THE 2015 INNATE IMMUNITY SUMMIT

ABSTRACTS

17th - 19th November 2015
London, UK

EuroSciCon

This international interdisciplinary event is an open forum for discussion of current research and thinking regarding an organisms first line of defence. Using a multi-professional and inter-specialty approach this event promises plenty of opportunity for discussion and debate set in an informal atmosphere.

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Invited Speakers Abstracts

The role of microRNA and autophagy in innate immune responses to pathogens

Dr Amal Amer, Associate Professor, Department of Microbial Infection and Immunity, Center for Microbial Interface Biology, Ohio State University

Cystic fibrosis (CF) is a fatal genetic disorder that is caused by malfunction of CFTR chloride channel. In addition, autophagy is impaired in CF macrophages, yet the underlying mechanism is unknown. Here, we show that defective CFTR function increases the expression of specific microRNAs that target autophagy molecules reducing their expression and thus impairing autophagy activity. In vitro and in vivo down-regulation of these elevated microRNAs ameliorated CFTR function, improved bacterial clearance and reduced inflammation via restoring autophagy activity. Our findings provide mechanistic insight into the connection between CFTR function and autophagy in CF.

Chemoattractant chemerin, a novel player in cutaneous defence

Dr Joanna Cichy, Faculty of Biochemistry, Biophycics & Biotechnology, Jagiellonian University, Krakow, Poland

Chemerin is a chemoattractant protein with adipokine properties. First identified as tazarotene- (retinoic acid-analog) induced gene 2 (TIG2) in tazarotene treated psoriatic skin, it has gained more attention over the past few years due to its multilevel impact on metabolism and immune responses. The pleiotropic actions of chemerin include triggering chemotaxis of dendritic cell, macrophage and NK cell subsets, as well as regulation of adipogenesis and glucose metabolism. Despite the fact that chemerin is a prominent protein in healthy but not diseased epidermis, the role of chemerin in skin remains elusive. We recently demonstrated that either recombinant chemerin or endogenous chemerin in exudates from organ cultures of primary human skin keratinocytes has antimicrobial activity. Here we show that chemerin-deficient mice are not able to efficiently clear S. aureus in an experimental cutaneous infection model, and that a specific chemerin-derived peptide can restore the ability of these mice to restrict infection. Together, our findings suggest that chemerin plays a role in cutaneous defence. We will discuss how this previously uncharacterized antimicrobial property merges with other chemerin functions to respond to microbial threats.

Endocannabinoids are master regulatory bioactive lipids of innate immunity-driven neuroinflammation

Dr Valerio Chiurchiù, European Center for Brain Research; Laboratory of Neurochemistry of Lipids, IRCCS Santa Lucia Foundation, Rome, Italy

The immune system can be modulated not only by foreign antigens but also by other humoral factors and metabolic products. Among these, endocannabinoids are a group of endogenously produced bioactive lipids (N- or O-derivatives of polyunsaturated fatty acids) produced on demand that serve as secondary immune-modulators. Innate immune cells, together with their receptors and enzymes regulating their synthesis and degradation, particularly express endocannabinoids. These lipids exert a plethora of homeostatic and anti-inflammatory effects, regulating the immune responses of monocytes, M1/M2 macrophages and dendritic cells, with a particular impact on neuroinflammation and neurodegenerative diseases, including Multiple sclerosis and Alzheimer's disease.

Novel Mediators and Mechanisms in Resolution of Inflammation and Tissue Regeneration: Immunoresolvents

Dr Jesmond Dalli, Brigham's and Women's Hospital and Harvard Institute of Medicine, Boston MA, USA

Resolving inflammatory exudates produce chemical mediators that regulate inflammation, stimulate resolution and tissue regeneration. These mediators include the lipoxin, resolvin, protectin and maresin families and are collectively called specialized pro-resolving mediators. Identification of novel signals in these processes and their pathways is of interest. These mediators actively regulate leukocyte responses counter-regulating the production pro-inflammatory signals, promoting their differentiation to a protective phenotype and orchestrating their trafficking in and out of injured tissues. Most recently in regenerating planaria, infectious murine exudates, human milk and macrophages, we identified novel potent molecules that stimulated tissue regeneration in planaria and mice. These molecules were peptido-maresins coined MCTR1 and MCTR2, they carried potent anti-inflammatory and proresolving actions, limited neutrophil infiltration and stimulated macrophage phagocytosis in vivo and in vitro. Together these novel mediators represent novel, conserved chemical signals and pathways (in planaria, mice and humans) that regulate host responses to control infection, tissue regeneration and organ protection.

Tityus serrulatus venom induces inflammation dependent on TLRs recognition and inflammasome activation

Lucia Helena Faccioli, Universidade de São Paulo - Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Brazil

Envenomation by Tityus serrulatus (Ts) results in systemic inflammatory reaction that may results in death. We have studied the activation of cells in response Ts venom (TsV), in order to understand the mechanisms responsible for the induction of inflammatory mediators released after envenomation. Our studies revealed that TLR2, TLR4 and CD14 receptors sense TsV facilitating the production of eicosanoids (LTB4 and PGE2) and cytokines; formation of lipid bodies (LBs); and activation of PPARγ and NFκB. Moreover, we find that Nlrp3 inflammasome is activated in response to TsV, resulting in IL-1β production. Administration of TsV to inflammasome-deficient mice resulted in death abrogation. Based in our data, we propose that during envenomation, TLRs recognize TsV resulting in inflammasome activation and generation of inflammatory mediators responsible for systemic inflammation and death. Finally, we suggest the term venom-associated molecular pattern (VAMP) to indicate molecules that are introduced into the host by stings and are recognized by PRRs, resulting in inflammation.

Collagenous complement-activating pattern recognition molecules: structure, mode of activation, and involvement in antimicrobial defense and homeostasis

Professor Jens Christian Jensenius, Department of Biomedicine, Aarhus University, Denmark, Germany

These pattern recognition molecules (PRMs) form part of the innate immune defence through their recognition of so-called Pathogen-Associated Molecular Patterns or PAMPs. After binding to their ligands they will activate complement through their associated serine proteases (MASPs), thus initiate mechanisms for the elimination of the pathogen including inflammatory reactions. The complement activating PRMs encompass the two collectins: mannan-binding lectin (or mannose-binding lectin), MBL, and CL-LK, and the three ficolins, H-, L-, and M-ficolin. While aiding elimination of pathogens they also initiate destructive inflammatory reactions following ischemia-reperfusion, and inhibition of this is attracting considerable clinical interest.

Heat shock proteins in inflammations

Dr Gaetan Jego, Associate professor, University of Burgundy, France

Heat shock proteins (HSPs) are powerful chaperones. Their expression is induced in response to a wide variety of physiological stress: increased temperature; cell differentiation; viral infections; pro-inflammatory conditions. Intracellular HSPs allow cell to survive potentially lethal conditions, while extracellular HSPs mediate cell-to-cell signals, either alone or through antigen chaperoning. Excessive expression or mislocalization of HSPs has been described in a broad variety of pathological conditions including oncogenesis and inflammatory diseases, leading to either blockage or exacerbation of inflammatory responses. Therefore, modulation of HSPs has emerged as an exciting therapeutic option to prevent inflammatory damages or conversely to restore immune responses.

Ocular Surface Defence Mechanisms and How they are Compromised by Contact Lens wear

Professor Alison M McDermott, Golden-Golden Professor, Professor of Optometry and Vision Sciences, Professor of Biology and Biochemistry, The Ocular Surface Institute (TOSI), University of Houston, College of Optometry, TX, USA

The ocular surface uses a plethora of innate defence mechanisms to ward off invading pathogens and so prevent infection of the cornea, the major refractive surface of the eye. Despite improvements in materials and cleaning solutions, corneal infection remains the most serious complication of contact lens wear and may result in permanent loss of vision. In this presentation the effects of contact lens wear on the normal ocular surface innate defence mechanisms will be discussed and novel strategies to prevent contact lens related infection described.

A newly identified innate immune response to HIV infection

Professor Áine McKnight, Professor of Viral Pathology, Queen Mary University of London, School of Medicine and Dentistry, Blizard Institute, London, UK

When an infecting virus breaches the plasma membrane and enters the cell it encounters innate immune factors or restriction factors that detect it and prevent its replication. Most of this anti-viral activity is orchestrated by interferon alpha/beta. We describe a novel cellular factor called RNA -associated-Early-Antiviral -Factor (REAF) that interacts with HIV within an hour of its entry into the cell. REAF consequently halts HIV replication at the stage of reverse transcription required by HIV to insert its genome into the host DNA. REAF is constitutively expressed in human macrophages and T-cells. So far our results suggest that unlike the majority of innate restriction factors REAF expression is not controlled by IFN.

Potentiation of the CD8 antiviral activity (CAF) by Thymosin alpha 1: new opportunities for immunomodulation during antiretroviral infections

Dr Claudia Matteucci, University of Rome "Tor Vergata", Rome, Italy

Thymosin alpha-1 (Ta1) is a molecule retaining pleiotropic effects towards several pathological conditions, especially acting as a modulator of immune response and inflammation, that exploits a specific action on lymphoid cells and induces in PBMCs a strong transcriptional response. CD8 antiviral factor (CAF) activity plays a role in the control or prevention of HIV-1 infection by a non-cytolytic mechanism. The ability of Ta1 to modulate, in vitro, the release of soluble factors with potential antiretroviral activity by CD8+ lymphocytes was investigated. New findings suggest Ta1 as a re-evaluated approach to antiretroviral therapy in combination with innovative treatments and with vaccine administration.

Macrophages: The Origin and Purveyor of Immunity in Animals

Dr Charles D. Mills, PhD, BioMedical Consultants, Marine on St. Croix, MN USA

Macrophages are the primary host defense in all animals. These multitalented cells perform their necessary protective roles through 4 basic and very different SHIP functions: Sample the environment; Heal (M2); Inhibit (M1), and finally in vertebrates to Present (antigen) to T and B cells. Thus, "immunity" throughout the animal kingdom is primarily a macrophage-centered system that makes necessary and critical decisions to help repair or directly (or indirectly) kill pathogens. Imbalances in macrophage functions contribute to (or cause) many diseases including cancer, atherosclerosis and autoimmunity. Understanding that modulating macrophages is the key to better health is a breakthrough in immunology.

Macrophage plasticity in normal and tumor-reprogrammed immune responses: the key role of the ramification of the signalling mechanisms

Dr Igor Malyshev, Head of the Department of Pathophysiology, Moscow State University of Medicine and Dentistry, NJ, USA

Immune response considerably depends on macrophages which may acquire either the pro-inflammatory M1 or the anti-inflammatory M2 phenotype. M1 contributes to destruction of bacteria, viruses and tumor. M2 contributes to destruction of parasites, angiogenesis and tissue repair. The process of changing the cell phenotype is called reprogramming. Reprogramming of macrophages plays an important role in many physiological and pathological processes. The presentation will describe key phenomena of macrophage reprogramming, analyze signalling mechanisms of reprogramming and give an idea of the specific features of these mechanisms, which provides a fundamental characteristic of macrophages and immune response in general - plasticity.

Surfactant Protein A and D: To bind or not to bind?

Dr Jens Madsen, Sir Henry Wellcome Laboratories, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK

Collectins are lectins with collagenous regions. They are secreted proteins found on mucosal surfaces (surfactant protein A and D (SP-A and SP-D) and in serum (Mannose Binding Lectin (MBL)). They promote phagocytosis of bacteria, viruses and other particulate matter and have both pro- and anti-inflammatory effects on the immune system. Facilitating uptake of pathogens is an efficient way of removing potential damaging micro-organisms but in some cases this is exploited by the micro-organism for its survival. This talk will discuss the role(s) of collectins in the innate immune system focusing on SP-A and SP-D.

Small molecule modulators of Toll-Like Receptor 4 (TLR4): a new generation of therapeutics

Dr Francesco Peri, MSCA-ETN action, Horizon 2020 programme, Department of Biotechnology and Biosciences, University of Milano Bicocca, Italy

Toll-like receptor 4 (TLR4) detects minute amount of bacterial endotoxin and activate the immune and inflammatory responses to pathogen infections. However, deregulated or excessively potent TLR4 activation and signaling generates serious syndromes such as septic shock, autoimmune, inflammatory and circulatory diseases and some types of diabetes and tumors. Small molecules active in modulating TLR4 activity are promising lead compounds for developing specific therapeutics against infectious and inflammatory pathologies. We synthesized cationic and anionic glycolipids that are active in modulating the TLR4-mediated inflammatory and innate immunity responses to infections and to endogenous stimuli. The mechanism of action of synthetic compounds and nanoparticles active on TLR4 will be discussed in detail.

New insights into the regulation of ILC2s – The enhancement and suppression of allergic lung inflammation

Dr Grace Poon, University of British Columbia, Canada

Allergic asthma is a chronic inflammatory disease with high morbidity and mortality in the industrialized countries. Group 2 innate lymphoid cells (ILC2s) play an essential role in allergic lung inflammation. Here we show that ILC2s display antigen non-specific memory-like functions, which enhance lung allergic responses. Allergen-experienced ILC2s may make individuals sensitive to a broad range of allergens. On the other hand, intranasal LPS pretreatment of mice suppressed both papain- and IL-33-induced ILC2 activation and eosinophilic lung inflammation. Therefore, identifying the inhibitory signal induced by type 1 inflammation may provide a way to limit allergic diseases.

Regulation of the innate immune response by sterile injury and inflammasomes

Pablo Pelegrin, PhD, Principal Investigator Inflammation and Experimental Surgery Research Unit Murcia Biomedical Research Institute (IMIB) Hospital Virgen de la Arrixaca - Foundation for Healthcare Training & Research of the Region of Murcia (FFIS), Murcia, Spain

Inflammatory diseases affect >80 million people worldwide, being the majority infection-free conditions. Innate immunity is the main coordinator and driver of inflammation through the secretion of cytokines upon innate immune cell activation. The activation of purinergic P2X7 receptors in immune cells by extracellular ATP is a novel and increasingly validated “sterile” pathway to initiate inflammation through the activation of the NLRP3 inflammasome and the release of IL-1beta and IL-18 cytokines. Extracellular ATP is a crucial danger signal released by injured cells, and one of the most important mediators of infection-free inflammation.

Innate Immunity in Atherosclerosis

Professor Alexander N. Orekhov, Institute of General Pathology and Pathophysiology, Director Institute for Atherosclerosis Research (Skolkovo), Moscow, Russia

Innate immunity-associated chronic reaction in arterial wall may trigger formation of atherosclerotic lesion. The main participants of innate immunity in atherosclerosis are multiple modified low-density lipoprotein, hematogenous inflammatory cells as well as pluripotent resident cells of the arterial wall also involved in the immune response. Modifies lipoprotein particles form a self-associates that arterial immune cells erroneously recognize as a foreign pathogen and trigger the innate immune response. If the mechanism of the innate immune system is working properly process terminates quickly. In case of breakdown of this mechanism reparative reaction may continue for a long time and transforms into chronic form. Chronic reaction is accompanied by local accumulation of lipids, increased proliferation of arterial cells, hypersecretion of extracellular matrix and violation of cell-to-cell communication that leads to the formation of atherosclerotic lesions.

Ocular Surface Innate Immunity and Dry Eye Disease

Dr Rachel Redfern, Assistant Professor, University of Houston, College of Optometry, Houston, The Ocular Surface Institute, Texas, USA

Dry eye is a common disease that leads to ocular surface discomfort, inflammation and visual disturbances. Despite disruption of the ocular surface, these patients often do not present with ocular infections, suggesting the role of the innate immune sytem to protect the ocular surface from invading pathogens once compromised during dry eye. This presentation will provide an overview of the ocular surface innate immune response and alterations that occur in the dry eye and experimental dry eye animal models.

In vivo expansion of regulatory neutrophils with a GPCR19 agonist ameliorates systemic inflammation

Dr Seung yong Seong, Department of Microbiology and Immunology, Department of Biomedical Sciences, Seoul National University College of Medicine, Korea

Clinical application of the immune regulatory cells is limited by low efficacy of their expansion. In this paper, we demonstrate the dramatic expansion of regulatory neutrophils (RN) in vivo by injecting G-protein coupled receptor 19 (GPCR19) agonist, sodium taurodeoxycholate (STDC), which prolongs survival of mice by suppressing systemic inflammation incurred by LPS. Gene expression pattern and proteome profile of RN from mice under sepsis drastically changed upon STDC treatment. Upregulation of Slamf4 in RN by STDC is involved in their protective roles in septic mice. GPCR19 agonist can be considered as potential therapeutics in sepsis by increase of RN.

Toll-like receptors in Helicobacter pylori infection & immunity

Dr Sinead Smith, Ussher Assistant Professor, Dept. of Clinical Medicine, Dublin, Ireland

Helicobacter pylori infects the stomachs of approximately half of the world's population and is the causative agent of stomach ulcers and gastric cancer. Pathogen recognition receptors of the Toll-like receptor (TLR) family are involved in the innate immune response to H. pylori infection. This presentation will address the role of TLRs in the recognition of H. pylori in distinct cell types, describe the TLRs responsible for the recognition of individual H. pylori components and outline the influence of innate immune activation on the subsequent development of the adaptive immune response.

Oral Presentation Abstracts

Oral presentations will be added after the submission deadline

HEPARIN EFFECT IN ALVEOLAR MACROPHAGES IN ACUTE LUNG INJURY MODEL

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Acute Lung Injury (ALI) is characterized by a promptly release of proinflammatory mediators started by macrophages that propagate the coagulant response. Previous studies have presented the beneficial effect of anticoagulants, not only for their anticoagulation activity but also for their antiinflammatory action.
The aim of the study was to evaluate the effect of local heparin administration in alveolar macrophages (AM) in ALI model.

Male Sprague-Dawley rats (250-300g) underwent intratracheal administration of Lipopolysaccharide (LPS 10 µg/g body weight.) or Saline (0.9%) in control animals. Saline or heparin (1000 IU/kg body weight) were nebulized at constant oxygen flow (2 L/min) before and after LPS administration to evaluate its preventive and therapeutical effect. Animals were sacrificed 24h after the injury and AM from all groups were isolated with a bronchoalveolar lavage. Heparin effect was assessed by the expression of TNFα, iNOS Arginase-1 and IL10. Moreover, neutrophil and monocytic chemoattractant activity of AM was evaluated by CXCL1 and CCL2, respectively. Data are expressed as media ± SEM, and the expression is relative to GAPDH and fold over saline group (n=8 for all the study groups). Statistical analysis was performed using One-Way-ANOVA and Newman Keuls post-hoc test. Statistical significance $p \leq 0.05$ is considered.

In ALI model the expression of all the evaluated markers were increased. Heparin was able to reduce significantly the expression of TNF-alpha (Control: 1±0.4, LPS: 19.1±6.4, Heparin pre: 0.5±0.1, Heparin post: 9±6.4) and Arginase-1 (Control: 1±0.5, LPS: 5.7±1.2, Heparin pre: 0.7±0.2, Heparin post: 2.9±1.1) showing a decrease in the AM activity. Also, AM were implicated with the novo monocytes recruitment into the lung and heparin treatment reduced this recruitment decreasing CCL2 expression (Control: 1±0.4, LPS: 19.7±3.8, Heparin pre: 7.3±4.4, Heparin post: 11.2±4.7). Additionally, iNOS and IL10 were reduced with heparin treatment and no changes were observed in CXCL1.

Altogether indicate that AM have a major role in the ALI development and resolution. During ALI, AM present more activity and the treatment with heparin is able to attenuate this response diminishing the lung injury.

Poster Presentation Abstracts

Poster abstracts will be finalised weeks before the event

INDUCTION OF TYPE I INTERFERON RESPONSES UPON STAPHYLOCOCCUS AUREUS INFECTION IN HUMAN MONOCYTES

Jana Musilova, Trinity Biomedical Sciences Institute, Trinity College Dublin

TLR2 recognizes a wide repertoire of pathogens including Gram-positive Staphylococcus aureus, a major pathogen causing nosocomial infections in hospitals worldwide. Upon recognition of pathogen, TLR2 elicits strong proinflammatory responses. Recently, purified TLR2 ligands and Listeria monocytogenes have been shown to induce type I interferon (IFN) in mouse cells. Similarly, we previously showed that TLR2 activation triggered TRAM-dependent endosomal signalling via a MyD88-IRF7 axis leading to type I IFN in mouse BMDMs. However, in human cells, the TLR2-mediated type I IFN response to S. aureus remains poorly defined, as do the nature of the signalling components downstream of TLR2. Hence, we investigated whether and how S. aureus stimulates type I IFN in human monocytes.

S. aureus infection of THP-1 cells elicited TLR2-dependent TNF and type I IFN. Both cytokine responses were blocked by the viral peptide inhibitor VIPER, which targets Mal and TRAM. Type I IFN but not TNF production required endocytosis and Syk kinase activity. Furthermore, using small molecule inhibitors of IKK-related kinases we showed that S. aureus-triggered type I IFN was dependent on IKK-related kinases. Surprisingly, SA-LTA but not Malp2 mimicked S. aureus-induced responses.

We report an unconventional TLR2-mediated type I IFN response that is relevant for staphylococcal infections in human monocytes suggesting a novel mechanism for S. aureus-induced pathogenesis. Moreover, we demonstrated that considerable differences exist in signalling responses amongst TLR2 ligands in human monocytes.

P2X7 RECEPTOR REGULATES INTERNALIZATION OF ANTIMICROBIAL PEPTIDE LL-37 BY HUMAN MACROPHAGES THAT PROMOTES INTRACELLULAR PATHOGEN CLEARANCE.

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Bioactive peptide LL-37/hCAP18, the only human member of the cathelicidin family, plays important roles in killing various pathogens, as well as in immune modulation. We demonstrate that LL-37 is internalized by human macrophages in a time-, dose-, temperature-, and peptide sequence-dependent endocytotic process. Both clathrin- and caveolae/lipid raft-mediated endocytosis pathways are involved in LL-37 internalization. We find that the P2X7 receptor (P2X7R) plays an important role in LL-37 internalization by human macrophages because significantly less internalized LL-37 was detected in macrophages pretreated with P2X7R antagonists or, more specifically, in differentiated THP-1 cells in which the P2X7R gene had been silenced. Furthermore, this P2X7R-mediated LL-37 internalization is primarily connected to the clathrin-mediated endocytosis pathway. In addition, our results demonstrate that internalized LL-37 traffics to endosomes and lysosomes and contributes to intracellular clearance of bacteria by human macrophages, coinciding with increased reactive oxygen species and lysosome formation. Finally, we show that human macrophages have the potential to import LL-37 released from activated human neutrophils. In conclusion, our study unveils a novel mechanism by which human macrophages internalize antimicrobial peptides to improve their intracellular pathogen clearance.

THE THERAPEUTIC EFFECTS OF MARINE COMPOUND (W) ON INNATE IMMUNITY DISORDER: THE ROLE OF MACROPHAGE IN ATOPIC ECZEMA

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Atopic eczema (AE) has increased in frequency recently. It is a common inflammatory and innate immunity disorder. Pruritus is also regarded as a hallmark of AE. However, there were few medications which can attenuate all forms of AE because the mechanisms remain unclear. Marine compound (W) which is derived from the Formosan soft coral Cladiella australis, was found to exert potent therapeutic ability in the treatment of neuropathic pain, atherosclerosis, and multiple sclerosis in vivo. The aim of the study is to examine the therapeutic effect of W on atopic eczema. In the present study, we used RAW264.7 macrophage cell as in vitro model to examine the possible mechanisms of W in AE by proteomics. BALB/c mice were used to evaluate the effect of W on cutaneous inducers, scratching behavior and the molecule mechanisms. In our data, we found that W significantly inhibited the expression of the pro-inflammatory protein, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), in the lipopolysaccharides (LPS)-stimulated RAW 264.7 cell. The proteomics results showed that W may increase activity of lysosomal-associated membrane protein 1 (LAMP-1) to degrade the unnecessary proteins and activate nucleophosmin (NPM) to exert anti-inflammation. On the other hand, the compound W could alleviate the scratch number in the induction of scratching behavior on BALB/c mice. W also decreases the factors of neuropathic itch such as substance P, transient receptor potential cation channel subfamily V member 1 (TRPV1), histamine and the scoring of AE lesion. Finally, we demonstrated that the anti-atopic eczema of W could be disrupted by inhibiting NPM. Taken together, the present study suggested that W significantly inhibit the symptom of atopic eczema by regulating immunity with anti-inflammation, anti-itch, and anti-oxidative stress. Based on above findings, we suggested that W may become a novel therapeutic potential which have multiple properties to relieve atopic eczema.

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INVESTIGATION OF THE ROLE AND EFFECT OF TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS (TREM-1) IN PARKINSON'S DISEASE IN VIVO AND IN VITRO.

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Abstract:
Parkinson's disease (PD) is one of the most common age-related neurodegenerative diseases after Alzheimer's disease. To date, there are still no entirely effective pharmacological agents or strategies against PD. Several lines of evidences have clearly demonstrated that inflammatory processes contribute to neuronal death in most neurodegenerative diseases including PD. The inflammatory response could amplify neuronal death through triggering receptor expressed on myeloid cells 1 (TREM-1). Previous studies also indicated that attenuation of TREM-1 activity could produce cytoprotective effects through reduced inflammation. Preliminary observations suggest that both 6-hydroxydopamine-induced neuronal cell death and lipopolysaccharide-induced microglial inflammation are significantly inhibited by a synthetic peptide blocker of TREM-1, LP17. Thus, we strongly suspect that the modulation of TREM-1 activity may be a novel therapeutic pathway for PD. We attempt to clarify the cellular mechanisms of the neuroprotective or anti-neuroinflammatory effects of attenuation of TREM-1. Furthermore, the present study also aims to evaluate the effects of the TREM-1 antagonist peptide, LP17, in PD-rats. Finally, we hope to offer experimental evidence to support future pre-clinical or clinical studies of PD.

INTRATHECAL CORAL-DERIVED COMPOUND PROMOTES AN ALTERNATIVE PATHWAY OF MACROPHAGE ACTIVATION AFTER EXPERIMENTAL SPINAL CORD INJURY IN RAT

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Traumatic spinal cord injury (SCI) often leads to permanent disability to the patients. The marine-derived natural cembranolide compound NERV-1 was isolated from the Formosan soft coral. In our previous studies NERV-1 significantly decreases the expression of the pro-inflammatory proteins inducible nitric oxide synthase and cyclooxygenase-2 in activated microglial cells. Moreover, it attenuates neuronal cytotoxicity and apoptosis. NERV-1, MP, or saline (control) were administered by intrathecal injection to female Wistar rats after a thoracic spinal cord (T10) contusion injury induced by NYU impactor. Compared to MP- or saline-treated animals, NERV-1-treated animals showed significantly better locomotor function recovery after SCI. The M1 (inducible nitric oxide synthase (iNOS)-positive, CD16/32-positive) and M2 (arginase 1-positive, CD206-positive) phenotype of microglia was determined by immunohistology. After SCI the iNOS-positive, CD16/32-positive M1 microglia was the predominant phenotype in the injured spinal cord of saline-treated animals, NERV-1 treatment promoted arginase 1-positive, CD206-positive M2 microglia activation. In addition, NERV-1 attenuated the SCI-induced upregulation of pro-inflammatory proteins (ICAM-1, iNOS, TNF-alpha, and IL-1beta) and attenuated SCI-induced microglia activation at 7 days after SCI. Moreover, NERV-1 enhanced BDNF, GDNF and VEGF expression in normal and injured spinal cord. We suggestion the benefit effects of NERV-1 may related to the alternative pathway of macrophage activation, and we believe this compound may be a promising therapeutic agent for SCI.

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TLR2-DEPENDENT TYPE I INTERFERON INDUCTION BY STAPHYLOCOCCUS AUREUS IN HUMAN MONOCYTES

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TLR2 recognizes a wide repertoire of pathogens including Gram-positive Staphylococcus aureus, a major pathogen causing nosocomial infections in hospitals worldwide. Upon recognition of pathogen, TLR2 elicits strong proinflammatory responses. Recently, purified TLR2 ligands and Listeria monocytogenes have been shown to induce type I interferon (IFN) in mouse cells. Similarly, we previously showed that TLR2 activation triggered TRAM-dependent endosomal signalling via a MyD88-IRF7 axis leading to type I IFN in mouse BMDMs. However, in human cells, the TLR2-mediated type I IFN response to S. aureus remains poorly defined, as do the nature of the signalling components downstream of TLR2. Hence, we investigated whether and how S. aureus stimulates type I IFN in human monocytes.

S. aureus infection of THP-1 cells elicited TLR2-dependent TNF and type I IFN. Both cytokine responses were blocked by the viral peptide inhibitor VIPER, which targets Mal and TRAM. Type I IFN but not TNF production required endocytosis and Syk kinase activity. Furthermore, using small molecule inhibitors of IKK-related kinases we showed that S. aureus-triggered type I IFN was dependent on IKK-related kinases. Surprisingly, SA-LTA but not Malp2 mimicked S. aureus-induced responses.

We report an unconventional TLR2-mediated type I IFN response that is relevant for staphylococcal infections in human monocytes suggesting a novel mechanism for S. aureus-induced pathogenesis. Moreover, we demonstrated that considerable differences exist in signalling responses amongst TLR2 ligands in human monocytes.

ENTRY OF FRANCISELLA TULARENSIS INTO B CELLS

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Francisella tularensis, the causative agent of tularemia and a potential biowarfare agent, is a facultative intracellular pathogen causing zoonotic disease in a wide variety of species. Both in vitro and in vivo, Francisella spp. infect and proliferate inside phagocytic cell types. Francisella tularensis also infects non-phagocytic hepatocytes, epithelial cells, and B cell lines. Information available about immune response to Francisella spp. comes from studies on natural human infections or immunizations as well as from animal model studies.

Francisella tularensis enters into B cells. B cells have a wide range of functions within the immune response, including recognition of antigens, antigen presentation to competent T cells, production of cytokines and antibodies, and contribution to the development of immunological memory. The entrance of F. tularensis into B cells requires both the active participation of bacteria and engagement of the B cell receptor and the other receptors we analyzed. Entrance of the bacteria into B cells occurs through ligation of B cell receptor and complement receptor 1/2. Our experiments have shown that B cell receptor and complement receptor 1/2 are involved in ex vivo recognition and engulfment of Francisellae into B cells. The complement receptors 3 and 4 and the FcγR receptor are not involved in these processes. The effect of entry of the blocking B cell receptor on CD19+ cells or separate B cell subsets demonstrated that B cell receptor alone is sufficient for the entry of F. tularensis into B-1a cells only. Intracellular trafficking of F. tularensis inside B cells is distinct from intracellular trafficking within other antigen-presenting cells. Once inside phagocytic cells, Francisellae escape from the phagosome into the cytosol where they proliferate. On the other hand, living Francisellae can be localized in the membrane surrounding the vacuole inside B cells. They do not proliferate there. The trafficking of F. tularensis was determined by detecting colocalization of the bacteria with the early endosome antigen EEA-1, late endosomal/lysosomal membrane marker LAMP-1, and cathepsin D.

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DISTRIBUTION OF AN OPHAN CYTOSINE METHYLASE (CPG) GENE IN LISTERIA MONOCYTOGENES 4B STRAINS

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L. monocytogenes is an intracellular pathogen that is responsible for the life threatening disease listeriosis in humans. Due to its ubiquitous nature and transmission in food, human exposure to L. monocytogenes is high but incidence of the disease is low. However it is a major health threat because of the high mortality rates; listeriosis has the highest fatality rate (16.9%), and caused 30% of deaths due to food-borne pathogens between 1996 and 2005 (FoodNet US). Individuals with an intact immune system will clear that bacterium rapidly, experiencing only mild flu-like symptoms or gastroenteritis. It is the immuno-compromised, including pregnant women, infants, the elderly and those with underlying disease that are most commonly affected. Previous studies in our laboratory have identified an orphan cytosine methylase gene (designated lcmA) in some serotype 4b strains of L. monocytogenes which is associated with a unique partial CpG DNA methylation pattern. Since unmethylated bacterial DNA is recognised by the host as a Pathogen-Associated Molecular Pattern (PAMPs), it is possible that this phenotype represents a mechanism by which the bacterium can avoid the cell-mediated immune response of the host, resulting in acute infection.

The methylase gene responsible for this phenotype was cloned and was found to be acquired as a three gene insertion in the bacterial genome. In this study PCR has been used to determine if (a) other serotype 4b isolates that demonstrate partial CpG methylation contain the same lcmA gene, and (b) if it is located at the same site in all strains. Results showed that the lcmA gene sequence was detected in four other CpG methylation-positive strains, but a gene homologue was not detected in all strains tested. In addition analysis of published genome sequence found that that this gene has been identified in other L. monocytogenes genomes located at the same site.

THE DECAPEPTIDE PROTHYMOSIN α(100-109) AS A POTENTIAL BIOMARKER OF BACTERIAL INFECTION

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The aim of this study was to develop a sensitive and specific immunoassay for the quantification of proTα(100-109), the C-terminal decapeptide of the TLR-4 agonist prothymosin alpha. Using high affinity purified polyclonal antibodies, we developed a competitive ELISA for proTα(100-109) and determined its concentration: (1) in vitro, in supernatants of cells driven to apoptosis; (2) in vivo, in the serum of mice infected with two different strains of Klebsiella pneumoniae, and (3) ex vivo in human sera. Our results showed that the levels of proTα(100-109) were higher in culture supernatants of apoptotic cells, whereas low concentrations were detected in the corresponding control supernatants. In the in vivo model of lethal septicemia, CD-1 (ICR) mice were intraperitonealy injected with a clinical isolate of Klebsiella pneumoniae (L-78) that is endocytosed by monocytes/macrophages, or a prototype Klebsiella pneumoniae strain (ATCC43816), which is not endocytosed. In the serum of L-78-infected animals, we observed a gradual increase of the concentration of proTα(100-109) and the highest levels were detected 48 h post-infection. Spleen macrophages from these mice showed increased percentages of early apoptotic cells at initial time points post-infection and massive apoptosis/necrosis at later time points. On the contrary, in sera of mice infected with the ATCC strain, an increase in proTα(100-109) concentration was detected 3 h post-infection and the levels of the decapeptide remained stable during the course of infection. Spleen macrophages of these mice were mainly driven to necrosis. Finally, we assessed the levels of proTα(100-109) in sera of healthy individuals, pediatric patients and patients hospitalized in intensive care units (ICU). The levels of proTα(100-109) in the serum of healthy individuals were very low and independent of age or sex. Children hospitalized due to infection had higher concentrations of the decapeptide in their blood serum, compared to children diagnosed with autoinflammatory diseases, who had low proTα(100-109) concentration. Serum samples from ICU patients were serially collected from admission to exit and showed variable proTα(100-109) concentrations during the hospitalization only in patients who were admitted with or developed bacterial infections. These high proTα(100-109) levels correlated with clinical symptoms and laboratory markers of sepsis. In contrast, the concentration of proTα(100-109) was minimal in the serum of ICU patients who did not develop an infection. Our results suggest that proTα(100-109) could be used as a sepsis biomarker. Moreover, the concentration of proTα(100-109) in blood serum may be considered as a surrogate marker for the differential diagnosis between septic and aseptic inflammation.

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ESCULENTIN-1a(1-21)NH2: A PROMISING PEPTIDE FOR PREVENTION AND ERADICATION OF PSEUDOMONAS AERUGINOSA BIOFILM FORMATION ON SOFT CONTACT-LENSES

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Contact lens wear is an important risk factor for microbial keratitis, a potentially vision threatening infection of the eye (1). Adverse events associated with microbial adhesion and colonization of lenses, especially by the biofilm forming Gram-negative bacterium *Pseudomonas aeruginosa* remain a major safety issue. This is further complicated by the increase in bacterial resistance to traditional antibiotics. Therefore, novel strategies to prevent and treat contact lens-associated keratitis are greatly needed. An important approach is the development of agents that hamper pathogen attachment and biofilm formation in the first place. Naturally occurring antimicrobial peptides (AMPs) hold particular promise in this regard. Esculentins are a family of AMPs derived from amphibian skin with a wide spectrum of antimicrobial activity. Esculentin-1a(1-21)NH2 [Esc(1-21)], that consists of the first 20 amino acids of the native Esculentin-1a, with a glycinamide residue at the C-terminus, is a novel AMP with a potent activity against

both free-living and sessile forms of *P. aeruginosa* (2). Previous studies have shown its anti-*Pseudomonal* activity in the presence of human basal tears as well as a reduction of infection in a mouse model of *P. aeruginosa* keratitis (3). Here we investigated the peptide's ability to inhibit and/or to disrupt biofilm formation on soft contact lenses using both reference strains and clinical isolates of *P. aeruginosa*. The percentage of surviving cells was evaluated by the 3(4,5-dimethylthiazol-2yl)2,5-diphenyltetrazolium bromide (MTT) assay. Our results indicate that Esc(1-21) is able to eradicate biofilm cells from contact-lenses at a concentration range of 4-16 μM, and to inhibit *Pseudomonas* biofilm when used at lower concentrations. The effects of Esc(1-21) on the morphology of biofilm cells on contact lenses were also visualized by scanning electron microscopy. Overall, our data suggest that Esc(1-21) has great potential for development as a novel pharmaceutical for prevention and treatment of contact lens-associated *P. aeruginosa* keratitis.

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HELICOBACTER PYLORI--INDUCED TIGHT JUNCTION DISRUPTION IN GASTRIC EPITHELIAL CELLS

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Disruption of gastric epithelial cell-cell tight junctions is considered to be an initial event contributing to gut pathogenesis. Aberrant expression and redistribution of various tight junction proteins are hall marks in gastric cancer tissues. Though H. pylori has been shown to cause tight junction disruption, the mechanism remains elusive. This study examines the host and bacterial factors involved in H. pylori-induced tight junction disruption leading to host pathogenesis.
The highly expressed tight junction protein in gastric tissues, Claudin-4 was found to be mis-localized from the cell junctions to cytoplasm and nucleus in H. pylori-infected polarized and non-polarized gastric epithelial cells. In the process, activation of ERK was found to induce barrier dysfunction, claudin-4 delocalization and deregulation of claudin-4 expression in H. pylori- infected cells. Concurrently, Gamma-Glutamyl Transpeptidase (GGT), a H. pylori virulence factor, was found to collaborate with claudin-4 in compromising barrier integrity. Following RNA-Seq, differential gene expression analyses showed the involvement of GGT in regulating gene expression of tight junction proteins and associated signalling molecules. The interactome analyses revealed sets of highly interacting complexes vital in determining host cell fate in response to H. pylori-infection. Taken together, this study reveals the role of H. pylori GGT-induced activation of ERK-assocaited signalling pathways.in tight junction disruption.

A NOVEL NATURAL COMPOUND IS EFFECTIVE IN SEVERE SEPSIS

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The incidence of sepsis has steadily increased during the last few decades. However, the development of targeted therapy has been hampered because bacteria trigger the activation of multiple pro-inflammatory pathways. Here, we have investigated the effects of SNU304, a novel compound derived from natural products, on mouse models of sepsis. SNU304 has shown to protect mice against sepsis induced by LPS or CLP (cecal ligation and puncture). SNU304 administration also reduced blood pro-inflammatory cytokines level, such as TNF-α, IL-1β, IL-6, MCP-1, and IFN-γ. Blood pressure of the SNU304-treated mice showed a significant recovery from 4 hours after LPS challenge. SNU304 was also able to inhibit the expression of costimulatory molecules on DCs in LPS-induced sepsis mice, which might be involved with alleviation of the physiological outcome of sepsis. In conclusion, SNU304 mitigated the development of severe sepsis by modulating immune system.

FUNCTIONAL VARIANTS OF TLRs AS A TOOL FOR THE CONTROL OF BOVINE INFECTIONS BY BREEDING

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Historical breeds of farm animals, conserved in frame of the genetic resources programmes, are considered to be a source of utilizable allelic variants of disease resistance genes. In contrast to modern production breeds, the spectrum of alleles is supposed to reflect infection pressure specific for the geographic region and breeding conditions. Screening for the diversity of innate immunity receptors belonging to the Toll-like receptor (TLR) family has been carried out in two historical cattle breeds (Czech Red and Czech Red Pied). The advantages of the PacBio NGS platform were used for facilitated discovery of sequence polymorphism and haplotype structure in pooled amplicons from the coding regions of ten bovine TLRs. The validation of detected SNPs was performed with Sanger sequencing and genotyping techniques (PCR-AFLP, ARMS, SnapShot primer extension). In the limited population of two herds, most of the known variability of TLR genes has been detected, comprising 150 SNPs distributed in ten genes. The phasing of SNPs was based both on the calculation by the PHASE programme and on the long reads that are characteristic for the PacBio platform. The length of most of the reads corresponded to the length of amplicons, varying from 700 to 1200 nt. Partially, in the case of TLR4 eight SNPs were validated in the coding and adjacent regions, yielding 18 haplotypes. This represents a markedly higher diversity than reported for the European production breeds. Moreover, differences in the frequency of haplotypes were observed between the two breeds. This finding corresponds to the phenotypic differences of the local breeds and to the speculative association of the Czech Red breed with the short-horn aurochs subspecies. A significant proportion of the observed variants is associated with predicted changes in protein structure or implies other consequences for function. These include Thr674Ile change in the transmembrane/TIR border of TLR4 and a deletion in the TLR5 promoter region. In a number of SNPs present in the followed population, a beneficial effect on infection resistance has been demonstrated in published studies. An additional guide how to get the animals from the genetic resources to the commercial breeding schemes will be obtained from the survey of TLR polymorphism in the production populations of the Czech Red Pied breed. The results also confirmed the advantages of using the PacBio technology for resequencing of the genes of interest. The limited capacity of runs is outweighed by the length of reads, single-strand sequencing and a low error rate, which are helpful in SNP discovery and phasing.