organ. Studies with Mx2-luciferase reporter mice, which express luciferase upon IFNAR triggering, revealed significant IFNAR triggering within the liver and intermediate triggering in secondary lymphoid organs. Of note, viral titers in the liver of infected IFNAR−/− mice were increased when compared to WT mice. Additionally, VACV infected IFNAR−/− mice showed dramatically enhanced cytokine responses as well as elevated liver enzyme levels in the serum. Histological analysis of liver of VACV infected WT mice revealed that such mice developed mild hepatitis around day 5 post infection. In contrast, livers of infected IFNAR−/− mice showed signs of severe hepatitis, massive influx of lymphocytes and inflammatory islets including acute necrotic areas. VACV infection of macrophage-specific IFNAR−/− (Ly5M-Cre+×IFNARfl/fl) mice revealed that IFNAR signaling of macrophages was needed to protect mice from severe liver damage, whereas IFNAR signaling of hepatocytes (Alb-Cre+×IFNARfl/fl) [mice] was dispensable. Collectively our results indicated that locally induced IFN-γ plays a crucial role in balancing cytokine responses and were necessary to trigger macrophage-mediated liver protection.

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13 Crosstalk between interferon-beta, foam cell formation and inflammatory responses

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Macrophage-derived foam cells are critical components of atherosclerotic lesions and the ways in which the inflammatory response of foam cells influences atherogenesis is of great interest. Previously we demonstrated that interferon-beta (IFN-β) promotes atherogenesis. But how IFN-β influences foam cell formation and inflammation is not understood yet. Hence, we assessed the functional involvement of IFN-γ in these processes in a normal versus high cholesterol environment. First, we performed a microarray study on IFN-β-stimulated bone marrow-derived macrophages (BMDM5) under normocholesterolemic conditions, which showed upregulation of immune response pathways and downregulation of cholesterol biosynthesis. Secondly, we loaded BMDMs with acLDL followed by 6 h IFN-β treatment, which surprisingly impaired the induction of IFN-γ target genes, like CCL5 and CXCL10. To validate these findings in vivo, LDLR−/− mice were put on normal chow (NC) or a high cholesterol diet (HCD) for 10 weeks. Peritoneal macrophages (PEMs) were collected 4 days after intraperitoneal thioglycolate administration, combined with IFN-β (5000 U/ml) or PBS administration 24 and 8 h before sacrifice. Lipid loading increased following IFN-β treatment, accompanied by increased scavenger receptor-A (SR-A) gene expression. This lipid loading also resulted in PEM IFN-γ-hyporesponsiveness, since several IFN-γ target genes were again less expressed compared to NC PEMs. In addition, ex vivo culturing of PEMs from IFN-β-treated animals on HCD versus NC showed an overall decreased inflammatory activity, as gene expression of inflammatory markers was reduced and secretion of IL-6, TNF and NO was decreased. Currently we assess how HCD interferes with IFN-γ-induced effects by investigating the IFN-γ-induced activation of transcription factors like STAT1, IRF3 and IRF9 in control and acLDL loaded BMDMs. Altogether, IFN-β promotes foam cell formation, possibly by increased lipid influx. Interestingly, lipid loading results in hyporesponsiveness to IFN-γ. More research is needed to reveal the biological relevance for this hypercholesterolemia-induced hyporesponsive state.

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14 Previous food allergy aggravates allergic markers and intestinal damages in a mouse model of asthma

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Aims: Increasing clinical data suggest a link between food allergy and the later development of respiratory allergy. This progression may be triggered by exposures to different allergens but the mechanism implicated remains unknown. This study aimed to identify the impact of a first exposure to food allergen on the development of a new form of allergy caused by exposure to a novel allergen using a mouse model.

Method: In our model, mice were intraperitoneally sensitized to wheat proteins (modified gliadins) to induce a systemic response, then they were exposed orally to the same allergen and finally they were intranasally exposed to HDM (Dermato-phagoides farinaceae extract) a respiratory allergen without adjuvant to assess an impact on lung mucosa.

Results: After food and respiratory allergen exposures, mice displayed stronger amount of blood markers: IgE specific and histamine. Moreover, splenocyte secretion of IL-4 and IL-17 were increased whereas weaker levels of IFNγ were observed in parallel Peyer patches lymphocytes secreted higher amount of IL-4 with a decreased in IFNγ, IL-10 and TGF-β productions. These mice exhibited intestine damages, higher paracellular flux and modification of transcellular permeability. In contrast, hyper-responsiveness, inflammatory cells and cytokines in lung remained unchanged compared to the respiratory allergy model.

Conclusion: We show that dual exposure induces a raise in specific IgE and local Th2 and Th17 cytokines secretion before triggering phase. During the latter, gut morphology and functions were affected but not lung in dual exposed mice compared to single one underlying the organospecific impact. Altogether, our data make a step further in the elucidation of the mechanisms linking allergy history to immunological and clinical status potentially linked to atopic March development.

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15 The role of cytokines in the cerebrospinal fluids of patients with Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME)

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Objectives: Previous research has provided evidence for a dysregulation in cytokine levels in the periphery of patients with Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME). To date few studies have examined cytokines in the cerebrospinal fluid. The purpose of this research is to examine the role of cytokines in the symptom presentation of CFS/ME patients.

Methods: Cerebrospinal fluid (CSF) was collected from 18 CFS/ME patients and 5 healthy controls. The CSF samples were examined for the expression of 27 cytokines [interleukin (IL)-1β, IL-1α, IL-2, IL-4, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17, basic FGF, eotaxin, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1 (MCAF), MIP-1α, MIP-1β, PGDF-BB, RANTES, TNF-α and VEGF] using the bio-plex human cytokine 27-plex assay.

Results: Of the cytokines examined, only four were significantly reduced in the CFS/ME patients in comparison to the controls.

Conclusions: The results show a decrease in pro-inflammatory cytokines in the CSF of CFS/ME patients and this may contribute to the clinical disease progression.

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16 Deregulated gp130/Stat3 signalling in lung cancer development

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Lung cancer (LC) is the most common and lethal cancer worldwide and, in Australia, accounts for ~7500 deaths annually. A causal correlation between LC and cigarette smoking is well established, however only 10–15% of smokers develop LC, suggesting there are other ill-defined genetic, epigenetic and/or environmental factors which predispose individuals to LC. Regarding the former, interleukin (IL)-6 signal via the gp130 signal-transducing receptor subunit to primarily activate the latent transcription factor STAT3, as such, the pro-inflammatory and oncogenic properties of the IL-6 gp130/STAT3 signalling axis can be established. Since IL-6 expression and STAT3 activity are up-regulated in human LC, we explored the downstream consequences of increased IL-6/STAT3 activity in the well documented Kras[G12D] mouse LC model. For this purpose, we utilised gp130[F/F] (FF) mice that carry a knock-in mutation in gp130 leading to deregulated IL-6/STAT3 signalling, and crossed these mice with the LoxP-Stop-LoxP Kras[G12D] mice which harbour a conditionally-activated oncogenic Kras allele. Inheritance of Fc:Kras[G12D] and WT:Kras[G12D] mice with a Cre recombinase adenovirus activated the Kras allele at 6 week of age, and mice were observed over 6 weeks. There was a severe and diffuse development of invasive adenocarcinoma in situ (AIS) in the lungs of Fc:Kras[G12D] mice only. The percentage density of lesions in the lungs of Fc:Kras[G12D] mice was increased compared to WT:Kras[G12D] littermate controls, and associated with an increase in the number of proliferative cells and inflammatory infiltrates (determined by immunohistochemistry). In contrast, partial suppression of deregulated IL-6/STAT3 signalling by crossing Fc:Kras[G12D] mice onto an IL-6−/− or Stat3−/− background led to a recovery of normal lung tissue and a decrease of both proliferative and inflammatory cells.
These data show that deregulated gp130/STAT3 signalling in this mouse model drives the development of AIS and invasiveness in the lungs, and that this increased severity of AIS is proliferation-driven.

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17 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

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Interleukin (IL)-3, granulocyte–macrophage colony stimulating factor (GM-CSF) and IL-5 are important cytokines that control production and function of myeloid cells and dendritic cells. Over-expression of these cytokines or their receptors can lead to chronic inflammatory diseases and myelo- and dendritic cell leukemias. These cytokines signal through heterodimeric specific receptors consisting of cytokine-specific a chains, and a signalling subunit b, shared by all three receptors. We have determined the 3D crystal structure of the binary GM-CSF/GM-CSF receptor a chain (GMR a) complex to 2.8 Å resolution, which reveals for the first time all three extracellular domains of the GMR a receptor chain. The structure shows a striking resemblance to the related IL-3R, IL-5R and IL-13R a/b subunits; however, the differing positions of the N-terminal domains suggest that this domain may play a cytokine-specific role in cell signalling. We have used mutagenesis, ligand binding and functional studies to examine residues identified through higher order complex assembly. Furthermore, the complete structure of the GMR a receptor chain from the binary complex was used to improve the electron density maps of the partial GMRs in the previously published ternary complex, elucidating additional GM-CSF/GMR a interactions and the position of the N-terminal domain of GMRs in the ternary complex. A structural comparison of the GM-CSF binary and ternary complexes revealed numerous major, as well as subtle, conformational changes in cytokine and both receptor chains. These studies enable us to identify differences and similarities in the way GM-CSF, IL-3 and IL-5 interact with their receptors and facilitate different types of cell signalling events.

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18 The inflammasome adaptor ASC mediates gastric tumourigenesis independent of inflammation

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Pre-cancerous inflammation is well established in the pathogenesis of gastric cancer, however the individual molecular pathways involved in this process are yet to be fully elucidated. Individually, both the potent pro-inflammatory cytokine IL-1b and the oncogenic latent transcription factor STAT3 are known to play a role in the development of gastric cancer. Using a STAT3-driven preclinical mouse model for gastric tumourigenesis (gp130(−/−)), human gastric cancer specimens and in vitro techniques, we have established a link between STAT3-driven tumour burden and the inflammasome, the latter representing a multi-protein inflammatory complex that controls the production of mature, biologically-active forms of IL-1b and IL-18. The effector molecules of inflammasome activation, namely activated caspase-1, IL-1b and IL-18 proteins, are upregulated in STAT3-driven gastric tumours. Genetic ablation of the inflammasome adaptor ASC reduced tumour burden by up to 50%, independent of inflammation and tumour cell proliferation. Rather, we have determined that the suppressed tumourigenesis is associated with increased tumour (epithelial) cell apoptosis in gp130(−/−) mice null for the Asc gene. Furthermore, using bone marrow chimeras we have shown that the reliance on ASC for gastric tumourigenesis is independent of bone marrow-derived hematopoietic (immune) cells expressing ASC. Finally, in tumours from gp130(−/−) mice, as well as in human gastric tumour biopsies, the expression of specific inflammasome-activating pattern recognition receptors are elevated overall, our results uncover a role for the inflammasome in the development of gastric cancer.

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19 Ellagic acid hydroxypropyl-ß-cyclodextrin inclusion complex alleviates adjuvant-induced arthritis: Attenuation of oxidative stress and inflammatory mediators

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Aim: The present study was designed to investigate the effect of ellagic acid hydroxypropyl-ß-cyclodextrin inclusion complex (EAHßCD) in adjuvant-induced arthritis in rats.

Methods: Adjuvant arthritis (AA) was induced by single subplanter injection of 0.1 ml Freund’s complete adjuvant (FCA) into foot pads of left hind paw of Wistar rats. Thirty-six healthy male rats were randomly divided into six groups with six rats each. From day 0 after FCA injection, EAHßCD group (10 mg/kg and 20 mg/kg), ellagic acid group (10 mg/kg) and standard diclofenac group (5 mg/kg) were administered by oral gavage for 28 consecutive days. The disease progression was examined by analysis of arthritis score, paw oedema volume, body weight, joint diameter, index of thymus and spleen, locomotor function, hyperalgesia and histological changes in joints. Further serum levels of nitric oxide (NO), glutathione (GSH), superoxide dismutase (SOD), IL-6 and TNF-α were evaluated.

Results: Subplanter injection of FCA significantly (P < 0.001) increase in arthritis score, paw volume and joint diameter, while daily oral administration of EAHßCD (10 mg/kg and 20 mg/kg) significantly (P < 0.001) attenuated the disease progression and associated hyperalgesia. The macroscopic and histopathological evaluation of knee joints were also improved in EAHßCD and diclofenac treated AA rats. The serum levels of oxidative stress markers (NO, GSH and SOD) and cytokines (TNF-α and IL-6) were restored in treatment groups.

Conclusion: The present study provides evidences of anti-arthritic potential of EAHßCD, which is mediated by attenuation of hyperalgesia, oxidative stress and pro-inflammatory cytokines (TNF-α and IL-6) in FCA-induced arthritic rats.

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20 Neutralising anti-G-CSF monoclonal antibody blocks neutrophil-driven inflammatory joint disease

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Granulocyte colony-stimulating factor (G-CSF) is a haemopoietic cytokine first identified for its role in steady state granulopoiesis but is now also recognised as a proinflammatory mediator of mature neutrophil activation and function. G-CSF binds to the G-CSF receptor (G-CSFR), which is present on neutrophils and haematopoietic progenitors. G-CSF-deficient mice are protected from disease in several models of rheumatoid arthritis, and blockade of G-CSF is also protective in those models. To further investigate the actions of blocking G-CSF/G-CSFR signalling in inflammatory disease, we developed a neutralising monoclonal antibody to mouse G-CSFR which prevents the binding of G-CSF and inhibits G-CSFR signalling. In the anti-collagen antibody induced arthritis model, anti-G-CSF treatment rapidly halved the progression of established disease. This therapeutic effect was observed at low dose and was associated with transient suppression of peripheral blood neutrophilia. Anti-G-CSFR treatment significantly inhibited neutrophil accumulation in the joints without rendering animals neutropenic, suggesting an effect of G-CSFR blockade on neutrophil homing to inflammatory sites. Consistent with this, neutrophils in the blood and arthritic joints of anti-G-CSF treated mice showed alterations in homing receptors, with reduced CXCR2 and increased CD62L expression. Microarray analysis of neutrophils from joints and peripheral blood showed a common up-regulation of 6 genes and down-regulation of 22 genes following anti-G-CSF treatment. Blocking neutrophil traffic to joints with anti-G-CSF suppressed local production of proinflammatory cytokines (IL-1b and IL-6) and chemokines (KC and MCP-1) known to drive tissue damage. These data show for the first time the effect of blockade of G-CSFR in a therapeutic model of inflammation and provide further support for utilising this approach in the treatment of inflammatory diseases such as rheumatoid arthritis.

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