer (GC) is the second most common cause of cancer-associated mortality. It has a poor prognosis, with new treatment and prevention strategies focused on impeding the role of chronic inflammation in disease progression. We present a new mouse model of invasive GC based on the loss of the NF-κB family member, NF-κB1. We show that loss of NF-κB1 results in the induction of inflammation (gastritis), gastric atrophy and a series of malignant changes that culminate in invasive gastric adeno-carcinoma. Importantly, disease manifestation occurs independently of *H. pylori* infection and even in the complete absence of commensal microbiota. This is the first GC model that recapitulates all stages of invasive human disease and pertinently, reduced *nfκb1* promoter activity due to polymorphisms/mutations are associated with a significant fraction of human GC in North East Asia

We have generated bone marrow chimeric mice to demonstrate that loss of NF-κB1 in both inflammatory and gastric epithelial cells (GECs) is required for the development of GC. We also demonstrate that nuclear extracts from gastric epithelial cells of wt mice predominantly contained NF-κB1 homodimer complexes, which were of course absent from the cells of *nfkb1*$^{-/-}$mice. NF-κB1 homo-dimers are known to associate with Histone deacetylase-1 (HDAC-1) to function as a repressor of NF-κB driven activation of several inflammatory genes within T cells and macrophages. Pertinently we found elevated levels of several pro-inflammatory cytokines (e.g. IL-1β, IL-6, IL-8, IL-17 and TNF-α, all known NF-κB targets) in the sera and GC lesions of *nfkb1*$^{-/-}$ mice. RNAseq analysis of the lymphoid, myeloid and epithelial cells isolated from the stomachs of young *nfkb1*$^{-/-}$mice identified myeloid cells as the dominant source of these cytokines. Collectively, our findings demonstrate that NF-κB1 functions as a tumour suppressor in GC and provides anovel model to test emerging therapeutic strategies for gastric cancer treatment.

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Sidt2 is required for innate immunity to extracellular dsRNA

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During viral infection, extracellular double stranded RNA (dsRNA) acts as a potent trigger of innate immunity via the production of type I interferons (IFNs). The detection of extracellular dsRNA involves different host cell sensors such as Toll-like receptor 3 (TLR3) and the RIG-I-like receptors (RLRs), RIG-I and MDA-5. TLR3 is located within endo-lysosomes, and is therefore well situated to detect dsRNA that has been taken up from the environment. Internalized dsRNA also activates the cytoplasmic RLRs, implying the existence of a mechanism to transport dsRNA across the endosomal membrane. Interestingly, the RLRs are functionally dominant over TLR-3 in the response to extracellular dsRNA and are critical for immunity to multiple viruses, but how dsRNA escapes from the endosome is unknown. Here we report that Sidt2, a mammalian ortholog of the *C. elegans* SID-1 dsRNA transporter, is present within the endosomal compartment and co-localizes with internalized poly(I:C), a synthetic dsRNA. Moreover, we generated Sidt2-deficient mice and found that loss of Sidt2 impairs the production of type I IFNs in response to extracellular poly(I:C) both *in vitro* and *in vivo*. This impairment in IFN production was not apparent when testing poly(A:U), a dsRNA ligand that activates only TLR-3 (and not the RLRs), and could be circumvented by delivering poly(I:C) with transfection reagents designed to facilitate endosomal escape. Together, these observations suggested a role for Sidt2 in transporting dsRNA from the endosome into the cytoplasm. In support of this, we subsequently found that loss of Sidt2 does not affect dsRNA internalization, but instead results in the accumulation of poly(I:C) within the endosomal compartment and a concomitant decrease in signaling events downstream of the RLRs. Finally, we show that Sidt2-deficient mice produce less type I IFNs and have increased mortality in response to infection with herpes simplex virus. Taken together, our findings demonstrate a key role for Sidt2 in the endosomal escape of dsRNA and the subsequent induction of innate immunity.

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Possible role of the NLRP3 inflammasome in the development of human atherosclerosis and myocardial infarction.

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Abstract

Objective- The NLRP3 inflammasome is an IL-1β and IL-18 cytokine processing complex that is activated in inflammatory conditions. The role of the NLRP3 inflammasome in the pathogenesis of atherosclerosis and myocardial infarction (MI) is not fully understood.

Approach and Results- Atherosclerotic plaques were analyzed for transcripts of the NLRP3 inflammasome, and for IL-1β release. The Swedish FIA cohort consisting of DNA from 555 MI patients and 1016 healthy individuals was used to determine the frequency of 5 SNPs from the downstream regulatory region of *NLRP3*. Expression of *NLRP3*, *ASC, CASP1, IL1B,* and *IL18* mRNA was significantly increased in atherosclerotic plaques compared to normal arteries. The expression of *NLRP3* mRNA was significantly higher in plaques of symptomatic patients when compared to asymptomatic ones. CD68 positive macrophages were observed in the same areas of atherosclerotic lesions as NLRP3 and ASC expression. Cholesterol crystals and ATP induced IL-1β release from LPS-primed human atherosclerotic lesion plaques. The minor alleles of the variants rs4266924, rs6672995 and rs10733113 were associated with *NLRP3* mRNA levels in PBMCs and plaques but not with the risk of MI.

Conclusions- Our results indicate a possible role of the NLRP3 inflammasomeand its genetic variantsin the pathogenesis of atherosclerosis.

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A type III effector antagonises death receptor signaling during bacterial gut infection

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Successful infection by enteric bacterial pathogens depends on the ability of the bacteria to colonise the gut, replicate in host tissues and disseminate to other hosts. Enteropathogenic *E. coli* (EPEC) is an attaching an effacing (A/E) pathogen that adheres intimately to the apical surface of host enterocytes and causes acute gastroenteritis in humans. Like other enteric bacterial pathogens such as *Salmonella* and *Shigella*, EPEC utilises a type III secretion system (T3SS) to deliver multiple effector proteins directly into host cells where they subvert various cellular processes including apoptosis and inflammatory signaling. Given their central role in the pathogenesis of many bacterial infections, elucidating the biochemical function of T3SS effectors is fundamental to understanding host-pathogen interactions. In this study, we found that the T3SS effector, NleB interrupted host extrinsic apoptotic signaling via the death receptors, TNFR1 and FAS. NleB expressed ectopically or delivered by the T3SS bound to the death domain (DD) proteins FADD, RIPK1 and TRADD in a DD dependent manner thereby preventing FasL and TNF-induced caspase-8 activation and cell death. Furthermore, NleB inhibited the formation of the death inducing signaling complex (DISC) during EPEC infection. This activity was dependent on the N-GlcNAc transferase activity of NleB1, which specifically modified Arg117 in the death domain of FADD. The importance of the death receptor apoptotic pathway to host defence was demonstrated using mice deficient in the FAS signalling pathway, which showed delayed clearance of the EPEC-like mouse pathogen *Citrobacter rodentium* and reversion to virulence of an *nleB* mutant. The activity of NleB suggests that EPEC and other attaching and effacing (A/E) pathogens antagonise extrinsic cell death pathways to prevent apoptosis of infected cells, thereby interfering with a major antimicrobial host response.

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The Pyrimidine Synthesis Modulon: A Stabilised Oscillatory Network Governed by Interferon Signalling.

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Background

Living cells simultaneously perform multitudes of precise, stringently regulated functions to co-ordinate interactions of the ~30,000 node gene-protein and protein-protein networks encoded by the genome, a feat achieved with remarkable efficiency (<8 pW per cell), using imprecise functional units with a large amount of internal redundancy and feedback. At all levels, noisy, overlapping and often contradictory input signals are integrated and processed to produce stable regulated outputs. Recognition of this striking resemblance to computing networks has inspired the exploration of a new and expanding area of biological electronics, which aims to use electrical engineering concepts to emulate complex biological processes.

Defining characteristics of complex biological networks, which have resulted from millions of years of evolutionary experiment, are self-organization, robustness and plasticity. They are stable to fluctuations (noise) in "expected" input, but prone to failure when challenged by unusually large or prolonged "unexpected" input, to which their response is a self-organized transition to a new meta-stable state, or failing to achieve that, to extinction. The transition phase is typically scale-free and describable by simple power laws of the form $y = ax^{b.}$ The network becomes refractory to further input and ultimately fails or shuts down if it lacks a required input, or if the amplitude or frequency of an input signal exceeds a critical threshold.

Modeling and Results

We devised an electronic analogue of the evolutionarily ancient biochemical pathway for de novo pyrimidine synthesis, which requires six enzymatic reactions, the fourth being coupled to mitochondrial respiration. Rate-limiting steps are directly or indirectly governed by interferon signaling which regulates flux through the pathway via control of gene expression and gene product stability. The model predicts that intermittent pulses of interferon stimulation are required to de-repress the pathway and that over-stimulation will cause mitochondrial "burn-out", consistent with many published observations.

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Interferon-β regulates Th17 polarization and dendritic cell migration in EAE

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To investigate the mechansim(s) of action of IFN-β in suppressing immune and inflammatory processes in multiple sclerosis (MS), we employ a myelin oligodendrocyte glycoprotein (MOG) peptide-induced experimental autoimmune encephalomyelitis (EAE) model of MS in IFN-$\beta^{+/+}$ and IFN-$\beta^{-/-}$ mice. IFN-$\beta^{-/-}$ mice exhibit earlier onset and more rapid progression of neurologic impairment compared with IFN-$\beta^{+/+}$mice. We provide MRI evidence for a rapid influx of cells into the brains of IFN-$\beta^{-/-}$ mice with EAE: increased ventricle volume compared with IFN-$\beta^{+/+}$ mice. Th17 cell numbers in the inguinal lymph nodes and in the CNS of IFN-$\beta^{-/-}$ mice with EAE are higher than for IFN-$\beta^{+/+}$ mice, yet Treg numbers are lower. Anti-CD3/anti-CD28 antibody stimulation of whole splenocytes or CD4+ T cells from IFN-$\beta^{-/-}$ mice results in production of IL-17A, whereas identical stimulation of cells from IFN-$\beta^{+/+}$ mice fails to increase IL-17A production. IFN-$\beta^{-/-}$ CD4+ T cells express higher levels of IRF-4 following anti-CD3/anti-CD28 antibody activation and increased expression of CCR6, IL-23R, IL-6R and CXCR4. Moreover, CD4+ T cells from IFN-$\beta^{-/-}$ mice exhibit a Th17 primed transcriptome. Increased Th17 cell polarization during EAE may be driven by dendritic cells (DCs), as DCs derived from IFN-$\beta^{-/-}$ mice induce greater proliferation of T cells derived from either IFN-$\beta^{+/+}$ or IFN-$\beta^{-/-}$ mice, compared with DCs derived from IFN-$\beta^{+/+}$mice. Additionally, IFN-$\beta^{-/-}$ DCs secrete cytokines associated with Th17 rather than Treg polarization. Adoptive transfer of MOG peptide-primed IFN-$\beta^{-/-}$ DCs into IFN-$\beta^{+/+}$ and IFN-$\beta^{-/-}$ mice with EAE resulted in their rapid migration into the brains and spinal cords (CNS) of recipient mice, visualized by fluorescence imaging. FACS analysis of DCs isolated from naïve IFN-$\beta^{-/-}$ mice revealed increased expression CCR7, CXCR4, and MHCII upon TLR4 activation compared to IFN-$\beta^{+/+}$ DCs. Taken together, our data indicate immunoregulatory roles for IFN-β in suppression of Th17 cells and in limiting the activation and trafficking of DCs during EAE.

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Targeting chemokines: Pathogens can, why can't we?

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Chemoattractant cytokines, or chemokines, are the largest sub-family of cytokines. About 50 distinct chemokines have been identified in humans. Their principal role is to stimulate the directional migration of leukocytes, which they achieve through activation of their receptors, following immobilsation on cell surface glycosaminoglycans (GAGs). Chemokine receptors belong to the G protein coupled 7-transmembrane receptor family, and hence their identification brought great promise to the pharmaceutical industry, since this receptor class is the target for a large percentage of marketed drugs. Unfortunately, the development of potent and efficacious inhibitors of chemokine receptors has not lived up to the early expectations. Several approaches to targeting this system will be described here, which have been instrumental in establishing paradigms in chemokine biology. Whilst drug discovery programs have not yet elucidated how to make successful drugs targeting the chemokine system, it is now known that certain parasites have evolved anti-chemokine strategies in order to remain undetected by their hosts. What can we learn from them?

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The Interleukin-11 signaling cascade: a promising target for cancer treatment

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Interleukin (IL)-11 is a pleiotropic member of the IL-6 family of cytokines. Despite its discovery over 20 years ago, characterisation of the function of IL-11 has been over shadowed by its famous sibling IL-6. We have recently solved the first crystal structure of human IL-11[1], which signals through specific a-subunit receptors and transmembrane b-subunit GP130 receptors. The formation of the IL-11 signaling complex results in the recruitment of JAKs and subsequent activation of the pro-tumourigenic transcription factor, STAT3. Constitutive STAT3 activation is a hallmark of gastrointestinal cancers, and associated with poor prognosis. We have shown that elevated IL-11 expression is linked to high STAT3 activation in human gastrointestinal cancers[2].

In order to compare the requirement of IL-6 and IL-11 signaling for gastrointestinal tumour progression we have coupled *il6*$^{-/-}$ mice, which lack both classic and trans-signaling, and *il11ra*$^{-/-}$ mice with a unique suite of gastric, inflammation-associated and sporadic colon cancer mouse models. Our results demonstrate that while both cytokines contribute to tumour progression, IL-11 has a dominant role in the development of gastrointestinal cancers[2]. We show that the production of IL-11 by cells within the tumour microenvironment promotes STAT3 activation in non-haematopoietic cells, which triggers the progression of tumours.

We have generated novel IL-11 signaling antagonists and demonstrate in numerous endogenous mouse and human xenograft models that therapeutic targeting of IL-11 signaling reduces inflammation in the tumour microenvironment and inhibits the progression, invasion and metastasis of tumour cells. Importantly, inhibition of IL-11 does not result in many of the side-affects commonly associated with inhibition of other components of the JAK/STAT pathway. Our results highlight an unappreciated hierarchy between IL-6 and IL-11, and suggest that understanding the cross-talk between cytokines and the tumour microenvironment will prove critical to the design and success of anti-cytokine reagents as future cancer therapies.

1. Putoczki et al. (2014) Acta Cryst. D70, doi:10.1107/S1399004714012267
2. Putoczki et al. (2013) Cancer Cell, 24(2):257

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Novel insights into interferon regulated expression of the *c-MYC* gene in human cancer

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We have mined datasets from microarray analyses of gene expression from large numbers of cell lines of particular human cancer types to determine relationships between levels of particular transcription factors and their impact on target gene expression. Using a set of defined conditions and by excluding confounder data points, this enabled us to then clearly observe significant correlations existing between sets of transcription factors and their target genes (1). These results were consistently confirmed and validated using other independent methods for analysis of transcription factor binding, including chromosomal immunoprecipitation, RT-PCR, 5'-RACE, gene reporter, knockdown of gene expression or gel shift assays. We have applied our microarray analysis of several genes to provide novel links not previously recognised including some genes involved in, or regulated by the interferon response. For example, IFNg activation of STAT5 signaling was shown to bind and activate MYC gene expression in human colon cancer. We propose that our approaches may be more widely applicable, as a general method for refining and determining novel relationships existing between different transcription factors and the genes that they regulate.

1) REST negatively and ISGF3 positively regulate the human STAT1 gene in melanoma. Amalraj J, Cutler SJ, Ghazawi I, Boyle GM, Ralph SJ. Mol Cancer Ther. 2013 Jul;12(7):1288-98.

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Exploiting the type-1 Interferon pathway in the treatment and prediction of breast cancer metastasis

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Metastatic disease accounts for almost all breast cancer related deaths. The immune mechanisms that dictate this spread to key sites such as the lung and bone are only now emerging. Our recent work emphasizes the role of the host immune system in restricting breast cancer metastasis, and that the balance between pro- and anti-metastatic immune responses is a critical determinant of metastatic progression. Previous studies in our laboratory using the syngeneic 4T1.2 breast cancer model have shown that breast tumour cells suppress their intrinsic type-1 interferon (IFN) pathway, and hence type-1 IFN secretion, in order to evade host immunosurveillance and metastasise to bone. These findings are supported by a significant correlation between patients with low primary tumour expression of this pathway and increased risk of bone relapse. Taken together, this highlights the type-1 IFN pathway as an attractive target for novel anti-metastatic agents.

In order to stimulate a systemic IFN response, we have utilized toll-like receptor (TLR) agonists and are currently evaluating their potential as anti-metastatic agents. Treatment of tumour bearing animals with the double-stranded RNA mimetic, Poly(I:C), potently suppressed metastasis to bone and lung and this was associated with enhanced activation of the NK cell compartment. These effects were superior to that observed by direct administration of IFN alpha. In addition to a closer examination of the cellular and molecular mechanisms that underpin the efficacy of these anti-metastatic agents, we are investigating the relevance of combining such therapies with common chemotherapeutics.

Finally, we have uncovered that the expression of a novel set of interferon-regulated genes in a patient's primary tumour is able to accurately predict metastasis-free survival. Our data suggest that utilisation of these biomarkers and IFN-based therapies may have a significant clinical impact in reducing rates of metastatic breast cancer.

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Changes in microglial proliferation rate in GFAP-IL10Tg mice following perforant pathway transection

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Activation and proliferation of microglia are key events in the response of CNS against inflammatory diseases. Cytokines play an important role in the control of these processes. Although production of Interleukin-10 (IL-10) by activated astrocytes and microglia has been demonstrated after different CNS injuries, the specific role played by IL-10 in modulating microglial proliferation remains unclear.

Aims. Hence, the objective of this study was to evaluate the effects of local IL-10 production on microglial proliferation in normal conditions and associated to axonal anterograde degeneration.

Methods.For this purpose, unilateral perforant pathway transection (PPT) was performed in transgenic mice with astrocyte-targeted production of IL-10 (GFAP-IL10Tg) and their corresponding wild types (WT) littermates. At 2, 3 and 7 days post-lesion (dpl), animals were intracardially perfused with 4% of paraformaldehyde and brains processed for immunohistochemical studies. Microglial cell number was quantified using the myeloid-specific transcription factor PU.1 and microglial proliferation was evaluated using the mitotic marker Phospho Histone H3 (pH3).

Results. Our results showed a significant increase in the number of microglial cells in GFAP-IL10Tg mice compared with WT, in both non-lesioned and lesioned animals. The peak of proliferation after PPT lesion was delayed in GFAP-IL10Tg mice showing the maximum number of pH3+ cells at 3dpl, while in WT the peak was observed at 2dpl. Moreover, the number of proliferating cells was lower in Tg mice at the different time points analyzed.

Conclusions. In conclusion, this study demonstrated that local production of IL-10 in the CNS modifies the microglial proliferation pattern associated to PPT. Further studies are necessary to understand the mechanisms involved in microglial proliferation in this paradigm.

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Role of *Bacillus infantis* NRRL B-14911 in the mediation of autoimmune myocarditis

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Environmental microbes can trigger autoimmune responses through multiple pathways, and one mechanism is antigenic mimicry. We recently reported that a mimicry epitope for cardiac myosin heavy chain (Myhc)-α 334-352, designated BAC 25-40, from *Bacillus* sp. NRRL B-14911 can induce myocarditis in A/J mice by generating cross-reactive T cells for cardiac myosin. By 16S rRNA phylogenetic analysis, we determined the species to be *Bacillus infantis* NRRL B-14911. Since this microbe was originally isolated from ocean water, we sought to evaluate the expression of N-carbomoyl L-amino acid amidohydrolase (NCAA), the source protein for BAC 25-40, under various growth conditions. Unexpectedly, NCAA was found to be expressed in low-salt conditions when bacteria were grown at 37^{o}C, but not in high salt-containing marine medium, suggesting that the conditions of mammalian systems may favor the expression of NCAA in this bacterium. Furthermore, we demonstrate that RAW 264.7 cells can phagocytose the bacteria and produce reactive oxygen- and nitrogen intermediaries to levels comparable to lipopolysaccharide. Likewise, primary macrophages obtained from A/J mice can produce proinflammatory cytokines, notably, tumor necrosis factor-α, and interleukin-6 in response to bacterial exposure. More importantly, A/J mice infected with *B. infantis* show the generation of CD4 T cells that cross-react with both BAC 25-40, and Myhc-α 334-352, as evaluated by major histocompatibility complex class II dextramer staining. Together, the data suggest that *B. infantis*may have a potential to induce myocarditis. The establishment of this disease model enables the study of the immune mechanisms of myocardial injuries of bacterial origin.

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Serum interleukin 38 is associated with disease severity and organ involvement in systemic lupus erythematosus.

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Aims: Interleukin (IL)-38 is a member of the IL-1 cytokine family. Although knowledge on IL-38 is sparse, IL-38 gene polymorphisms have been associated with inflammatory diseases, and recombinant IL-38 inhibits IL-17 and IL-22. Intriguingly, both IL-17 and IL-22 play a role in systemic lupus erythematosus (SLE), a severe autoimmune disease. We therefore set out to investigate IL-38 in SLE.

Methods: IL-38 and IL-10 were quantified by ELISA in serum from SLE patients at admission (baseline, 142 patients) and two subsequent clinic visits (115/142). SLE disease activity index-2000 (SLEDAI-2k) and treatment details were recorded. Serum of 28 healthy donors served as controls. Moreover, we silenced IL-38 in PBMC from healthy volunteers by siRNA (siIL-38) and measured IL-6 by ELISA.

Results: IL-38 (63-5928pg/ml) was detectable in 59 out of 345 patient samples (17.1%) and IL-38 abundance in SLE samples was significantly higher than in healthy controls (p=0.003). Moreover, patients with active disease (SLEDAI-2k≥4) had 18.4-fold higher serum IL-38 than patients with non-active disease (p=0.044). Importantly, IL-38 was associated with increased risk of renal (RR=1.6, 95% CI 1.2-2.2, p=0.028) and CNS disease (RR=2.2, 95% CI 1.2-4.3, p=0.035), and when IL-38 was detectable at baseline, patients had a 1.7-fold increased risk of developing persistently active disease (RR=1.7, 95% CI 1.3-1.9, p=0.01).Remarkably, siIL-38-treated PBMC from healthy volunteers produced up to 30-fold more IL-6 than control-transfected cells when stimulated with CpG or imiquimod (n=3). Similarly, in SLE patients, the anti-inflammatory cytokine IL-10 was 5-fold higher when IL-38 was detectable, suggesting that IL-38 may be protective in SLE.

Conclusions: This study is the first to demonstrate the anti-inflammatory activity of endogenous IL-38. We moreover reveal IL-38 as the first mediator that exhibits an association with markers of SLE disease activity and renal and CNS involvement; IL-38 may thus become the highly sought-after first biomarker for SLE.

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COLONY-STIMULATING FACTOR-1 AND TUMOR NECROSIS FACTOR- α IN ARTHRTIC PAIN

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Colony-stimulating factor-1 (CSF-1) is a key cytokine that has been linked to the development of arthritis in animal models. Its role in neuropathic pain has been studied by several laboratories; however, its potential involvement in arthritic pain has not received attention. Tumor necrosis factor-alpha (TNF-α) is a proinflammatory cytokine which has been implicated in the pathogenesis and progression of RA, as well as the generation of pain. Thus, the objectives of the current study were as follows: 1) to examine the role of CSF-1 in arthritic pain using an acute monoarticular methylated bovine serum albumin (mBSA)-induced arthritis model; 2) to compare the TNF-mediated pain to the CSF-1-induced pain; and 3) to elucidate the mechanisms by which CSF-1 can mediate arthritic pain. Results from this study showed that systemic administration of CSF-1 can induce pain in the mBSA model. Preliminary data showed that early CSF-1-induced pain was not reversed following indomethacin administration, suggesting that CSF-1-mediated pain is cyclooxygenase-independent. Systemic administration of TNF-α could also induce pain in the novel mBSA model. Unlike CSF-1, early TNF-mediated pain was abolished following the administration of indomethacin. This indicated that the TNF-mediated pain is mediated through the production of eicosanoids. Thus, both cytokines were able to induce pain; however, each cytokine mediated arthritic pain via a different pathway. Further insight into the mechanisms by which CSF-1 mediates pain could determine its contribution to arthritic pain and may provide novel therapeutic strategies for joint pain.

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The RIG-I like receptor pathway: New insights in the subcellular localization of the components

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Aims: The RIG-I like receptor (RLR) signaling is an essential pathway for the initiation of host IFN-mediated antiviral responses. The activation of this pathway is complex and well characterized, but most of the spatio-temporal events, and the subcellular localization where the essential proteins interact, are still under interrogation.

Methods: The interaction of key partners in the RLR pathway was examined by Bimolecular Fluorescence Complementation (BiFC). This technique allowed us to isolate, visualize and analyze protein-protein interactions in living cells.

Results: We have defined spatially in the cell different complexes formed between RIG-I, TRIM25 and MAVS, in the presence and absence of viral IFN antagonistic proteins. Dimers and/or multimers of RIG-I, TRIM25 and MAVS localize into different compartments in the cytoplasm of the cell. The impact of several viral proteins in the RLR pathway was investigated through BiFC: HCV NS3/4A, IAV NS1, NiV V and SFTSV NSs proteins. In order to inhibit the IFN response, these viral proteins target specific complexes and interact in defined areas in the host cell.

Conclusion: For the first time, the interactions among proteins formed along the RLR pathway have been tracked and visualized in living cells. We found different complexes localized in concrete subcellular compartments not known before. This method has provided us new information to further understand the mechanisms that regulate the specific distributions of these complexes in the activation of the RLR pathway.

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Determining the source of Type III IFNs (IFNλs) in vivo using a novel IFNλ reporter mouse model

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Similar to Type I IFNs (IFNα/β), Type III IFNs (IFNλ1,2,3,4) exert antiviral and antiproliferative effects through a JAK-STAT mediated pathway that activates ISGF3 (STAT1/STAT2/IRF9) and consequently induces the expression of interferon-stimulated genes (ISGs). IFNλs signal through a receptor complex distinct from that of IFNα/β, composed of IL10R2 and IFNλR1, which appears to be preferentially expressed on epithelial cells and certain immune cell subsets. Accumulating evidence suggests that IFNλs play a major role in protecting the host from viruses targeting the mucosal surfaces of the respiratory and gastrointestinal tracts as well as the liver. Like type I IFNs, IFN-λs are produced by various cell types in response to virus infection or TLR ligand stimulation, via mechanisms that appear to be similar but not identical. The major cell populations contributing to the production of IFNλs in vivo, in response to specific viral pathogens, remain unclear. To gain a clearer picture of the source(s) of type III IFNs in the course of virus infection, we have generated a novel IFNλ reporter mouse (IFNλ2$^{+/eGFP}$), in which one allele of the IFN-λ2 coding sequence has been replaced with *eGFP* by homologous recombination, while maintaining the IFN-λ2 promoter regions and UTRs intact. Thus, any cell from this mouse that produces IFN-λ2 will also express *eGFP*, allowing us to identify and isolate IFNλ-producing cells present within tissues as an infection progresses. Preliminary studies using FLT3L and GMSCF cultured, bone marrow-derived dendritic cells from these mice show that a subpopulation of dendritic cells produce IFN-λ in response to Newcastle disease virus infection, and characterization of these cells, including their role as type III IFN producers, is now underway. It is anticipated that the availability of the IFNλ2$^{+/eGFP}$ reporter mouse will help in the identification of IFN-λ producing cells in response to various viral infections *in vivo* and ultimately aid in understanding the distinct role IFNλs play in antiviral immunity.

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Type I IFNs display differential antiviral potency in cells with JAK-STAT signaling deficiencies

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A multitude of type I IFNs signal through a shared heterodimeric receptor complex. The binding of any one leads to the expression of hundreds of IFN-stimulated genes that render cells highly resistant to virus infection. Type I IFNs display a marked difference in their receptor binding affinities, and at varying concentrations, give rise to quantifiable differences in gene induction; however, whether or not type I IFNs can give rise to gene induction profiles that are qualitatively distinct from one another and whether diverse classes of viruses are differentially susceptible to their antiviral properties remain unanswered questions. Here, we present data on the characterization of several type I IFNs and synthetic IFN variants with respect to their effect on gene expression and their ability to restrict diverse viruses.

In physiological settings, biologically important differences in signaling by type I IFNs may arise from variation in the intrinsic abundance and availability of signaling components. We find that in the presence of high IFN concentrations, *STAT1*$^{-/-}$ cells are capable of launching an effective antiviral response; however, the IFNs tested are indistinguishable with respect to antiviral potency. In stark contrast, in *TYK2*$^{-/-}$ cells, differences in the antiviral potencies are substantially exaggerated. Despite the large variation in potencies among the IFNs in different cellular environments, the relative differences are consistent across viruses. These results provide additional support for a model where a low level of type I IFN signaling is sufficient to elicit a potent pan-antiviral state and suggest that variations in the level available signaling components may be important for distinguishing type I IFN signaling and fine tuning the IFN response in biologically relevant cellular environments. In ongoing work, we aim to determine whether distinct gene expression profiles are induced by type I IFNs in cells with deficiencies in JAK-STAT signaling components.

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IRF6: a key regulator of oral epithelial cell responses to the human pathogen *Porphyromonas gingivalis*

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Porphyromonas gingivalis is a major pathogen in periodontitis, a prevalent chronic inflammatory disease of the tissues supporting the teeth and which also affects systemic health. *P. gingivalis* forms microcolonies in the superficial layers of the polymicrobial biofilm accreted to the teeth such that it can dysregulate the host immune response to the biofilm resulting in dysbiosis and chronic inflammation. In addition to functioning as a physical barrier to prevent infection, the epithelial cells abutting the teeth utilize toll-like receptors (TLRs) to detect and respond to pathogens. The interferon regulatory factor (IRF) transcription factors are key components of TLR signaling as they impart specificity to downstream responses. In contrast to the other IRFs, IRF6 expression appears to be restricted to epithelial cells, including those of the oral epithelium. Here, we investigated a role for IRF6 in regulating the responses of oral epithelial cells to *P. gingivalis*. The novel IL-1 family cytokine IL-36γ was shown for the first time to be induced in response to *P. gingivalis*infection. IL-36γ expression was also induced by a TLR2 agonist. Gene silencing experiments and gene promoter assays demonstrated that IRF6 functions downstream of IRAK1 to mediate IL-36γ expression. IL-36γ exerts potent inflammatory effects on dendritic cells and macrophages, such as induction of the Th17 chemokine CCL20. Significantly, the ability of IL-17 to up-regulate IL-36γ expression in oral epithelial cells may further amplify the *P. gingivalis*-triggered IL-36γ response. IRF6, which we have shown to also be an important regulator of oral epithelial cell differentiation, is transcriptionally up-regulated in response to *P. gingivalis* infection. Collectively, our data therefore indicate that IRF6 functions as an important signaling nexus for pathways regulating both the inflammatory and barrier functions of oral epithelial cells.

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Changes in RNA expression pattern in neutrophils of patients with Indian Visceral leishmaniasis

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Aim- Visceral leishmaniasis (VL), is a disease caused by *Leishmania donovani* in India. Neutrophils act as first line of defense against invading microorganisms and their interaction with other immune cells and environmental signals during disease can influence overall immune response. We observed HLA DR expressing low density "neutrophil like cells" which stain CD66b in PBMC and whole blood samples in active VL subjects by flowcytometry previously. In continuation to our previous study we also tried to look at changes in mRNA expression in active VL pre and post treatment (paired) groups and endemic healthy controls (EHC) to understand the contribution of these cells during active disease.

Methods –

Patient Selection:All patients presented with VL symptoms at KAMRC, Muzaffarpur, India were confirmed to be VL positive by detection of amastigotes in splenic aspirates and/or by positive rk39 test and EHCs were enrolled from local area.

Gene Expression: Human neutrophils were isolated using Militenyi CD66abce microbead kit to get highly pure neutrophils (> 97%). cDNA was prepared using High capacity cDNA archive kit. Real time PCR was performed using ABI-Prism 7500 using specific Sybr Green primers for mRNA of interest.

Results- Differences were observed in expression of cytokine and chemokine mRNAs in the same subjects (paired) in pre and post treatment(n=8). Some, but not all differences mirrored profiles in EHCs (n=5).

IFN γ,IL10, were significantly up regulated during Active VL while cytokine IL1β and βdefensins and chemokines CXCL8,CCR4,CCR5,CXCR1,CXCL9 were found to be down regulated. We looked at some important markers like MPO, PD1, Arginase 1, HLA DR which were found to be upregulated during active VL. These markers were studied to address functional competency of neutrophils during active VL at mRNA levels.

Conclusions- Neutrophils from subjects with active disease differ from the healthy subjects and from the same subjects after drug induced cure showing neutrophils during active disease are capable of extensive changes in gene expression and can influence development of overall immune response.

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Interleukin-1 receptor type 2 suppresses collagen-induced arthritis by inhibiting interleukin-1 signal on macrophages

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Background and aims

Interleukin-1 plays important roles in host defense against infection and inflammatory diseases. IL-1 receptor type 1 is the receptor for IL-1, and IL-1 receptor type 2 (IL-1R2) is suggested to be a decoy receptor, because it lacks signal transducing TIR domain in the cytoplasmic part. The roles of IL-1R2 in health and diseases, however, remain largely unknown. Rheumatoid arthritis (RA) is a typical autoimmune disease affecting approximately 1% of people worldwide independently of races. Because the concentration of IL-1R2 protein is increased in the synovial fluid and plasma in RA patients, the involvement of IL-1R2 in the pathogenesis is suggested. In this report, we demonstrated the roles of IL-1R2 in the development of arthritis.

Methods and results

We generated EGFP-knock-in *Il1r2*$^{-/-}$ mice and showed that *Il1r2*$^{-/-}$ mice were highly susceptible to collagen-induced arthritis, an animal model for rheumatoid arthritis. *Il1r2* was highly expressed in neutrophils but only low in other cells including monocytes and macrophages. Antibody production and T cell responses against type II collagen were normal in *Il1r2*$^{-/-}$ mice. In spite of the high expression in neutrophils, no effects of *Il1r2* deficiency were observed in neutrophils. On the other hand, we found that the production of inflammatory mediators in response to IL-1 was greatly enhanced in *Il1r2*$^{-/-}$ macrophages.

Conclusion

IL-1R2 is an important regulator of arthritis by acting specifically on macrophages as a decoy receptor for IL-1.

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Local overproduction of IL10 in astrocyte targeted IL10Tg mice modulates microglial response by modulating regulatory receptors TREM2 and CD200R following perforant pathway transection.

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Introduction: Interleukin-10 (IL-10) is a general anti-inflammatory cytokine that has been demonstrated in activated astrocytes and microglia after CNS injury but its specific role still remains ambiguous. One of the endogenous mechanisms regulating inflammation following brain injury is the expression of modulator and/or inhibitor membrane receptors. Recent studies demonstrate that TREM2-mediated phagocytic function of microglia/macrophages (MM) is required for debris clearance and maintenance of CNS tissue homeostasis. Moreover, CD200 receptor expressed on microglia is involved in maintaining the microglia in quiescent state.

Aim: We therefore, aimed to study the role of IL10 in modulating microglia using the axonal anterograde degeneration paradigm and to monitor TREM2 and CD200R expression as an effect of local astrocyte-targeted production of IL-10. We performed a perforant pathway transection (PPT) on adult GFAP-IL10 transgenic (Tg) mice and corresponding wild types (WT) littermates. Animals were intracardially perfused at 2,3,7,14 and 21 days post-lesion using 4% paraformaldehyde, brains frozen and sections cut in a cryostat. We performed single and double immunohistochemistry for microglia expressing TREM2 and CD200R.

Results: Our results show an increase in TREM2 and CD200R expression on microglia in the ipsilateral hemisphere of the Tg and WT animals especially in the molecular layer of the dennervated dentate gyrus after PPT and the expression was always higher in the Tg animal. We noticed that their expression is upregulated via local production of IL10 as seen by the activated phagocytosing phenotype (CD68^{high} and CD16/32^{high}) of microglia in the GFAP-IL10Tg animals. Furthermore, we found a possible feedback inhibition of IL10 as observed by a constant pSTAT3 expression in Tg mice.

Conclusions: Hence, specific modulation of these receptors and the anti-inflammatory cytokine IL10 with pharmacological tools represent a promising therapeutic strategy against CNS pathologies. Future studies are concentrated on evaluating the mechanism of action of IL10 in modulating these regulatory receptors following CNS injury.

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Epithelial IFN-λ and IFN-α/β constitute a compartmentalized mucosal defense system that restricts virus infection of the intestinal and respiratory tract

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Strawberry notch homolog 2 is a novel nuclear IL-6 primary response gene in glial cells

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Aims: An investigation of potential transcriptional mechanisms underlying interleukin (IL)-6 actions identified a novel putative murine transcriptional regulator called strawberry notch homolog 2 (*Sbno2*) as a prominent IL-6 target gene. We hypothesise that *Sbno2* is a key regulator of IL-6 and other gp130 family cytokine actions in the CNS and other tissues. The aim of this study was to better define the hyper-IL-6-induced regulation of *Sbno2* in astrocytes and microglia, as well as to examine its localisation using lentiviral transduction.

Methods: Murine primary cultured astrocytes and the C8-B4 microglial cell line were treated with 25 ng/mL hyper-IL-6 and 10 µg/mL cycloheximide, and astrocytes were treated with 25 ng/mL hyper-IL-6 and 10 µg/mL actinomycin D. To determine SBNO2 localisation, lentiviral vectors were produced to deliver an HA epitope-tagged *Sbno2* gene and a bicistronic fluorescent marker into the NIH-3T3 murine fibroblast cell line. Fluorescence immunocytochemistry was performed using an antibody against the HA epitope to visualise the transgenic SBNO2.

Results: Treatment with the protein synthesis inhibitor cycloheximide alone had little to no effect, but superinduced *Sbno2* expression over 8-fold upon hyper-IL-6 treatment in both cell types. Previous results showed that hyper-IL-6-induced *Sbno2* expression lasts for at least 12 h in astrocytes. Inhibition of transcription by actinomycin D revealed *Sbno2* mRNA has a half-life of 51 min in these cells. In NIH-3T3 fibroblasts the transgenic SBNO2 was found to localise exclusively to the nucleus but not the nucleoli.

Conclusions: (1) *Sbno2* is a primary response gene to hyper-IL-6 and is able to be regulated by hyper-IL-6 in the absence of additional protein synthesis, (2) its mRNA levels are tightly regulated, and (3) transgenic SBNO2 localises exclusively to the nucleus consistent with a role as a transcriptional regulator.

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Innate cell-like function of T cells in modulation of inflammatory response to infection and tissue injury

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Innate cells sense and respond to pathogens, tissue damage and stress by producing pro-inflammatory cytokines and chemokines (PICC), which are physiological steps to mobilize immune defense, tissue repair and restoration of homeostasis. Abnormal inflammatory reponses lead to autoimmunity, fibrosis and other autoinflammatory diseases. Thus inflammatory response is tightly controlled both locally and systemically.

We have previously shown that T cells are both necessary and sufficient to temper Toll like receptor (TLR) mediated inflammatory response to pathogens, in a TCR-independent manner. TCR-independency thus highlights that T cells may possess evolutionally conserved characteristics of innate immune cells. Herein I will also discuss another feature of how pro-inflammatory CD8+ T cells activate macrophages to initiate myocardiac injury casued by hypertension. Macrophages infiltration and activation in myocardium is a pivotal immunopathological lead to hypertensive cardiac micro-injury, but underlying mechanisms remain elusive. We have found that CD8+ T cells are essential for the process. CD8 gene targeting (CD8 KO) or CD8+ T cells depletion by antibody significantly reduced cardiac pro-fibrotic inflammatory responses induced by angiotensin II (Ang II) infusion, whereas CD8 KO mice reconstituted with CD8+ T cells became sensitive to Ang II. More importantly, CD8+ T cells were required for macrophage infiltration in myocardium and subsequent activation to express PICC. Furthermore, macrophage activation required direct contact with CD8+ T cells, but independent of T cell receptor (TCR), both in vitro and in vivo. Finally, hypertension may casue myocardium or myofibroblast cells to produce IFNγ, which is responsible to chemoattract CD8+ T cells to the left ventricles in the onset of cardiac inflammation. Thus, TCR independent innate nature of CD8+ T cells are both necessary and sufficient to induce hypertensive cardiac inflammation.

In conclusion, innate cells, conventional and non-conventional, seem to be both sensors and effectors that determine the magnitude and duration of inflammatory response to tissue damage.

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Characterizing the Molecular Players Involved in Lipopolysaccharide Inflammatory Responses

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The human body relies on its ability to sense invading microorganisms in order to mount an effective immune response. A primary player in the immune systems response to gram-negative bacterial invasion is lipopolysaccharide (LPS). LPS is a critical component of the outer membrane of gram-negative bacteria, and its endotoxin properties can elicit a large inflammatory response that can induce sepsis and subsequent death. Presently, we know that the binding of LPS to CD14 initiates signal transduction; however, the molecular processes immediately following this event remains unclear.

Previously, Chinese hamster ovary (CHO)-K1 cells that stably express CD14 were mutagenized and exposed to LPS. Two complementation groups were isolated from this screen, both of which fail to response to LPS stimulation but still respond to TNF stimulation. Complementation Group A was further characterized and found to contain mutations in MD-2. Soluble MD-2 has since been shown as a critical regulator of cellular responses to LPS through TLR4 signaling. Here, I have utilized whole transcriptome sequencing to characterize all mutations in Complementation Group B. I am currently using wild-type expression constructs isolated from the other complementation group to verify the gene required to induce a LPS non-responsive phenotype in Complementation Group B. After *in vitro* confirmation, these mutations will be incorporated into engineered mouse models in order to characterize the *in vivo* response of this mutation.

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Characterisation of human metapneumovirus infection of primary human bronchial cells

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Aims: Human metapneumovirus (HMPV) is a recently discovered respiratory pathogen that presents with similar clinical indications to respiratory syncytial virus (RSV), ranging from mild respiratory symptoms to severe cough, wheezing, bronchiolitis and pneumonia. There is mounting evidence suggesting an association between HMPV infection and asthma exacerbation in both adults and children. Our study examines HMPV infection of primary human bronchial airway cells, comparing responses from normal and asthmatic patients.

Methods: We infected primary human bronchial epithelial cells, fibroblasts and macrophages with HMPV and examined viral replication, production of inflammatory mediators and anti-viral responses.

Results: HMPV can infect human bronchial epithelial cells, fibroblasts and macrophages; however, we have observed several differences between the cell types. Viral replication peaks at 48 hours post infection in epithelial cells, whereas replication is still increasing at 72 hours post infection in fibroblasts. Epithelial cells express MIG, whereas fibroblasts and macrophages release MCP-1. All cell types have a strong anti-viral response, expressing IP-10 and RANTES and the interferon-stimulated gene, ISG56.

Conclusions: Our data demonstrate that HMPV infects and replicates in primary human bronchial epithelial cells, fibroblasts and macrophages, and induce a strong inflammatory and anti-viral response. Future studies are looking at these indices in cells obtained from asthmatic patients to identify a mechanism to explain the link between HMPV infection and asthma etiology and exacerbation.

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A flow cytometric assay for ASC speck formation in inflammasome responses

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Inflammasomes are protein complexes that form in response to infection, cell damage and environmental stress. Inflammasome activation leads to the processing of proinflammatory cytokines and pyroptotic cell death through the recruitment and activation of caspase 1, and apoptosis via caspase 8 activation. A common component of many inflammasomes is the adaptor molecule “apoptosis associated speck-like protein containing a caspase activation recruitment domain” (ASC). ASC is normally spread throughout the cell, however, upon inflammasome activation the vast majority of ASC is recruited into a single dense speck in the cytosol. We considered that the dramatic relocalisation of ASC should be detectable by flow cytometry. An assay has been developed that differentiates cells with ASC specks from cells with diffuse ASC by comparing time of flight parameters (height, width and integral/area) of the fluorescence pulse generated as a cell passes through the laser beam. This analysis has been applied to native ASC within immunostained macrophages and monocytes, as well as to ASC-GFP overexpressed in HEK293 cells. Applications for this method of speck detection include examination of early events in inflammasome formation, and detection of inflammasome responses within mixed cell populations using lineage markers. In addition this will allow dissection of structural requirements for ASC speck formation using cells reconstituted for inflammasome formation.

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Mast cell differentiation is operated by a T cell response

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After exposure to an allergen, mast cells (MCs) accumulate at the allergic site and then act as effector cells of inflammation. Despite many investigations into of the role of MCs in allergic inflammation, the mechanism underlying the expansion of these cells under allergic inflammatory conditions remains unclear.

When splenocytes from mice immunized with OVA or KLH were repeatedly stimulated *in vitro* with antigens, expansion of MCs was greater than that of antigen-specific T cells for up to 3 weeks. This observation raised the possibility that T cell responses elicited by the antigens were associated with MC development. To verify this, purified splenic T cells from immunized mice were cocultured with MCs or various progenitor populations and repeatedly stimulated with antigens. The results obtained from these coculture experiments suggested that T cell responses could have an effect on myeloid progenitors and act to drive the MC differentiation. Additionally, IL-3 from T cells was crucial for MC differentiation. To validate whether MC differentiation that we observed in *in vitro* culture experiments might also occur in allergic disease, we used a murine food allergy model that was dependent on T cells and displayed an accumulation of MCs the colon, and found that this murine model exhibited the accumulation of myeloid progenitors and the up-regulation of IL-3.

Collectively, our study suggests that T cell responses against allergens may drive MC differentiation to generate effector cells of allergic inflammation. Blocking this differentiation pathway may be an attractive therapeutic strategy for treating allergic inflammation.

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Apoptotic Cell Recognition Receptors, TYRO3, AXL and MER (TAM), Demonstrate Distinct Patterns and Complex Regulation of Ligand-Induced Activation and Signaling

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Efficient recognition and removal of apoptotic cells by phagocytes lead to the active suppression of inflammatory responses and the induction of tolerance. Apoptotic cell (AC) recognition receptors, TYRO3, AXL and MER, share similar structural organization of their extracellular and intracellular domains, and form a subfamily of receptor tyrosine kinases (RTKs), abbreviated as TAMs. Activation of TAMs is triggered by secreted glycoproteins, growth arrest-specific gene 6 (GAS6) or protein S (PROS1), that bind to TAMs through their C-terminal Laminin G (LG) domains. In turn, GAS6 and PROS1 are γ-carboxylated on glutamic acid residues in their N-terminal Gla domains and interact in a Ca^{2+}-dependent manner with externalized phosphatidylserine (PS) on the surface of ACs and enveloped viruses. Therefore, GAS6 and PROS1 act as bridging factors: their γ-carboxylated Gla domains bind to PS and opsonize ACs, whereas their LG domains interact with TAMs, enabling TAMs to bind indirectly to ACs and act as immunoregulatory receptors that dampen inflammation. Although TAMs share significant similarity, very little is known about the specificity of interaction between TAMs and their ligands in the context of apoptotic cells, and about downstream signaling cascades. We generated and utilized a set of reporter cell lines expressing chimeric TAM receptors to demonstrate that each TAM has a unique pattern of interaction with GAS6 and PROS1. These interactions are also differentially affected by the presence of ACs, PS liposomes, and enveloped viruses. We also demonstrated that γ-carboxylation of ligands is essential for the full activation of TAMs. In addition, we found that TAM-mediated signaling up-regulates expression levels of programmed death-ligand 1 (PD-L1) and PD-L2 that play an important role in immunosuppression. Overall, these studies reveal that, despite their similarity, TYRO3, AXL and MER perform distinct but overlapping functions in the recognition and removal of ACs, and in the regulation of inflammatory and immune responses.

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Poor outcome in rheumatoid arthritis patients switching from etanercept to tocilizumab therapy

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At present, there is no standard by which to pre-qualify rheumatoid arthritis (RA) patients for tocirizumab therapy among those who have failed previous anti-cytokine therapy (non-naïve). In this study, forty-one biologic non-naïve patients who had failed one to three prior treatments with methotrexate combined with anti-TNF-a therapy were enrolled in this study. Prior to receiving tocilizumab treatment, serum cytokine, chemokine and soluble receptor levels were measured using Bio-Plex human cytokine/chemokine 27-Plex panel and Millipore soluble receptors panel (sIL-6R, sgp130, sTNFR-I and sTNFR-II). Patients were treated with 8mg/kg of tocilizumab once every 4 weeks. After 16 weeks of therapy (4 administrations), clinical efficacy was judged based on patients' disease activity score (DAS28-CRP), and whether patients experienced complete remission or non-remission.

All non-naive RA patients except one showed improvements in their DAS28-CRP score at week 16 of therapy and 9 out of 40 (22.5%) non-naïve patients experienced clinical efficacies that were judged as complete remission. The remaining 31 patients experienced non-remission (symptom levels: low=6, median=21, and high=4). In the same study, complete remission (DAS-28-CRP≧2.3) was achieved by 56 % of naïve patients. Although 20 out of 21 patients who switched form etanacept to tocirizumab had improved DAS28 score only two of these patients achieved remission. On the other hand, 5 of 11 patients switching from infliximab reached remission. Single logistic regression analysis revealed that sgp130 and sIL-6R level played a role in these outcomes.

Additionally in comparing naïve and non-naïve RA patients' pre-treatment cytokine/chemokine levels, we observed that they were relatively similar except for sTNFR-II, which was higher than in non-naïve patients.

These results suggest that prior RA treatment may influence the outcome of further treatments using different biological drugs. Using prediction biomarkers may help clinicians to select more treatments with more favorable outcomes especially for patients who are switching from one treatment protocol to another.

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Procaspase-8 recruitment and activation at the AIM2 inflammasome

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Inflammasomes are macromolecular complexes that mediate inflammatory and cell death responses to pathogens and cellular stress signals. Inflammasome formation leads to activation of both caspase-1 and caspase-8. We have studied the molecular interactions important for assembly of the AIM2 inflammasome and for procaspase-8 recruitment and activation. AIM2 is a member of the PYHIN (pyrin domain (PYD) and HIN domain-containing) family and is activated in response to cytosolic DNA. Upon activation, AIM2 oligomerises and recruits the adaptor protein ASC. ASC is composed of an N-terminal PYD, with which it mediates a homotypic interaction with AIM2 PYD and a C-terminal CARD domain, with which it recruits procaspase-1. We show that procaspase-8 interacts with both ASC and AIM2 via interactions between the tandem DEDs of procaspase-8 and the PYDs of ASC and AIM2. Mutations on ASC PYD that disrupt its self-association also disrupt interaction with AIM2 and procaspase-8 suggesting that that either ASC PYD self-association is important for the different interactions or that the interaction mode between the three domains is similar. However, swapping of the PYDs between ASC and AIM2 disrupted inflammasome function arguing against identical surfaces being used for all three interactions. Furthermore, we show that procaspase-8 is efficiently recruited to specks formed by ASC alone but is only activated

in specks containing both ASC and AIM2. This indicates a more stringent requirement for procaspase-8 activation than for its recruitment. The inflammasome does not just cluster caspases, but also promotes a specific orientation of procaspase-8 that permits activation.

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DYSTONIA-CAUSING FRAME-SHIFT MUTATION IN PACT ACTIVATES INTERFERON-INDUCED PROTEIN KINASE PKR BY AGGREGATE FORMATION

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PKR or Protein Kinase R is an interferon-induced, double stranded RNA (dsRNA)-activated eIF2α kinase that is a key regulator of cellular stress response pathways. PACT (also known as PRKRA, and DYT16) activates PKR in response to oxidative stress, endoplasmic reticulum (ER) stress, and serum starvation. Prolonged PKR activation in response to cellular stress leads to cell death by apoptosis. An inherited form of early-onset dystonia has been identified to be associated with multiple mutations in the coding region of PACT. Dystonia is a neurological disorder defined by an occurrence of sustained involuntary muscle contractions, often leading to disabling and abnormal postures. Several recessive missense mutations and one dominant frame-shift mutation in PACT have been identified to result in dystonia. A German patient was recently identified as having a heterozygous mutation in exon 3 of PACT (AT deletion at c.266-267) creating a premature stop codon, resulting in a truncation at AA position 109. **The main hypothesis** of this research is that the frame-shift mutation in PACT leads to production of a truncated protein that is misfolded and forms aggregates resulting in cellular stress and the disease phenotype. We compared the ability of wild type PACT and frame-shift (FS) PACT proteins to interact with and activate PKR using *in vitro* biochemical assays. In addition, we have investigated changes in sub-cellular localization, protein aggregation, and cell death resulting from FS PACT mutant. Our results indicate that although FS PACT lacks the PKR activation domain, it activates PKR robustly *in vitro* because it forms aggregates. In addition, the truncated FS mutant protein also exhibits altered sub-cellular localization and forms aggregates in mammalian cells. These results raise a possibility that the PKR activation observed in several neurodegenerative diseases that are caused by protein aggregation may be an important contributing factor for the disease pathology.

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Guanylate-Binding Proteins (GBPs) and cytoskeleton regulation

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Guanylate-Binding Proteins (GBPs) are a family of pro-inflammatory cytokine-induced genes. These proteins have been best studied for their anti-microbial activities against pathogens sensitive to IFN-gamma. However, GBPs have a number of significant functions not directly related to their antimicrobial activities. One of these is regulation of the cytoskeleton. Integrins, TNF-alpha, and PDGF all promote rearrangement of the actin cytoskeleton, in part through the activation of the master cytoskeleton regulator Rac. mGBP-2 inhibits these rearrangements by inhibiting their ability to activate Rac. The inhibition of Rac is accompanied by the binding of mGBP-2 to the catalytic subunit of PI3-K and inhibiting its function. mGBP-2 also protects cells from killing by the cytotoxic chemotherapeutic drug, paclitaxel. Paclitaxel is proposed to kill cells by inhibiting the dynamic instability of microtubules. Cells can be protected from paclitaxel by restoration of this dynamic instability. Forced expression of the human ortholog of mGBP-2, hGBP-1, also protects tumor cells from killing by paclitaxel. hGBP-1 mRNA is expressed at greater than 2-fold higher levels than in benign ovaries in only 11% of new ovarian tumors. This number was at least 40% for tumors that recurred after a therapeutic regime containing a taxane. While the mechanism(s) by which hGBP-1 protects cells from paclitaxel is still under investigation, one possibility is that this occurs through hGBP-1-mediated alterations in cytoskeletal dynamics.

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ASTROCYTE-TARGETED PRODUCTION OF IL-10 INDUCES CHANGES IN MICROGLIAL REACTIVITY AND REDUCES MOTOR NEURON DEATH AFTER FACIAL NERVE AXOTOMY

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Interleukin-10 (IL-10) is a cytokine that plays a crucial role in regulating the inflammatory response and immune reactions. In the central nervous system (CNS), IL-10 is mainly produced by astrocytes and microglia and its upregulation has been demonstrated after various insults, such as experimental autoimmune encephalomyelitis, middle cerebral artery occlusion, excitotoxicity and traumatic brain injury. To better understand the effects of IL-10 in the normal and injured CNS, we generated transgenic mice expressing the murine*IL10* gene under the transcriptional control of the GFAP promoter (GFAP-IL10Tg). Previous studies by our group demonstrated specific changes in microglial phenotype under basal conditions in GFAP-IL10Tg mice. The objective of the present study was to investigate the effects of local astrocyte-targeted IL-10 production on microglial activation, neuronal degeneration and leukocyte recruitment after experimental axotomy. Our results showed that after facial nerve axotomy, GFAP-IL10Tg mice showed remarkable changes in the expression of different molecules associated with the microglial activation pattern, such as CD16/32, CD18 and MHC-II, as well as in the number of microglial clusters and in microglial cell density. Furthermore, a higher CD3+ lymphocyte infiltration was observed on the axotomized FN of GFAP-IL10Tg mice. These changes in microglial activation and lymphocyte recruitment correlated with a reduced motor neuron death at 21 dpi. Altogether, our findings suggest that astrocyte-targeted production of IL-10 impacted the microglial response and lymphocyte recruitment and culminated in a beneficial effect on neuronal survival.

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Cytoplasmic DNA induces rapid cell death in Drosophila cells

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Eukaryotic cells sequester their DNA in the nucleus and organelles, leaving cytosol DNA-free. The presence of DNA in the cytosol indicates a danger, such as infection, activity of retrotransposons or DNA damage. We hypothesized that defence against cytosolic DNA is a feature of all eukaryotic cells, and fundamental to maintenance of genome integrity as well as recognition of infection. In mammals cytosolic DNA is recognized by AIM2, a member of the HIN-200 family, leading to formation of a multimolecular inflammasome complex, that in turn induces a rapid lytic death in macrophages, termed pyroptosis. There is no data about such a system for invertebrates, and AIM2 itself is a mammalian-restricted protein. Moreover there is no indication of active necrotic cell death in insects. To investigate whether an analogous system operates in *Drosophila*, cells were transfected with different types of DNA via electroporation and viability was assessed either by MTT assay or by exclusion of membrane-impermeable DNA stains. We have found that both primary hemocytes, and the Drosophila S2 macrophage-like cell line are sensitive to cytosolic DNA. Results showed that DNA from various sources, but not synthetic double stranded RNA caused rapid cell death. Unlike mammalian DNA-dependent cell death, denatured DNA was also toxic. Experiments with synthetic DNA showed that the response was sequence-specific. The mode of cell death was investigated using flow cytometry. Rapid loss of membrane integrity, lack of a population of cells with sub-G0/G1 levels of DNA, and insensitivity to the pancaspase inhibitor z-VAD-fmk showed that cell death was not apoptotic. We conclude that sensing of cytosolic DNA in fruitfly induced a novel lytic pathway of cell death. Elucidation of the molecular basis for this DNA recognition will allow determination of whether it plays a role in defence against infection or in guarding genome integrity.

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Regulation of inflammatory cytokine responses by a new TLR4 regulator complex of Rab8a/PI3Kγ

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Introduction: Toll-like receptor 4 (TLR4) is activated by bacterial lipopolysaccharide (LPS) to mount innate immune responses that involve the initial release of pro-inflammatory cytokines, followed by the regulator, or anti-inflammatory, cytokines. Failure to constrain inflammatory responses results in acute and chronic inflammatory disease. Multiple molecular mechanisms are emerging to control the cytokine profile that is released after receptor activation.

Results: Here we find TLR4 complexes localised in LPS-induced dorsal ruffles on the surface of macrophages. The small GTPase Rab8a is similarly enriched in these membrane domains. By using mass spectroscopy and biochemical mutational analysis, we have established a direct interaction between Rab8a and the phosphatidylinositol 3-kinase γ (PI3Kγ). Knockdown of Rab8a or PI3Kγ, pharmacological inhibition, or genetic ablation of PI3Kγ all serve to reduce Akt signalling in response to LPS/TLR4. The cytokine profiles in cells depleted of Rab8a and mice lacking PI3Kγ were examined and we show that in both cases there is a dramatic increase in the production of the pro-inflammatory cytokines IL-6 and IL-12p40 with a concomitant decrease in the production of the anti-inflammatory cytokine IL-10. This was mirrored by inhibition of mTORC1 by rapamycin, illustrating that Rab8a/PI3Kγ function as a novel amplifier of the Akt/mTOR pathway downstream of TLR4.

Conclusions: Our studies introduce Rab8a and PI3Kγ as a novel complex and provide the first biochemical evidence for a Rab interacting with the immune-specific class IB PI3K. This positions PI3Kγ in a TLR4 cross-talk pathway that may serve as a pharmacologically relevant target during the innate immune response.

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Regulation of embryonic hematopoiesis by a cytokine inducible SH2-domain (CISH) homologue in zebrafish

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Cytokine-inducible SH2-containing protein (CISH), a member of the suppressor of cytokine signaling (SOCS) family of negative feedback regulators, is induced by cytokines that activate signal transducer and activator of transcription 5 (STAT5) and can inhibit STAT5 signaling *in vitro*. However, demonstration of a definitive *in vivo* role for CISH during development has remained elusive. This study employed expression analysis and morpholino-mediated knockdown in zebrafish in concert with bioinformatics and biochemical approaches to investigate CISH function. Two zebrafish *CISH* paralogues were identified, *cish.a* and *cish.b*, with high overall conservation (43-46% identity) with their mammalian counterparts. The *cish.a* gene was maternally derived, with transcripts present throughout embryogenesis, and increasing at 4-5 days post fertilization, while *cish.b* expression commenced at 8 hours post fertilization. Expression of *cish.a* was regulated by the JAK2-STAT5 pathway via conserved tetrameric STAT5 binding sites ($TTCN_3GAA$) in its promoter. Injection of morpholinos targeting *cish.a* – but not *cish.b* or control morpholinos – resulted in enhanced embryonic erythropoiesis, myelopoiesis and lymphopoiesis, including a 2-3 fold increase in erythrocytic markers. This occurred concomitantly with increased activation of STAT5. This study indicates that CISH functions as a conserved *in vivo* target and regulator of STAT5 in the control of embryonic hematopoiesis.

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Critical role of cytokine and growth factor receptors in AML identified using an RNAi functional screen

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Methods: Acute myeloid leukemia (AML) is one of the most common hematologic malignancies affecting both children and adults. Because cytokine and growth factor receptors contribute to cancer pathogenesis by regulating growth, differentiation, and survival[1 2 3], we designed a functional RNAi screen targeting 188 cytokine and growth factor receptors that we found highly expressed in primary leukemia specimens. Using this screen we identified interleukin-2 gamma receptor (IL2Rγ) and Colony Stimulating Factor 2 Receptor Beta (CSF2RB)as critical growth determinants for JAK3 mutation-positive AML cells.

Results: We observed that knockdown of IL2Rγ reduced the growth of JAK3 mutation-positive AML cells and abrogates phosphorylation of JAK3 and downstream signaling molecules, JAK1, STAT5, MAPK and pS6 ribosomal protein. Overexpression of IL2Rγ in murine cells increased the transforming potential of activating JAK3 mutations, whereas absence of IL2Rγ completely abrogated the clonogenic potential of JAK3A572V mutation-positive cells. In addition, mutation at the IL2Rγ interaction site in the FERM domain of JAK3 (Y100C) completely abrogated JAK3-mediated leukemic transformation. Mechanistically, IL2Rγ and JAK3 contribute to constitutive JAK/STAT signaling through their reciprocal regulation.

Additionally, CSF2RB was identified as a secondary hit in CMK cells and siRNA-mediated knockdown reduced cellular viability and phosphorylation of JAK2, STAT5, ERK1/2 and S6 ribosomal protein. CSF2RB is highly expressed in 30% of AMLs[4] and preliminary data shows some primary AMLs may depend on CSF2RB expression, indicating a potential therapeutic target.

Conclusions: We have demonstrated: 1) A novel role for IL2Rγ in potentiating oncogenesis in the setting of JAK3-mutation-positive leukemia. 2) CSF2RB is a potential functional target in AML and future studies investigating this possibility may yield novel therapeutic strategies. 3) RNAi-based functional assays can be used to facilitate the identification of non-kinase cytokine and growth factor receptor targets for inhibiting leukemic cell growth.

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Syk signaling in dendritic cells orchestrates innate resistance to systemic fungal infection

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Host protection from fungal infection is thought to ensue in part from the activity of Syk-coupled C-type lectin receptors and MyD88-coupled toll-like receptors in myeloid cells, including neutrophils, macrophages and dendritic cells (DCs). Given the multitude of cell types and receptors involved, elimination of a single pathway for fungal recognition in a cell type such as DCs, primarily known for their ability to prime T cell responses, would be expected to have little effect on innate resistance to fungal infection. Here we report that this is surprisingly not the case and that selective loss of Syk but not MyD88 in DCs abrogates innate resistance to acute systemic *Candida albicans* infection in mice. We show that Syk expression by DCs is necessary for IL-23p19 production in response to *C. albicans*, which is essential to transiently induce GM-CSF secretion by NK cells that are recruited to the site of fungal replication. NK cell-derived-GM-CSF in turn sustains the anti-microbial activity of neutrophils, the main fungicidal effectors. Thus, the activity of a single kinase in a single myeloid cell type orchestrates a complex series of molecular and cellular events that underlies innate resistance to fungal sepsis.

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The role of PTPN2 in early thymocyte development and JAK/STAT signaling

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The role of common γ-chain receptor cytokines in the promotion of thymocyte development and peripheral T cell survival is well characterized. However, the mechanisms by which cytokine signaling is attenuated during T cell development, particularly at the double-negative (DN) stages, remain unclear. We have shown previously that the protein tyrosine phosphatase PTPN2 acts as a key negative regulator in T cells and contributes to central tolerance and the prevention of excessive responses to self-antigen in the periphery.1,2 The Janus kinases (JAK) 1 and 2 and the transcription factors STAT1, STAT3 and STAT5 are bona fide substrates of PTPN2.

Our studies indicate that global PTPN2 deficiency in mice enhances STAT5 signaling during early thymocyte development. DN thymocytes isolated from Ptpn2–/– mice exhibited increased STAT5 phosphorylation at DN stages 2 and 3 resulting in elevated Bcl-2 levels and enhanced thymocyte survival. Studies with mixed bone marrow chimeras demonstrated that PTPN2-deficient thymocytes outcompeted their wild type counterparts in lethally irradiated congenic wild type hosts at DN stages 3 and 4. In addition, T cell progenitors isolated from the bone marrow of Ptpn2–/– mice transitioned faster through the DN-stages when compared to wild type control cells using the OP9-DL1 system ex vivo. In contrast, retrovirally transduced over-expression of PTPN2 in bone-marrow chimeras prevented progression beyond DN stage 2.

These results indicate that PTPN2 is required to restrain thymocyte selection at the DN stages in a cell-intrinsic manner through the inhibition of the JAK/STAT5 pathway. These findings are consistent with studies showing that Interleukin-7 (IL-7)-induced STAT5 signaling is crucial for T cell development and survival and the progression of DN thymocytes to the double-positive (DP) stage. Our results identify PTPN2 as a critical regulatory element in early T cell development.

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The BTB-ZF transcriptional regulator, PLZF, modifies chromatin to restrain inflammatory cytokines signaling programs

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Inflammation is critical for host defense, but without appropriate control it can cause chronic disease, or even provoke fatal responses. Here we identify a mechanism that limits the inflammatory response. Probing the responses of macrophages to the key sensory Toll-like receptors, we identify that the BTB/POZ, zinc finger transcriptional regulator PLZF limits the expression of inflammatory cytokines products. In accord with this, PLZF-deficient animals express higher levels of potent inflammatory cytokines and mount exaggerated inflammatory responses to infectious stimuli. We show that this protective effect of PLZF is mediated via modulation of macrophage activity. Temporal quantitation of inflammatory gene transcripts shows more rapid gene induction in the absence of PLZF. Genome-wide analysis of histone modifications distinguish that PLZF establishes basal activity states of early response genes to maintain immune homeostasis and limit damaging inflammation. We show that PLZF stabilizes a co-repressor complex that encompasses histone deacetylase activity to control chromatin. Together with our previous demonstration that PLZF promotes the antiviral response, these results suggest a strategy that could realize one of the major goals of immune therapy to retain immune resistance to pathogens while curbing damaging inflammation.

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Pronuclear injection of circular plasmids expressing hCas9 and gRNA generates mutant mice in genes involved in immune responses.

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Recently, the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated (Cas) 9 system have been found in some kind of bacteria's defense system like a mammalian acquired immune system. This system develops as powerful tools to modify the genome in many species including mammalian. In this system, gRNA recruits hCAS9 nuclease to the specific genomic locus according to the base-pairing rule and then, introduces double strand breaks (DSBs). After the DSBs, non-homologous end-joining is occurred and causes small insertion or deletion at the specific site. Thus, in order to rapidly obtain gene-deficient mouse in several genes involved in immune responses, we designed over 10 gRNAs and validated their working with hCas9 expression in cultured cells. After checking, we injected two plasmids expressing hCas9 and single gRNA, which is targeted at an inflammatory cytokine gene, into the pronucleus of mouse zygote and transferred with pseudopregnant mice. Finally, we succeeded in getting several lines of the gene-deficient mouse.

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Brain derived neurotrophic factor regulated immune responses in vivo

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It is well known that Brain derived neurotrophic factor (BDNF) is a one of neurotrophic factors and a key player for sustaining of homeostasis in brain. On the contrary, there are few reports whether BDNF affects immune cells. We previously reported that BDNF affects immune cells and induced regulatory T cells (Treg). In this study, we investigated whether BDNF has effects on immune cells in vivo. At 24 hours after the BDNF administration by i.n., splenocytes were prepared. Flow cytometry demonstrated that BDNF administration induced Foxp3 positive cells in splenocytes. To determine whether BDNF administration affected splenocytes, proliferation of splenocytes were examined. The proliferation of splenocytes stimulated with mitogen (LPS) was attenuated. Additionally, TNF-α production induced by LPS was lower than control in splenocytes. These results suggested that BDNF contributes immune suppressive event in vivo.

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Kupffer cell derived IL-1a promotes acetaminophen-induced liver injury

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Cell necrosis can induce profound inflammation and potentially increases overall tissue injury. One important example is acetaminophen-induced liver injury (AILI), in which the initial tissue injury caused by the toxicity of acetaminophen (APAP) is followed by the second wave of tissue injury caused by cell necrosis and inflammatory responses. In the present study, we show that the TLR4-dependent activation of IL-1a in kupffer cells is critical for the progression of AILI. Notably, interference of IL-1R1 or IL-1a, but not IL-1b or NLRP3 inflammasome, exhibited reduced hepatic injury, as evidenced by decreased serum levels of alanine and aspartate aminotransferases, decreased hepatic necrosis, decreased neutrophils and monocytes infiltration and improved survival of experimental animals. The secreted IL-1a was derived from kupffer cells and the maturation and activation of IL-1a was dependent on TLR4/MyD88 signaling. Moreover, the infiltrated Gr-1 positive myeloid cells serve as target cells in response to IL-1a to contribute to liver injury. Taken together, these findings indicate that inhibition of IL-1a signaling can alleviate the acute inflammatory responses and tissue injury, and represent a potential therapeutic method in AILI.

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