10

Inflammation

10-01/P

OPPOSITE REGULATION OF IL-1β AND SIL-1RA PRODUCTION BY PI3KS IN HUMAN MONOCYTES ACTIVATED BY LPS OR CELL-CONTACT WITH T CELLS

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The unbalanced production of IL-1β and its secreted inhibitor sIL-1Ra plays an important role in chronic inflammation. Relevant to this condition, direct cellular contact with stimulated T cells is a potent inducer of cytokine production in human monocytes/macrophages. We previously demonstrated that activation of PI3 kinases (PI3Ks) controls the transcription of sIL-1Ra gene in IFNβ-activated monocytes. Here we assess the involvement of PI3Ks in the production of IL-1β and sIL-1Ra in monocytes activated by cellular contact with stimulated T cells (mimicked by CHAPS-solubilized membranes of stimulated T cells, CE_{sHUT}), or LPS, in order to compare stimuli involved in conditions relevant to chronic and acute inflammation, respectively. In both conditions, PI3Ks controlled the induction of sIL-1Ra gene transcription. In contrast, the PI3K inhibitor, Ly294002, increased the production of IL-1β protein, in both CE_{sHUT}- and LPS-activated monocytes, the enhancement being drastically higher in the former condition. This was not due to changes in IL-1β mRNA steady-state levels and transcript stability. The determination of intracellular and secreted IL-1β levels suggests that PI3Ks were mainly implicated in the repression of IL-1β release when cells were activated by LPS, whereas in CE_{sHUT}-activated monocytes PI3Ks repressed both IL-1β protein secretion and yet-undefined mechanism. Together, the present results demonstrate that PI3Ks differentially regulate IL-1β and sIL-1Ra production in isolated human blood monocytes by controlling different mechanisms in conditions mimicking chronic/sterile (CE_{sHUT}) and acute/infectious (LPS) inflammation. Thus PI3Ks represent key effectors that might be uncontrolled in pathological conditions. This further suggests that the stimulation of PI3K pathways may be an effective approach for preventing or treating chronic/sterile or dysregulated acute inflammation.

10-02/O

DIFFERENT MOLECULES AT THE SURFACE OF STIMULATED T CELLS INDUCE IL-1B, TNF AND SIL-1RA IN HUMAN MONOCYTES

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Imbalance in cytokine homeostasis plays an important part in the pathogenesis of chronic inflammatory diseases such as multiple sclerosis and rheumatoid arthritis. Stimulated T cells exert a pathological effect through direct cellular contact with monocytes/macrophages, inducing a massive up-regulation of IL-1 and TNF in the latter cells. This mechanism that might be relevant to chronic inflammation is inhibited by high-density lipoproteins (HDL). Like many other stimuli, besides pro-inflammatory cytokines, contact-mediated activation of monocytes induces the production of cytokine inhibitors such as sIL-1Ra. Here we demonstrate that HDL inhibit the production of IL-1β and TNF but not that of sIL-1Ra in monocytes activated by membranes isolated from stimulated T cells to mimic cellular contact. Similarly, sIL-1Ra mRNA expression was not inhibited contrary to IL-1β and TNF mRNA. This demonstrates that different molecules at the surface of stimulated HUT-78 cells are involved in the induction of IL-1β, TNF and sIL-1Ra in monocytes, IL-1β and TNF being activated by ligand(s) of HDL. Separation of CHAPS-solubilized membrane molecules by liquid isoelectric focusing showed that 2 activity peaks were present, one activating IL-1β, TNF and sIL-1Ra production, the other inducing the production of sIL-1Ra in the absence of IL-1β and TNF. Further isolation of these two types of factor by gel filtration demonstrated that factor(s) inducing IL-1β, TNF and sIL-1Ra displayed a M_r around 40,000 kD, whereas factors inducing sIL-1Ra in the absence of IL-1β and TNF displayed M_r around 30,000 kD, the latter being unaffected by HDL. Thus different factors are expressed at the surface of stimulated T cells that differentially trigger the production of pro- and anti-inflammatory cytokines and are differently affected by HDL. This further confirms the anti-inflammatory function of HDL.

10-03/P

CORTICOSTEROIDS IN THE COMPLEX MANAGEMENT OF NECROTIZING PANCREATITIS: INFLUENCE ON CYTOKINES AND ADHESION MOLECULES

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Despite the potent anti-inflammatory and immunosuppressive properties of glucocorticoids its applying in the management of severe necrotizing pancreatitis is still controversial.
The plasma levels of interleukins (IL-6, IL-8, and IL-18) and adhesion molecules (E-selectin and ICAM-1) were measured in 48 patients with necrotizing pancreatitis who admitted in clinic within 48 hours after disease onset. The measurement was performed immediately after admission, at the 3, 7, and 14 day. All patients were divided on two groups: first group compiled 26 patients, in which dexamethasone (24 mg/day during 4-6 days) was applied in the complex management of acute pancreatitis, and control group – 22 patients that did not receive corticosteroids. All patients received the initial therapy, which included adequate fluid replacement, pain medication, proteases inhibitors, pentoxifylline, as well as the administration of antibiotics. The increased levels of IL-6, IL-8, IL-18, ICAM-1, and E-selectin were noted in both groups of patients at the time of admission. The signs of MODS were present in the 10 patients of the first group and in the 9 patients of the control group. The gradually increase of all proinflammatory mediators plasma levels up to seventh day was noted in patients of the control group. Its levels clear correlated with the severity of MODS and spreading of necrotic processes confirmed by CT. Starting from the third day the gradually decrease of mediators levels were noted in the patients of the first group. The incidence of contamination of necrotic foci had no difference in both groups of patients (23.1% in the first and 22.7% - in the control group).
The ability of glucocorticoids to inhibit expression of proinflammatory mediators due to the glucocorticoids-mediated repression of NF-kappa B pathway provide the pathogenetic substantiation for the applying of glucocorticoids in the complex management of necrotizing pancreatitis.

10-04/P

TNF-ALPHA IN ONTOGENIC AND RADICULAR CYSTS

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Odontogenic cysts are one of the commonest bone destroying lesions of the maxillofacial skeleton, while radicular cyst are result from inflammatory process in the periapical tissues. TNF-α is pleiotropic cytokine that is considered a primary modifier of inflammatory and immune reaction in response to various diseases. It is induce stimulation of osteoclastic bone resorption and helps fibroblast to release collagenase. Based on these multiple effects we investigated its values in 53 radicular cysts and 15 odontogenic keratocysts, obtained from patients undergoing surgery. The cysts were surgically enucleated under local anaesthesia, mucoperiostal flap was raised and cystic fluid was aspirated from non-ruptured cysts, than immediately centrifuged to remove cells and supernatant was stored at -70˚C until use in ELISA assay. TNF-α was analyzed in respect to clinical symptoms, cystic diameter, localization, presence fistules, radiographic and histological examinations as well (section from each cysts was stained with hematoxylin-eosin to access presence of inflammatory cells). We found that TNF-α is elevated in both cysts, but higher values was found in radicular cyst in comparison to keratocysts (Mann Whitney U-test, $p < 0.05$). Higher values of TNF-α was associated with smaller radicular cyst (diameter below 2 cm), higher protein concentration, and higher presence of inflammatory cells in peri cystic tissues (significant correlation was found between elevated TNF alpha and presence of leukocytes in inflammatory infiltrate under epithelia cell layer, Spearman correlation, $p < 0.05$). No correlation was found, based on these parameters in odontogenic keratocyst, but all cyst have high values of TNF-α. We concluding that determination of TNF-α simultaneous with other clinical parameters can help in classification, earlier and better diagnosis between these two cystic types.

10-05/P

CROHN'S DISEASE-ASSOCIATED NUCLEOTIDE OLIGOMERIZATION DOMAIN (NOD)2^{3020INSC} MUTANT IS A NOVEL REGULATOR OF IL-10 GENE EXPRESSION

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Nucleotide-binding oligomerization domain 2 (NOD2) is a monocyte/macrophage-restricted member of a protein family critically involved in host defense against enteric bacterial infection. Dysfunctional mutations in the *NOD2* gene are strongly implicated in the pathogenesis of Crohn's disease (CD), which is a Th1-medaited inflammatory disease in the gastrointestinal tract. One of the most common mutations in the *NOD2* gene associated with CD is an insertion and frame shift mutation in the leucine-rich repeat domain, designated NOD2^{3020insC}. Two recent human studies demonstrated defective IL-10 production in response to Toll-like receptor (TLR) 2-mediated signaling in mononuclear cells of CD patients homozygous for NOD2^{3020insC}, implying that it is a loss-of-function mutation. The impaired IL-10 production may contribute to the loss of control of chronic inflammation in the intestinal mucosa. However, our recent study indicates that NOD2^{3020insC} is a gain-of-function mutant that can actively suppress constitutive and microbe-stimulated IL-10 production in macrophages. We investigated how NOD2^{3020insC} affects the transcription of the IL-10 gene in macrophages, and have uncovered highly interesting mechanisms involved in this pathway. Our study reveals a novel gain-of-function of the disease-causing NOD2 mutant and the underlying molecular basis, which may have significant implications on the etiology and pathogenesis of CD.

10-06/P

SELECTIVE PROTECTION OF CD8+ T CELLS FROM ACTIVATION-INDUCED CELL DEATH BY IL-18: EFFECT OF IL-18 ON CD4/CD8 RATIO IN ACTIVATED T CELLS

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Effect of IL-18 on viability of activatedCD8+ T cells and on CD4/CD8 ratio was examined. Viability of spleen cells activated by immobilized anti-CD3 antibody(Ab) was much less in the cells from IL-18 knockout (KO) than in the cells from wild type (WT) mice, which was reversed by exogenously added IL-18. The decreased viability of IL-18KO cells was largely due to the decreased number of live CD8+ T cells. Since there was not significant difference in the viability of CD4+ T cells, the CD4/CD8 ratio in the culture was higher in IL-18KO than in WT cells. Expression of IL-18 receptor (R) β was strongly augmented in the activated CD8+ T cells, and correspondingly, the viability of WT CD8+ cells that were activated was impaired by blockade of IL-18 R. When examined by CFSE dilution test, it was shown that IL-18 alone failed to promote proliferation of naïve CD8+ T cells, but promoted viability of CD8+ T cells without affecting cell division and thus increased size of group of proliferating cells. Levels of phosphorylated Akt and synthesis of Bcl-2 in activated IL-18KO CD8+ T cells were augmented by exogenous IL-18, and inversely, they were reduced by blockade of IL-18R in WT cells. Blockade of IL-18R or removal of IL-18 from the culture decreased viability without affecting cell division, and the treatment altered surface markers such as CD94, NKG2A, CD122, and CD28 in CD8+ T cells. Correspondingly to these *in vitro* observations, CD4/CD8 ratio in T cells from IL-18KO mice treated with Propionibacterium(P.) acnes was shown to be much increased when compared with that from WT mice treated with P. acnes. Thus, IL-18 was shown to play a role in selective protection of activated CD8+ T cells from AICD. IL-18 may affect immune/inflammatory responses by influencing homeostasis of CD8+ T cells.

10-07/P

HYPERINSULINAEMIA UP-REGULATES IL-10 LEVELS DURING HUMAN ENDOTOXAEMIA

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Infectious diseases cause significant morbidity and mortality among type 2 diabetes patients. Evidence exists that intensive insulin treatment to obtain euglycaemia in septic patients on intensive care units reduces overall mortality. To investigate the selective effects of hyperglycaemia and hyperinsulinaemia on cytokine production in a human model of systemic inflammation, 24 healthy humans were studied for eight hours during one of the following experiments: (1) lower insulinaemic euglycaemic clamp, (2) lower insulinaemic hyperglycaemic clamp, (3) hyperinsulinaemic euglycaemic clamp and (4) hyperinsulinaemic hyperglycaemic clamp. In the hyperglycaemic clamps target levels of plasma glucose were 12 mmol/l *versus* 5 mmol/l in the euglycaemic clamps. In the hyperinsulinaemic clamps target plasma insulin levels were 400 pmol/l *versus* 100 pmol/l in the lower insulinaemic clamps. Three hours after initiation of clamping, 4 ng/kg of *E.coli* endotoxin was injected intravenously. Endotoxin injection induced sharp rises in all plasma cytokine concentrations. Hyperinsulinemia especially in the presence of euglycemia led to increased interleukin (IL)-10 levels. Between the four clamps we found no difference in plasma concentrations of tumour necrosis factor (TNF)-α, IL-6 and IL-8. As IL-10 is a major anti-inflammatory cytokine, these data imply that hyperinsulinemia may have an anti-inflammatory effect during a state of systemic inflammation.

10-08/P

CO-INFECTION WITH *BORRELIA BURGDORFERI* SENSU STRICTO AND *BORRELIA GARINII* ALTERS THE COURSE OF MURINE LYME BORRELIOSIS.

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Ixodes ricinus, the European vector for Lyme borreliosis, and also mammalian hosts can be simultaneously infected with *Borrelia burgdorferi* sensu stricto and *Borrelia garinii*. The effect of co-infection of these *Borrelia* species on the outcome of Lyme borreliosis has never been investigated. We investigated whether co-infection with *B. garinii* strain PBi - infectious but non-arthritogenic - and *B. burgdorferi* sensu stricto strain B31 - infectious and arthritogenic - alters Lyme borreliosis in Lyme susceptible C3H/HeJ and Lyme resistant C57BL/6 mice. We infected mice with either strain alone, simultaneously with both strains or with PBi followed by B31.
The level of B31 spirochetes in both C3H/HeJ and C57BL/6 mice was significantly higher in mice simultaneously or sequentially infected with PBi and B31 than in control mice infected with B31 spirochetes alone. In contrast, the number of PBi spirochetes was significantly lower in co-infected mice as compared to controls infected with PBi alone. Spirochete levels were measured by species specific quantitative PCR. Co-infected C3H/HeJ mice showed significantly more paw swelling and arthritis, and C57BL/6 mice simultaneously infected with PBi and B31 prolonged paw swelling. Anti-B31 total IgG measured in serum by ELISA and several pro-inflammatory and Th1/Th2 cytokines measured in serum by cytometric bead arrays showed similar patterns in co-infected mice and control mice.
We conclude that co-infection of PBi and B31 spirochetes results in more severe murine Lyme borreliosis. Moreover, in nature, competition between *B. burgdorferi* sensu stricto and *B. garinii* within the host could have important implications, since this could cause *I. ricinus* feeding on co-infected animal reservoirs to take up *B. burgdorferi* sensu stricto rather than *B. garinii*. This could contribute to the preferential maintenance of *B. burgdorferi* sensu stricto in European *I. ricinus* populations, increasing the risk of human *B. burgdorferi* sensu stricto infection over time.

10-10/P

INVOLVEMENT OF PAI-1 IN HOST DEFENSE AGAINST *MYCOBACTERIUM TUBERCULOSIS*

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Plasminogen activator inhibitor type 1 (PAI-1) is the main inhibitor of the fibrinolytic system. Recently, endogenous PAI-1 was demonstrated to influence the T helper 1/T helper 2 balance during allergic rhinitis. Aim of our study was to determine the contribution of PAI-1 to the immune response to *Mycobacterium (M.) tuberculosis*. Therefore, C57BL/6J mice (WT) and PAI-1 gene deficient ($PAI^{-/-}$) mice were intranasally inoculated with *M. tuberculosis*. At 2 and 5 weeks after infection lungs, tracheobronchial lymph nodes, liver and spleen were harvested for measurement of colony forming units (CFUs), lung cell differentiation, cytokine and chemokine levels. Although no differences in bacterial growth were detected 2 weeks post-infection, $PAI^{-/-}$ mice displayed higher mycobacterial loads in the lungs after 5 weeks of infection. Interestingly, $PAI^{-/-}$ mice demonstrated increased cell numbers in the lung early during infection. At both time points lung infiltrates of $PAI^{-/-}$ mice consisted of a reduced percentage of neutrophils and an increased percentage of lymphocytes, whereas the percentage of macrophages in $PAI^{-/-}$ mice was lower only late during infection. This was accompanied by relatively more $CD8^+$ cells in the lungs of $PAI^{-/-}$ mice, of which a higher proportion was activated ($CD69^+$). There were no differences in production of the protective cytokine IFN-γ at the site of the infection, however splenocytes harvested from $PAI^{-/-}$ mice 5 weeks after infection showed an increased capacity to produce IFN-γ upon antigen-specific recall stimulation. Interestingly, both pulmonary pro-inflammatory cytokine levels (tumor necrosis factor-alpha), as well as anti-inflammatory cytokine levels (interleukin (IL) -4 and IL-10), and chemokine levels (cytokine-induced neutrophil chemoattractant (KC)) were reduced in $PAI\text{-}1^{-/-}$ mice. Taken together, $PAI^{-/-}$ mice demonstrate an altered inflammatory response in the lung during tuberculosis which is associated with enhanced mycobacterial outgrowth.

10-11/P

IFN-ACTIVATED NEUTROPHILS PRODUCE SOLUBLE TNF-RELATED APOPTOSIS-INDUCING LIGAND (TRAIL/ APO-2 LIGAND)

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TNF-related apoptosis-inducing ligand (TRAIL/Apo-2 ligand) is a member of the TNF superfamily that is expressed either as a type II transmembrane protein, or as a soluble form (sTRAIL) generated through enzymatic shedding or released in association with microvesicles. TRAIL exerts selective apoptotic activities towards tumor and virus-infected cells, as well as immunoregulatory functions on activated T lymphocytes, by interacting with a complex system of two death receptors (DR4/TRAIL-R1 and DR5/TRAIL-R2).
In this study, we demonstrate that upon incubation with therapeutic concentrations of IFNα, human neutrophils are induced to accumulate TRAIL mRNA and release sTRAIL that fully retains pro-apoptotic

activities towards TRAIL-sensitive leukemic cell lines. We also show that other agonists such as TNFα, lipopolysaccharide, formyl-metionyl-leucyl-phenylalanine (fMLP), CXCL8/IL-8, G-CSF and GM-CSF fail to up-regulate TRAIL gene expression and soluble protein release in freshly purified neutrophils. More recent experiments reveal that only a minor fraction of the total TRAIL newly synthesized by IFN-activated neutrophils is released outside, the rest being retained in intracellular compartments. Studies aimed at determining the intracellular localization of TRAIL and its eventual mobilization are ongoing. Interestingly, we have also observed that sTRAIL serum levels as well as leukocyte-associated TRAIL significantly increase following IFNα administration to melanoma patients, further highlighting the possible participation of neutrophils to TRAIL-mediated tumor immunosurveillance *in vivo*. Collectively our findings indicate that sTRAIL released by IFN-activated neutrophils may contribute not only to the immunoregulatory actions but also to the therapeutic activities of IFNα.

10-12/O

REQUIREMENT FOR THE ADIPOCYTE FATTY ACID BINDING PROTEIN AP2 IN ALLERGIC AIRWAY INFLAMMATION

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The adipocyte fatty acid binding protein aP2 regulates systemic glucose and lipid metabolism. In addition to its abundant expression by adipocytes, we report that aP2 is also expressed by human airway epithelial cells, and shows a striking upregulation following stimulation of epithelial cells with the Th2 cytokines IL-4 and IL-13. Regulation of aP2 mRNA expression by Th2 cytokines was highly dependent on STAT6, a transcription factor with a major regulatory role in allergic inflammation. We examined aP2-deficient mice in a model of allergic airway inflammation, and found that infiltration of leukocytes, especially eosinophils, into the airways, was highly dependent on aP2 function. T cell priming was unaffected by aP2 deficiency suggesting that aP2 was acting locally within the lung, and analysis of bone marrow chimeras implicated non-hematopoietic cells, most likely bronchial epithelial cells, as the site of action of aP2 in allergic airway inflammation. Thus, aP2 regulates allergic airway inflammation and may provide a link between fatty acid metabolism, diet, and asthma.

10-13/P

COMBINED TREATMENT WITH INTERFERON GAMMA AND TUMOR NECROSIS FACTOR ALPHA INDUCES APOPTOSIS OF MOUSE OSTEOBLASTIC CELL LINE MC3T3-E1 VIA DOWN-REGULATION OF BLC-2

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Interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) are the pro-inflammatory cytokines that affect bone remodeling in chronic inflammatory diseases such as periodontal disease. In the case of inflammatory bone loss, the skeletal homeostasis balance between bone formation and bone resorption is disrupted. Excessive bone resorption by osteoclast and reduced bone formation by osteoblast have been shown to result in bone loss in the osseous tissue microenvironment. To determine the molecular mechanism by which pro-inflammatory cytokines reduce bone formation in osteoblast, we examined the effect of IFN-γ and TNF-α on the cell growth and survival of the mouse osteoblastic cell line MC3T3-E1. Although IFN-γ or TNF-α alone had only a marginal effect on the growth and viability of the cells, co-treatment with these cytokines synergistically inhibited cell growth and reduced cell viability. The diminished cell viability was due to apoptosis as demonstrated by increased Annexin V and TUNEL staining and increased caspase 3, 8, and 9 activities. The cytotoxic effect of co-treatment with IFN-γ and TNF-α was significantly blocked by *N*-acetyl-L-cysteine, a thiol antioxidant and a precursor of intracellular glutathione, suggesting that oxidative stress and/or redox status plays a role in apoptosis. Western blot analysis showed that co-treatment with IFN-γ and TNF-α down-regulated Bcl-2 protein levels without affecting Bax expression and induced cytochrome c release from mitochondria to cytoplasm. Furthermore, over-expression of Bcl-2 in a stable transfectant of MC3T3-E1 cells attenuated the cytokine-induced apoptosis. These results indicate that IFN-γ and TNF-α-induced apoptosis of the mouse osteoblastic cells is, at least partially, mediated by oxidative stress and/or intracellular redox status, which triggers down-regulation of Bcl-2 expression and the subsequent activation of caspases.

10-14/P

COAGULATING PLASMA RAPIDLY INDUCES IL-8 PRODUCTION BY HUMAN LUNG EPITHELIAL CELLS

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In pulmonary inflammatory diseases, like pneumonia and adult respiratory distress syndrome (ARDS), capillary damage can lead to plasma leakage into the alveolar compartment, causing coagulation on the pulmonary epithelial surface. Since activation of the coagulation system can induce inflammation, this process could result in an enhancement of the pulmonary inflammatory response. To investigate the potential of coagulating plasma to induce an inflammatory response in the pulmonary epithelial compartment we compared the production of the pro-inflammatory chemokine interleukin-8 (IL-8) by human alveolar epithelial cells (A549 cell line) after three hour incubation with heat killed bacteria, pro-inflammatory cytokines, thrombin, or eight-fold diluted coagulating plasma. While heat killed bacteria did not induce IL-8 generation and thrombin only elicited a small IL-8 response, coagulating plasma induced a production of IL-8 as potently as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). The anticoagulant proteins activated protein C (APC) and tissue factor pathway inhibitor (TFPI) had no effect on the responses elicited by IL-1β and TNF-α but inhibited the plasma induced IL-8 production, indicating involvement of the coagulation process. Plasma fibrinogen depletion potently inhibited IL-8 production, indicating a role for fibrin formation in the coagulating plasma induced IL-8 production. Fibrin clots generated by co-incubation of purified thrombin and fibrinogen failed to drive IL-8 production, indicating that a fibrin dependent product or process is involved, like fibrinolytic enzymes or products. In conclusion, coagulating plasma induces IL-8 production by human lung epithelial cells to the same extent as IL-1β and TNF-α. This process is dependent on the formation of fibrin and possibly on activation of the fibrinolytic system. We conclude that in inflammatory conditions in which plasma leakage occurs, like in pneumonia or ARDS, treatment with anticoagulant proteins like APC or TFPI may counteract, by preventing clot formation, the coagulation induced enhancement of pulmonary inflammation.

10-15/P

FAS LIGAND ELICITS A CASPASE-INDEPENDENT PRO-INFLAMMATORY RESPONSE IN HUMAN ORGANOTYPIC MODEL OF ECZEMATOUS DERMATITIS

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Fas ligand (FasL) causes apoptosis of epidermal keratinocytes and triggers the appearance of spongiosis in eczematous dermatitis. We demonstrate here that FasL also aggravates inflammation by triggering the expression of pro-inflammatory cytokines, chemokines, and adhesion molecules in keratinocytes. In HaCaT cells and in reconstructed human epidermis (RHE), FasL triggered a nuclear factor-kappaB (NF-kB)-dependent mRNA accumulation of inflammatory cytokines (TNF-alpha, IL-6, and IL-1beta), chemokines (CCL2/MCP-1, CXCL1/GROalpha, CXCL3/GROgamma, and CXCL8/IL-8), and the adhesion molecule ICAM-1. Inhibition of caspase activity prevented FasL-triggered apoptosis and epidermal barrier disruption; however, it failed to suppress the expression of FasL-induced genes. Our findings identify a novel pro-inflammatory role of FasL in keratinocytes that is independent of caspase activity and is separable from apoptosis. Thus, in addition to causing spongiosis, FasL may play a direct role in triggering and/or sustaining inflammation in eczemas.

10-16/P

TPA GENE DELETION LEADS TO HIGHER BACTERIAL LOAD AND HIGHER CYTOKINE LEVELS IN EXPERIMENTAL PYELONEPHRITIS

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Acute pyelonephritis, most frequently caused by *E. coli*, forms a considerable health problem and can lead to end stage renal failure. Tissue-type plasminogen activator (tPA) is a potent fibrinolytic agent, but can exert immunomodulatory effects as well. Recently we described impaired neutrophil migration in tPA knock-out (tPA-/-) mice during renal ischemia reperfusion injury. Since data on the role of tPA during pyelonephritis are lacking, we investigated the role of tPA in experimental urinary tract infection.
We induced pyelonephritis in tPA-/- (n=8 per time point) and C57BL/6 wild type (WT) mice (n=8 per time point) by intravesical inoculation with 10^9 CFU uropathogenic *E. coli 1677*, isolated from a febrile uroseptic patient. Mice were sacrificed 24 and 48 hours after inoculation. Kidneys were harvested, homogenized partly in sterile saline for determination of bacterial outgrowth on blood agar plates and partly in lysis buffer for measurement of TNF-α, IL-1β and myeloperoxidase by ELISA.
At both time points tPA-/- mice showed significantly more bacterial outgrowth in kidney homogenates compared to WT mice (24 hrs: $8.6x10^3 \pm 4.2x10^3$ CFU/mL *versus* $0.2x10^3 \pm 0.1x10^3$ CFU/mL ($p < 0.005$); 48 hrs: $9.2x10^2 \pm 4.9x10^2$ CFU/mL *versus* $0.7x10^2 \pm 0.5x10^2$ CFU/mL ($p < 0.05$), mean ± SEM). At both time points, concentrations of TNF-α and IL-1β in kidney homogenates were significantly higher in tPA-/- mice compared to WT mice. Despite more bacteria and inflammatory cytokines in tPA-/- mice, neutrophil influx was not different between both types of mice. Blood cultures remained negative in all mice.
The present study shows that deletion of the tPA-gene in mice leads to a relative impairment in neutrophil influx, associated with significantly more bacterial outgrowth and higher levels of TNF-α and IL-1β during experimental pyelonephritis.

10-17/P

SERUM CYTOKINE EXPRESSION ACCORDING TO TREATMENT RESPONSE OF CHRONIC HEPATITIS B VIRUS INFECTION

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Cytokines are known to play pivotal roles in the pathogenesis of chronic Hepatitis B virus (HBV) infection. The association between the cytokine profiles and the sequence of the disease phases or clinical factors has been studied in detail so far. However, the relationship between cytokines and treatment response of drugs for chronic hepatitis B is not clearly defined. In the present study, we measured the serum cytokine levels of IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, VEGF (Vascular Endothelial Growth Factor), INF-γ, TNF-α (Tumor Necrosis Factor-alpha), MCP1 (Macrophage/Monocyte Chemotactic Protein 1), and EGF (Epidermal Growth Factor) to elucidate the cytokine expression pattern according to the patients' response to Lamivudine, one of the most common therapeutic agents for chronic hepatitis. Fifty-nine specimens from twenty-seven patients with chronic HBV infection and specimens from 20 healthy individuals were tested. The patients were classified into four groups: patients before treatment, patients with ongoing treatment, patients maintaining remission, and patients with recurrence due to resistance against Lamivudine. A CCD (Charge Coupled Device) which quantifies cytokines by detecting signals from the commercially available protein chip (Randox, Antrim, UK) was used for the detection of serum cytokines. All cytokine values were higher in the patient groups than in the control group. IL-6, IL-8, IL-10, and TNF-α were elevated in patients with recurrence due to resistance against Lamivudine compared to patients maintaining remission and patients with ongoing treatment. Serial analyses for the cytokine values in the same patients showed similar tendencies. In summary, serum cytokine values well reflected the pathological differences of the individual treatment phases, and may become useful indices in monitoring the treatment response of chronic hepatitis B virus infection.

10-18/P

ACUTE PHASE RESPONSE IMPAIRS HOST DEFENSE AGAINST ENTEROCOCCUS FAECIUM PERITONITIS IN MICE

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Multiresistant *Enterococcus faecium* is an important cause of hospital-acquired infections. In particular patients with a pre-existing illness are susceptible to infections with *E. faecium*. Such predisposing diseases are almost invariably associated with an acute phase protein response. We here tested the hypothesis that a sterile acute phase response renders the host more vulnerable to *E. faecium* infection. Therefore, C57BL/6 mice were injected subcutaneously with turpentine or saline (control) in both hind limbs one day before intraperitoneal infection with 10^8 CFU *E. faecium*. Mice were sacrificed at different time points up to one week after infection to determine bacterial loads and immune responses. Turpentine induced a sterile acute phase protein response as reflected by a transient weight loss and strong increases in the plasma levels of serum amyloid P and C3. Whereas control mice cleared the enterococci from the primary site of infection (peritoneal fluid), the liver, lungs and blood within 72 hours, turpentine-injected mice demonstrated a strongly delayed clearance with positive cultures from all body compartments except blood up to one week after infection. The turpentine-induced acute phase response resulted in a reduced influx of granulocytes to the peritoneal cavity during *E. faecium* peritonitis. Both turpentine and saline injected mice showed a modest cytokine response that was not different between groups with the exception of IL-6, which was slightly increased in the former group at 48 hours. These data suggest that a pre-existing sterile acute phase protein response, such as occurs after trauma or major surgery, impairs host defense against *E. faecium* peritonitis at least in part by attenuating granulocyte recruitment to the primary site of infection.

10-19/O

CHARACTERISATION OF THE ROLE OF 12/15-LIPOXYGENASE IN REGULATING MACROPHAGE PHENOTYPES IN INFLAMMATION

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Growing evidence indicates that 12/15-lipoxygenase (LOX) plays important roles in immunobiology. In this study, we characterized the role of 12/15-LOX in regulating leukocyte migration in murine peritonitis *in vivo*. Basally, 95% of resident peritoneal macrophages of wild type C57BL/6 mice ($CD11b^{hi}$) express 12/15-LOX while 5% are negative. Phenotypic characterisation by FACS showed that 12/15-LOX^{-ve} cells express higher levels of mannose receptor, dectin-1, CX_3CR1, CSF-R1 and -R2, CCR5, MHC II, CD11c and MARCO, in contrast to 12/15-LOX^{+ve} cells which express lower levels of dectin-1, and CSF-R1/2, are very low/negative for the other markers, but express higher levels of F4/80 ($F4/80^{hi}$) and SR-A. Peritoneal lavages from naïve 12/15-$LOX^{-/-}$ mice had significantly higher numbers of $CD11b^{hi}$, $F4/80^{hi}$ peritoneal macrophages (149 %), but similar levels of $CD11b^{hi}$, $F4/80^{med}$ macrophages and lymphocytes to wild type mice. This suggests that expression of 12/15-LOX exerts selective negative effects on migration and/or differentiation of the resident macrophage population. Analysis of mononuclear cell infiltration during *Staphylococcus epidermidis* supernatant (SES)-induced peritonitis in wild type mice showed a decrease in 12/15-LOX-expressing macrophages during the first 3 hours followed by infiltration of 12/15-LOX^{-ve} monocytes/macrophages which peaked at 24 hrs. 12/15-$LOX^{-/-}$ mice displayed the same kinetics of macrophage migration, but with a 2-fold increase in the number of recruited 12/15-LOX^{-ve} monocytes/macrophages persisting from 1-7 days. In contrast, neutrophil and lymphocyte migration into the peritoneal cavity was unaffected by 12/15-LOX deletion. CCL2/MCP-1 and CCL5/RANTES were 50% decreased in peritoneal lavage of 12/15-$LOX^{-/-}$ mice during inflammation, indicating that suppression of macrophage migration by 12/15-LOX did not involve inhibition of macrophage chemokine generation. These data show two distinct resident macrophage phenotypes in the mouse peritoneal cavity, distinguished by differential expression of a number of immune regulatory molecules, including 12/15-LOX. Furthermore, 12/15-LOX inhibits monocyte/macrophage infiltration during inflammation, consistent with an immunosuppressive role for this enzyme.

10-20/P

IL-22 IS A REGULATOR OF INFLAMMATION

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IL-22 is a cytokine that is made by T cells and signals *via* its receptor into epithelia and some fibroblasts in solid tissues. The IL-22 receptor is not present on naïve or *ex vivo* activated immune cells from either blood or secondary lymphoid tissues. IL-22 effects gene expression indicative of an acute phase response and is reported to have an anti-inflammatory function in the liver. We report here that IL-22 can also be pro-inflammatory in function. With *i.p.* administration to mice, recombinant IL-22 transiently augmented within hours the number or amount of neutrophils, serum amyloid A (SAA) and GROα chemokine in the blood. We propose these to be direct effects of IL-22 since the induction of TNFα, IL-1β and IL-6 by IL-22 was not detected at either the RNA or protein level. Infection of mice with replication-defective adenovirus that expresses IL-22 demonstrated longer-term effects in the blood: subtle decreases in red blood cell parameters with a corresponding and sustained increase in platelets, SAA and fibrinogen levels in the blood. We asked whether IL-22 might have a role in the context of an autoimmune disease and determined that IL-22 gene expression and protein is detected in the paws and blood, respectively, from mice that have collagen-induced arthritis. To determine whether IL-22 has a pathogenic role in disease, two antibodies – a rat IgG1κ and a human IgG1λ – were made that neutralize the activity of murine IL-22 cytokine in cell-based assays. These IL-22 antibodies block the progression of arthritis in this model. We are currently looking for biomarkers that correlate with IL-22 activity and antibody treatment. In conclusion, our results demonstrate that IL-22 has pro-inflammatory properties and that blocking this cytokine is efficacious. From a clinical perspective, these results indicate that IL-22 represents a novel therapeutic target in autoimmune and inflammatory diseases.

10-21/P

AGING CAUSES DEFECTS IN MACROPHAGE SIGNALING THAT ARE LIMITED TO TLR-MEDIATED PATHWAYS.

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We recently reported that macrophages from aged mice produced less tumor necrosis factor (TNF)-α and interleukin-6 (IL-6) after stimulation with lipopolysaccharide (LPS) than macrophages from young animals. This diminished cytokine production correlated with decreased levels of phosphorylated and total p38 and c-Jun N-terminal kinase (JNK) mitogen-activated protein kinases (MAPKs). In the present study, we chose to determine if advanced age also affects other Toll-like (TLR) and non-TLR signaling pathways in macrophages. We found that LPS- and zymosan-stimulated TNF-α and IL-6 production is attenuated in splenic macrophages from aged mice compared to young. Conversely, LPS-treatment, but not zymosan-stimulation, resulted in elevated production of IL-10 by macrophages from aged mice relative to young. In contrast to LPS stimulation, IL-2 treatment induced production of TNF-α and IL-6 by macrophages was not affected by age. The age-associated changes did not correlate with alterations in the cell-surface expression of TLR2, TLR4, or IL-2Rβ. Macrophages from aged mice have lower levels of p38 MAPK and MAPK-activated protein kinase (APK)-2 activation. Protein expression of p38, but not MAPK-APK-2, was reduced with age. Additionally, nuclear factor (NF)-κB activation was significantly decreased in macrophages from aged mice after exposure to LPS, but not IL-2. These data reveal that age-associated changes in macrophage signaling transduction are pathway-specific and suggest that TLR-mediated pathways are impaired with age at the level of MAPK expression. (Supported by NIH AG18859, NIH AG18859-S1, an Illinois Excellence in Academic Medicine Grant, and the Dr. Ralph and Marion C. Falk Medical Research Trust).

10-22/O

A ROLE FOR THE PHAGOSOME IN TNF SECRETION

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Two critical events in innate immunity necessitate intracellular membrane traffic; phagocytosis of pathogens and secretion of proinflammatory cytokines. We have indentified a novel and shared membrane trafficking pathway that unites both of these actions – an adaptation designed to economize on membrane transport and enhance the immune response (Murray et al, Science 2005). Our approach to mapping the intracellular trafficking pathway for TNF was to identify specific vesicular machinery that functions in this pathway using gene expression data to screen for LPS-regulated trafficking proteins and a

series of assays to investigate the localisation, protein interactions and functional role (using siRNA and dominant negative proteins) of the candidate proteins in TNF secretion. We found TNF to be transported in a two-step process from the Golgi in vesicular carriers to the recycling endosome and then with recycling endosome membrane to the cell surface. We have identified key membrane fusion proteins (SNAREs) that regulate and are rate-limiting for this process, one such protein VAMP3 has also been shown to mediate the polarized delivery of recycling endosome membrane to the cell surface for phagocytic cup formation. We found that TNF is delivered with recycling endosome membrane specifically to the phagocytic cup and is rapidly cleaved prior to its closure and internalisation. This presents both an unexpected site for cytokine secretion and a rapid and efficient mechanism for release of an early response inflammatory mediator. Recent studies have also shown further specialisation of this site of fusion in the role of cholesterol dependent lipid rafts.

10-23/P

TISSUE FIBROSIS FOLLOWING RECURRENT INFLAMMATORY FLARES IS ASSOCIATED WITH A DISTORTION IN THE BRIDGE BETWEEN INNATE AND ACQUIRED IMMUNITY: A DEFINING ROLE FOR INTERLEUKIN-6

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The bridge between innate and acquired immunity represents a crucial checkpoint in the resolution of any inflammatory response. During acute inflammation this event is defined by a transition from neutrophil to mononuclear cell recruitment. Any distortion of this process is therefore likely to skew the immune response and affect the inflammatory outcome. To test this hypothesis we have developed a recurrent model of peritoneal inflammation in which to examine sequential changes in inflammatory activation and the development of tissue fibrosis. By following the profile of leukocyte infiltration through four rounds of peritoneal inflammation in wild type mice it is evident that the temporal appearance of the T-cell infiltrate becomes progressively quicker, and following repeated rounds of activation coincides with the initial neutrophil influx. This alteration in leukocyte trafficking is associated with increased fibrosis of the peritoneal membrane. Development of these inflammatory hallmarks was also accompanied by progressive changes in the profile of IL-1β, KC, IL-6, γ-IFN and TGFβ expression. We have previously reported that IL-6 promotes transition from innate to acquired immunity, whilst it is known that IL-6-deficient mice remain largely resistant to experimental models of chronic disease. Significantly, IL-6-deficient mice showed no evidence of peritoneal fibrosis follow repeated peritoneal inflammation, whilst the natural boundary between neutrophil and lymphocyte migration remained intact. Subsequent analysis of transcription factor activation within peritoneal membranes from wild type and IL-6-deficient mice provided a potential mechanism for these changes, and suggested that the balance between STAT1 and STAT3 activity is crucial for defining the inflammatory outcome. Consequently, progression to a chronic inflammatory setting is accompanied by a distortion in the IL-6 control of the transition from innate to acquired immunity.

10-24/P

MODULATION OF LEUKOCYTE RECRUITMENT BY IL-6 IN A REPEAT FLARE MODEL OF ARTHRITIS

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Although factors governing the chronic and sporadic nature of RA remain poorly defined, it is evident that recurrent inflammatory episodes and prolonged maintenance of leukocytes within the joint contribute to disease progression. We have previously shown that interleukin (IL)-6 in association within its soluble receptor promotes disease activity *in vivo* through regulation of mononuclear leukocyte infiltration. Using a repeat-flare model of antigen-induced arthritis we have examined how recurrent inflammatory episodes affect leukocyte trafficking and determined the involvement of IL-6 in this process. Consecutive rounds of AIA led to a significant exacerbation of joint inflammation in WT mice. This resulted in both increased joint swelling and histological changes in the profile of leukocyte recruitment and overall joint destruction. Specifically, following the second and third inflammatory episode leukocyte recruitment appeared to be distorted with evidence of increased maintenance within the joint. Such progressive changes in joint inflammation were not however seen in IL-$6^{-/-}$ mice. Alteration in disease progression however appeared to be driven by the inflamed synovium since markers of activation (including CD62L, CD44 and IL-6R) on circulating leukocytes remained unchanged in both WT and IL-$6^{-/-}$ mice. Induction of AIA did however cause a decrease in sIL-6R levels only in WT mice following inflammatory insult. Collectively, these data suggest that IL-6 (and sIL-6R) are important for regulating the degree of leukocyte infiltration into an arthritic joint, and that recurrent inflammatory episodes appear to distort or dysregulate the IL-6 control of these processes leading to chronic joint destruction and retention of the inflammatory infiltrate within the inflamed synovium. Such findings endorse a rationale for targeting IL-6 or its soluble receptor as a therapeutic strategy.

10-25/O

CONSTITUTIVE DELETION OF JUN PROTEINS IN THE EPIDERMIS LEADS TO A TNF-α DEPENDENT DISEASE

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We have recently shown that the inducible deletion of the Jun proteins, c-Jun and Jun B, in the epidermis of adult mice leads to a psoriasis-like disease with arthritis [1]. We next investigated the consequences of constitutive epidermal deletion of these two Jun proteins. Mice lacking both c-Jun and JunB die at post-natal day 1 with a phenotype similar to cachexia. Glycogen and fat reservoirs in the newborn mice were absent providing a possible explanation for the lethal phenotype.
Further analyses indicated that the pups apparently exhibit no skin barrier defect, however, increased levels of cytokines were detected in the serum as well as in the epidermis. It is known that elevated TNF-α secretion can give rise to cachexia, hemorrhage, necrosis and ultimately death. Since high levels of TNF-α were also detected in the skin of epidermal *c-Jun JunB*-deficient adult mice, we decided to analyze the role of TNF-α signaling in these mutant pups. The soluble form of TNF-α was increased in the serum of newborns as well as in the epidermis, although TNF-α transcription was unaffected. When proteins implicated in the conversion of the membrane bound to the soluble form of TNF-α were analyzed a drastic transcriptional down-regulation of TIMP-3, a potent and specific inhibitor of TACE (TNF-α Converting Enzyme) was detected. As a consequence, TACE activity was found increased. This molecular pathway could also be reproduced *in vitro* by Adeno-Cre-mediated deletion of both *c-Jun* and *JunB*. The skin of mutant newborns showed infiltrates of neutrophils, macrophages and T-cells. Moreover, increased levels of cytokines regulated by TNF-α as well as increased RelA phosphorylation were detected. Finally, the lethal phenotype could be genetically rescued in a TNFR1 null background. In conclusion, c-Jun and JunB proteins control TNF-α signaling through transcriptional regulation of TIMP-3 further demonstrating that these AP-1 proteins are essential regulators for skin homeostasis.

Reference

1. Zenz R, Eferl R, Kenner L, *et al*. Psoriasis-like skin disease and arthritis caused by inducible epidermal deletion of Jun proteins. *Nature* 2005; 437: 369-75.

10-26/P

EFFECT OF HYPOXIA ON MACROPHAGE POLARIZATION

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Cell adaptation to hypoxia requires activation of transcriptional programs that coordinate expression of genes involved in oxygen delivery (*via* angiogenesis) and metabolic adaptation (*via* glycolisis). During migration and invasion of normal and pathological tissues, cells may encounter different oxygen levels, due to poor or altered vascularization, and recent evidence have suggested that chemotaxis is a cell function which is affected by oxygen availability. We have recently described that oxygen availability is a determinant parameter in the setting of chemotactic responsiveness to the Stromal-Derived Factor 1 (SDF-1, CXCL12). Low oxygen concentration induces high expression of the CXCL12 receptor CXCR4, in myeloid cells, including: monocytes, monocyte-derived macrophages and tumor associated macrophages (TAM). Macrophages are functionally plastic cells, which express different functional programs in response to different microenvironmental signals. In particular, macrophage polarization is generally represented in their two extremes of functional polarization, M1 *versus* M2. While M1 macrophages are known to express inflammatory and cytotoxic functions, M2 polarized macrophages tune inflammation and immunity, promote angiogenesis and tissue remodeling. As TAM display an M2-associated phenotype and are characterized by their preferential localization in hypoxic areas of tumors, we have explored the effects of hypoxia on macrophage polarization in response to M1- and M2-inducing signals, LPS and IL-4 respectively. We observed that hypoxia strongly enhances the LPS-induced expression of M1-related cytokines (e.g. IL-12, TNF) and chemokines (e.g. CCL5, CXCL10), while it also upregulates the IL-4-induced expression of M2-related chemokines (e.g. CCL17 and CCL22). Thus hypoxia does not appear to significantly affect macrophage polarization, but rather plays an important role in setting the magnitude of polarized immune responses.

10-27/P

THE HUMAN IFN-INDUCIBLE IFI16-GENE TRIGGERS ENDOTHELIAL INFLAMMATORY ACTIVITY THROUGH THE NF-κB COMPLEX

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The human IFI16 gene is an interferon-inducible gene been implicated in the regulation of endothelial cell proliferation and tube morphogenesis. Immunohistochemical analysis has demonstrated that this gene is highly expressed in endothelial cells in addition to hematopoietic tissues. In this study, gene array analysis of human umbilical vein endothelial cells (HUVEC) overexpressing IFI16 revealed an increased expression of genes involved in immunomodulation, cell growth, and apoptosis. Consistent with these observations, IFI16 triggered the expression of genes encoding adhesion molecules (ICAM-1, E-selectin) or chemokines (IL-8, MCP-1). Functional analysis of ICAM-1 promoter demonstrated that NF-κB is the main mediator of IFI16 driven gene induction. NF-κB activation by IFI16 appears to be triggered by down-regulation of IκBα expression at both mRNA and protein levels. Suppression of IκBα gene transcription appears to be mediated by inhibition of Sp1 binding to the IκBα promoter, as shown by chromatin immunoprecipitation experiments. Taken together, these data implicate IFI16 as a novel regulator of endothelial proinflammatory activity through NF-κB activation and provide new insights into the function of the IFN-inducible gene IFI16.

10-29/P

FREUND'S ADJUVANT INDUCES OSTEOCLASTOGENESIS AND ARTHRITIS IN MICE LACKING A FUNCTIONAL IFN-γ RECEPTOR.

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Collagen-induced arthritis (CIA) in mice is an animal model for rheumatoid arthritis (RA) in man. To induce CIA, DBA/1 mice are immunised with heterologous collagen type II (CII) in complete Freund's adjuvant (CFA). Having observed that mice lacking a functional interferon-γ (IFN-γ) receptor (interferon-γ receptor knock-out [IFN-γR KO] mice) are more susceptible to CIA and that this is conditioned by the use of CFA, we considered the hypothesis that CFA plays an essential role in the induction of arthritis disease. Specifically we tested whether CFA by itself is able to induce arthritis in IFN-γR KO mice.

When IFN-γR KO mice were injected with a single intradermal injection of CFA, symptoms of arthritis appeared from day 16 onwards and reached an incidence of 55%. The onset coincided with expansion of $CD11b^+CD115^+$ spleen and blood cells, known to correspond to osteoclast precursor cells (OCP). QRT-PCR for receptor activator of NF-κB ligand (RANKL), tumour necrosis factor alpha (TNF-α) and osteoprotegerin (OPG), all known to be important in osteoclastogenesis, revealed a more than 100-fold increase in the RANKL/OPG ratio in the synovia of CFA-sensitised mice than in those of naive animals. TNF-α mRNA was present in synovia of CFA-sensitised mice, but the levels were not different from those of naive mice. However, $CD11b^+$ splenocytes from CFA sensitised mice spontaneously produced TNF-α. Treatment with the TNF-α antagonist etanercept, completely prevented the development of arthritis and mitigated the increased expansion of myeloid cells as well as the increase in OCP numbers in spleen and blood. These results suggest that CFA induces arthritis by an auto-antigen-independent pathway involving induction of TNF-α and stimulation of osteoclastogenesis.

10-30/P

IDENTIFICATION OF NOVEL ARTHRITIS-RELATED GENE BY TRANSCRIPTOME MAPPING ANALYSIS OF MODEL MICE FOR RHEUMATOID ARTHRITIS

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Rheumatoid arthritis (RA) is an autoimmune disease. Previously, we showed that human T cell leukemia virus type I transgenic (HTLV-I-Tg) mice and Interleukin-1 receptor antagonist knockout (IL-1Ra-KO) mice develop autoimmunity and joint-specific inflammation resembling RA in humans. To identify genes involved in the pathogenesis of arthritis, we analyzed the gene expression profiles of synovial tissues in these animal models using high-density oligonucleotide arrays. The gene expression profile between HTLV-I-Tg mice and IL-1Ra-KO mice were closely correlated, suggesting that many genes participate in the pathogenesis of both models in common. We extracted 554 genes of which expression significantly changed in both models, assuming that pathogenically important genes at the effector phase would change in both models. To determine the genomic distribution of the arthritis-related genes, we assigned these genes into the whole genome in a scale of the one megabase pairs. As a result, we have identified several arthritis-related gene clusters. The most significant peak of the transcript density was found on chromosome 17, which corresponds to the MHC gene cluster. Based on these results, we identified C-type lectin receptor (CLR) gene cluster on chromosome 6 was one of the arthritis-related gene cluster including attractive candidate genes. In order to confirm the involvement of CLR to the pathogenesis of arthritis, we generated KO mice of one of the CLR member. During collagen-induced arthritis, the KO mice exhibit markedly exacerbated response. Thus, this molecule is suggested to be involved in the development of arthritis as a negative regulator. The analysis of gene clusters using microarray data may be useful to elucidate the pathoetiology of diseases and find new targets for their treatment or biomarkers of diagnosis. And newly identeified CLR as arthritis-related gene could elucidate the novel mechanism of the development of autoimmune arthritis.

10-31/O

A C-TYPE LECTIN THAT INVOLVED IN THE DEVELOPMENT OF ARTHRITIS PLAYS AN IMPORTANT ROLE IN THE HOST DEFENSE

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We have produced two rheumatoid arthritis models using embryo manipulating techniques; one is transgenic mice carrying the *tax* gene of human T–cell leukemia virus type 1(HTLV-I-Tg) and the other is IL-1 receptor antagonist-deficient (IL-1Ra-KO) mice. Although the etiology is different between two models, both models show similar joint pathology closely resembling rheumatoid arthritis and develop autoimmunity. To elucidate the pathogenesis of arthritis and autoimmunity, we employed microarray analysis with two rheumatoid arthritis models, and found C-type lectin gene complex were up-regulated in the both arthritis models. Interestingly, deficient mice for the C-type lectin revealed to be resistant against CIA induced by immunization with chicken type II collagen with FCA supplemented additional heat killed Mycobacterium tuberculosis (H37Ra). Furthermore, the deficient mice showed tendency to reduction in type II collagen specific antibody production and cellular respons. On the other hand, the C-type lectin deficient mice were susceptible to fungal infection. TNF production with fungal cell wall component was significantly reduced in the deficient mice. These data indicates that this C-type lectin is involved in both innate and acquired immunity.

10-32/P

PROTEIN C/ACTIVATED PROTEIN C IS EXPRESSED BY AND STIMULATES THE PROLIFERATION OF HUMAN KERATINOCYTES

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Activated protein C (APC) is a natural anti-coagulant derived from its vitamin K-dependent plasma precursor, protein C. It possesses strong anti-inflammatory and anti-apoptotic properties and can promote wound healing and cell proliferation. We have recently reported that human keratinocytes express the specific receptor for PC/APC, endothelial protein C receptor (EPCR). In this study, using human keratinocytes isolated from foreskins, we firstly examined whether PC was expressed by keratinocytes. Using real-time PCR, western blot and immunohistochemistry, our results showed that human keratinocytes expressed PC at both gene and protein levels. The epidermis of foreskin also stained strongly for PC protein, which was mostly present in the basal and suprabasal layers of the epidermis and decreased towards the outer stratum corneum. We then investigated whether this endogenous PC/APC affected cell proliferation. Cells were treated with PC small interfering (si) RNA (500 nM) which caused an 80% reduction in PC mRNA expression. PC siRNA significantly inhibited keratinocyte proliferation as measured by MTT assay, suggesting that endogenous PC/APC is required for cell growth. This inhibitory effect on growth rate was restored by the addition of exogenous APC (10 μg/ml). We further determined whether PC/APC functioned through EPCR and epidermal growth factor receptor (EGFR). When unstimulated keratinocytes were pretreated with RCR252, an EPCR blocking antibody or PD153035, an EGFR inhibitor, their growth was inhibited by 20% and 30%, respectively. APC (10 μg/ml)-stimulated proliferation was also completely abolished by both inhibitors, indicating that APC acts *via* EPCR and EGFR. In summary, our data demonstrate for the first time that PC is expressed by human keratinocytes. Furthermore, endogenous PC/APC stimulates keratinocyte proliferation *via* EPCR and EGFR.

10-33/P

CYTOKINE-DRIVEN MONOCYTE DIFFERENTIATION AND MACROPHAGE POLARIZATION THROUGH THE LOOKING GLASS OF SYSTEM BIOLOGY

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Monocytes and tissue macrophages provide both immediate defence against foreign agents and assist during the setting off and development of the adaptive immune response. Their maturation and activation greatly depends on the influence of master cytokines. In this context M-CSF is considered the prototypic lineage determining cytokine, responsible for monocytes maturation. The subsequent activation with Th1 cytokines as IFN-γ or Th2 cytokines as IL-4, elicit the so called polarized classical (M1) or alternative (M2) activation phenotypes, respectively. Concordantly the goal of the present study was to comprehensively profile gene expression during human monocyte-to-macrophage differentiation driven by M-CSF, and their polarization towards the M1 and M2 phenotypes. The genome profiling strongly evinced that M-CSF-driven monocyte-to-macrophage differentiation is associated with the induction of a significant number of cell cycle-related genes, a finding substantiating the underestimated proliferation potential of monocytes. Interestingly, M-CSF and IL-4 induced expression profiles with a significant overlap, demonstrating a dual role on maturation and polarization for M-CSF, and suggesting that under homeostatic conditions a default shift towards M2 may occurs. Modulation of genes involved in metabolic activities stood out as a prominent feature of macrophage differentiation and polarization. The Gene Ontology category *lipid metabolism* is particularly over-represented in the dataset, and includes the previously unknown polarized regulation of the cycloxygenases COX1 and COX2 and the sphingolipids metabolism-related enzymes sphingosine and ceramide kinases (SPHK1 and CERk). Each differentiation step was characterized by a different repertoire of G protein-coupled receptors, highlighting in particular a cluster of nucleotide receptors as novel M2-associated genes. In addition the chemokine profile is profoundly diverse in polarized macrophages and new differentially expressed chemokines have been identified. Thus, transcriptome profiling reveals novel molecules and signatures associated with human monocyte-to-macrophage differentiation and polarized activation that may represent candidate tools and targets in pathophysiology.

10-34/O

IDENTIFICATION OF A NOVEL FUNCTION FOR HUMAN SARM

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TLR receptors are central components in the innate immune system where they detect pathogen associated molecular patterns (PAMPs). Engagement of these receptors leads to activation of NF-κB, IRF3 and IRF7, leading to pathogen elimination.
Located in the cytoplasmic tail of these receptors is the signature sequence for this family, the Toll-interleukin-1receptor-resistance (TIR) domain. Downstream signalling from these receptors is achieved by the presence of cytoplasmic TIR domain containing adaptor proteins which provides specificity in the response to the various invading pathogens. Five adaptor proteins exist, MyD88, Mal, TRIF, TRAM and SARM (sterile alpha and HEAT/armadillo motif protein). With the exception of SARM a role in TLR signaling has been described for all the adaptors. For example TRIF is involved in NF-κB and IRF3/7 activation by TLR3 and TLR4. Here we report that human SARM is a specific inhibitor of TRIF dependent TLR signaling. Transient expression of SARM inhibited both TLR3 and TLR4 gene induction and cytokine release, while signals dependent upon IL-1 or RIG-I were insensitive to SARM expression. We also demonstrate an interaction between TRIF and SARM and identify the motifs in SARM necessary for the observed inhibition. siRNA mediated knockdown of SARM message led to enhanced TRIF dependent gene induction and cytokine release both in transformed and primary human cells. Finally we show that SARM protein is rapidly stabilized by LPS treatment, providing a mechanism for negative feedback for TLR4/TRIF signalling.

Overall these findings demonstrate that SARM, the final mammalian TIR adaptor to be characterized, is a negative regulator of TRIF function.

10-35/O

INTERLEUKIN-31 AND ONCOSTATIN M: CHARACTERIZATION OF SIGNALING CAPACITIES AND RELEVANCE IN INFLAMMATORY SKIN DISEASES

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T lymphocytes contribute crucially to the pathogenesis of eczema such as allergic contact dermatitis or atopic dermatitis. Recently, interleukin (IL)-31 was identified which is produced by activated T lymphocytes, preferentially by Th2 cells. Particularly, the skin-homing $CD45RO^+$ CLA^+ T cell subset secrets elevated levels of IL-31. In contrast, a close relative of IL-31, oncostatin M (OSM), is preferentially secreted by Th1 cells. Both cytokines share the common signaling receptor subunit OSMRβ. OSM induces heterodimerization of this receptor with gp130, the common signal transducer of IL-6-type cytokines. In contrast, IL-31 first binds to the IL-31R, the closest relative of gp130. In this study we characterize in depth the molecular mechanisms underlying OSMR- and IL-31R-mediated signaling. We identified the residues in each receptor responsible for the activation of STAT1, 3 and 5 as well as the MAPK cascade. Additionally, we discovered a novel receptor tyrosine-independent mechanism of SOCS3-mediated feedback inhibition of signal transduction. Analyzing 162 skin biopsies from patients with atopic and allergic contact dermatitis as well as psoriasis we detected elevated IL-31 transcripts only in atopic and allergic contact dermatitis, but not in psoriasis. In contrast, OSM transcripts were found to be significantly upregulated in all three skin disorders. Expression of IL-31 correlates with the Th2-cytokines IL-4 and IL-13 in atopic and allergic contact dermatitis. For OSM, no clear correlation could be determined for IL-4/IL-13 or IFNγ. The function of IL-31 and OSM in the establishment or maintenance of inflammatory skin diseases is still unclear. However, it has recently been shown that IL-31 expression is also increased in the highly pruritic skin disease prurigo nodularis. Therefore, it was hypothesized that IL-31 might play a role in the development of itching, one of the characteristic features of atopic as well as allergic contact dermatitis.
This work was supported by the Deutsche Forschungsgemeinschaft (SFB 542) and by Fonds der Chemischen Industrie.

10-36/P

INGESTED (ORAL) SIRS PEPTIDE 1-21 SUPPRESSES TYPE 1 DIABETES (T1DM) IN NOD MICE

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Background: Ingested murine IFN-α administered to nonobese diabetic (NOD) mice decreased islet inflammation and suppressed type 1 diabetes (T1DM). Type I IFN activate human suppressor T cells that produce soluble immune response suppressor (SIRS). **Objectives**: We examined whether a protein induced by type I IFN would have similar effects to ingested IFN-α in the NOD mouse model of T1DM. **Methods**: NOD female mice were fed (gavaged) with saline or 1, 10 or 100 μg SIRS peptide 1-21{NH-MET-Thr-Glu-Glu-Asn-Gln-Gln-Ser-Ser-Gln-Pro-Lys-Thr-Thr-Ile-Asn-Asn-Ala-Gly-Asp-Ser-CysOH} daily. Mice were followed by weekly blood glucose determination. Mice were considered diabetic if two consecutive blood glucose determinations were above > 11.1 mmol/l or > 200 mg/ml. Splenocytes from non-diabetic saline (mock) fed or non-diabetic 1 mcg SIRS peptide fed mice were stimulated with Con A and cytokine responses were examined using mouse cytokine inflammatory antibody array. Mice were sacrificed after reaching endpoint, pancreata were harvested and islet inflammation (insulinitis) was graded. **Results**: Mice ingesting 1 μg SIRS peptide 1-21 showed significant delayed onset of T1DM and a decreased frequency of diabetes compared to mock fed, 10 mcg and 100 mcg fed mice ($p < 0.014$). One mcg SIRS peptide 1-21 also showed significant decrease in islet inflammation ($p < 0.001$). Splenocytes from SIRS peptide 1-21 treated mice showed increased production of IFN-γ, IL-1β, IL-6, RANTES, tissue inhibitors of matrix metalloproteinase (MP) (TIMP-2), and soluble extracellular domains of TNF receptors I (sTNF RI) and II (sTNF RII). **Conclusion**: Ingested (oral) SIRS peptide significantly inhibits both clinical T1DM and insulitis presumably *via* inhibition of MP and TNF-α despite increased peripheral expression of IL-1, IL-6, IFN-γ and RANTES.

10-37/P

INGESTED (ORAL) SIRS PEPTIDE 1-21 INHIBITS ACUTE EAE

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Background: Ingested type I IFN inhibits clinical attacks, relapses and inflammation in murine experimental autoimmune encephalomyelitis (EAE). Type I IFN activate human suppressor T cells that produce soluble immune response suppressor (SIRS). **Objectives**: We examined whether a peptide (SIRS) induced by type I IFN would have similar effects to ingested IFN-α in EAE. **Methods**: C57BL/6 mice were actively immunized with myelin oligodendroglial (MOG) peptide 35-55 (MEVGWYRSPFSRVVHLYRNGK) in IFA and followed for evidence of disease. Clinical severity was graded daily. For parenteral treatment, on day –7 preceding active immunization, and continuing through day +14 post immunization, B6 mice were injected with control saline (mock), 0.1, 1, or 10 mg SIRS peptide 1-21 (NH-MET-Thr-Glu-Glu-Asn-Gln-Gln-Ser-Ser-Gln-Pro-Lys-Thr-Thr-Ile-Asn-Asn-Ala-Gly-Asp-Ser-CysOH). For oral treatment, B6 mice were fed with 0.1 ml of saline (mock) or 1, 10 or 100 mg SIRS peptide 1- 21 daily. Following sacrifice, spinal cords were removed and evaluated independently for foci of inflammation by a blinded observer. Splenocytes from grouped saline (mock) fed or 100 μg SIRS peptide fed mice were stimulated with Con A and examined using mouse cytokine inflammatory antibody array. **Results**: S.C. 10 μg SIRS peptide 1-21 showed significant inhibition of EAE ($p < 0.001$). Ingested SIRS peptide at 10 and 100 μg SIRS peptide inhibited EAE compared to placebo ($p < 0.001$). There were significantly less inflammatory foci in the SIRS peptide fed group compared to the control mock saline group ($p < 0.03$). Splenocytes from SIRS pepide 1-21 fed mice showed increased production of CD30L, IL-6, IL-13, I-TAC, TCA-3/CCL1, TNF-α and decreased production of lymphotactin after Con A stimulation. **Conclusion:** Ingested (oral) SIRS peptide significantly inhibits both clinical EAE and inflammation predominately *via* counter-regulatory type 2-like cytokines and chemokines IL-13, CD30L and TCA-3 despite increased peripheral expression of TNF-α.

10-38/P

ADIPONECTIN DEFICIENCY DOES NOT ALTER THE INFLAMMATORY RESPONSE TO LPS OR CONCANAVALIN A IN MICE

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Adiponectin (APN) is an adipocyte-derived protein with insulin-sensitizing and anti-inflammatory activities. It has been suggested that APN modulates the response to LPS, possibly by binding LPS and thus preventing activation of TLR4. To investigate whether APN deficiency alters the response to LPS, WT and APN KO mice were injected with LPS at 100 µg/mouse and serum levels of cytokines (IL-6, IFN-gamma and TNF-alpha) and glucose as well as anorexia and body weight loss evaluated. No significant differences between WT and APN KO mice were observed for any of the parameters measured. To evaluate the role of APN in the response to low doses of LPS, WT and APN KO mice were injected with either 10 or 100 ng/mouse of LPS together with D-galactosamine, which acts as a sensitizer to LPS. Parameters evaluated included liver toxicity and lethality and no significant differences were observed between WT and APN KO mice.
An anti-inflammatory role for APN has also been suggested in the model of liver damage induced by administration of ConA. To verify whether APN deficiency alters the response to ConA, WT and APN KO mice receive an iv injection of ConA at 100 µg/mouse. Serum levels of ALT were measured as an indication of hepatotoxicity; serum levels of the cytokines TNF-alpha, IL-6, IFN-gamma, IL-4 and IL-10 were also evaluated. Our data indicate that APN deficiency does not significantly alter the response to ConA for any of the parameters investigated.
In conclusion, although APN plays an anti-inflammatory role in various experimental models, APN deficiency is not associated with altered inflammatory responses following administration of ConA or LPS at either high or low doses.
This work was supported by NIH grants DK61483 (to GF) and DK68037 and HL51586 (to LC).

10-39/P

A REGULATORY EFFECT OF THE BALANCE BETWEEN TUMOR NECROSIS FACTOR-ALPHA AND INTERLEUKIN-6 IN INFLAMMATORY RESPONSE.

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Following intravenous inoculation with *Rhodococcus aurantiacus* (*R. aurantiacus*), wild-type (WT) mice develop non-necrotic granulomas. As a high level of endogenous tumor necrosis factor-alpha (TNF-α) is observed at the initial phase post-infection in this murine model, we examined the extent to which TNF-α contributes to the granulomatous inflammation induced by *R. aurantiacus* using TNF-α gene-deficient (TNF-$\alpha^{-/-}$) mice. In spite of a lack of *R. aurantiacus* proliferation, TNF-$\alpha^{-/-}$ mice displayed high mortality rates, as well as a markedly high level of endogenous interleukin-6 (IL-6) was detected in their spleens. Histologic examination showed an absence of granuloma formation in the TNF-$\alpha^{-/-}$ mice. Administration of recombinant TNF-α (rTNF-α) to TNF-$\alpha^{-/-}$ mice failed to restore the granuloma formation, but accelerate bacterial removal and cellular recruitment into the liver. This rTNF-α administration also attenuated IL-6 production, resulting in an increased survival of TNF-$\alpha^{-/-}$ mice. Our previous study demonstrated that IL-6 gene-deficient (IL-$6^{-/-}$) mice induced an overproduction of TNF-α and interferon-gamma (IFN-γ) during the early phase of *R. aurantiacus* infection. In vitro, heat-killed *R. aurantiacus* induced enhanced mRNA expression and production of IL-6 in macrophages and dendritic cells from TNF-$\alpha^{-/-}$ mice compared with WT controls, and treatment of TNF-$\alpha^{-/-}$ mouse cells with rTNF-α decreased the IL-6 secretion. Moreover, anti-TNF-α or anti-IL-6 treatment increased IL-6 or TNF-α production by WT mouse cells, respectively. These data suggest that the production of TNF-α and IL-6 can be negatively regulated by each other. Administration of recombinant IFN-γ (rIFN-γ to TNF-$\alpha^{-/-}$ mice promoted bacterial clearance as well as the development of immature granulomas, and treatments with both rTNF-αand rIFN-γ led to the formation of mature granulomas. Collectively, TNF-α appears crucial for bacterial clearance, cellular recruitment and granuloma formation. Moreover, the balance between TNF-α and IL-6 during the early phase of infection controls the development of the granulomatous response to *R. aurantiacus* infection.

10-41/P

EFFECT OF SPELEOTHERAPY ON THE LEVELS OF CIRCULATING TGF-BETA1 IN PATIENTS WITH ANKYLOSING SPONDYLITIS

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Ankylosing spondylitis (AS) is a chronic inflammatory disease of the axial joints with no satisfactory therapy. A beneficial effect has been reported after 3-4-weeks of speleotherapy in "Gasteiner heilstollen" in Austria, which includes exercises, hyperthermia and exposure to radon in a former mine. The mechanisms underlying these beneficial effects are not clearly understood. Therefore, we studied the involvement of transforming growth factor-beta1 (TGF-beta1), a potent immunomodulator and antiinflamatory cytokine, in the responsiveness to therapy. We measured the serum levels of transforming growth factor-beta1 (TGF-beta1), in 83 patients with AS and ten patients with non-inflammatory low back pain (LBP, controls) before and after therapy.
The results demonstrated a significant increase in TGF-beta1 (total and active) after therapy. The mean concentration of total TGF-beta1 in AS was 28715 pg/ml and increased to 43136 pg/ml, $p < 0{,}0001$. Active TGF-beta1 increased from 77 to 1096 pg/ml, $p < 0.0001$ in AS patients and in LBP from 31 to 42 pg/ml, $p < 0.005$. When the AS patients were divided into two groups according to pain reduction, group 1 (responders: n=46) exhibited a 17 fold increase of active TGF-beta1 levels (96 to 1654 pg/ml) while group 2 (non-responders: n=37) showed only a 7 fold increase (53 to 402 pg/ml). There was a moderate increase in active TGF-beta1 in patients with LBP from 31 to 42 pg/ml ($p < 0.01$) and no significant change was observed in the patients treated with conventional therapy. These results demonstrate a significant increase in circulating TGF-beta1 in patients with AS after therapy in Gastein Heilstolen. Elevated TGF-beta may exert a beneficial effect through its anti-inflammatory function leading to reduction of joint pain.

10-42/P

SPHINGOSINE-1-PHOSPHATE TYPE 1 RECEPTOR AGONIST REDUCES CYTOKINE GENE EXPRESSION AND NEUTROPHIL AND MACROPHAGE INFILTRATES IN RENAL ISCHEMIA/REPERFUSION INJURY

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Acute renal failure due to ischemia/reperfusion (I/R) injury is a common clinical problem with a high morbidity and mortality. Recently, we have shown that a newly identified sphingosine 1-phosphate (S1P) type 1 receptor (S1P1)-selective agonist, SEW2871, ameliorates ischemic ARF. The improvement of renal function was demonstrated by a 40% reduction in plasma creatinine levels and a significant reduction in tubular necrosis scores 24 hours after ischemia. In this study, we further examined the expression profiles of proinflammatory cytokine/chemokine. Mice were subjected to bilateral ischemia for 30 min followed by reperfusion for 1 to 24 h. SEW2781 (20 mg/kg body weight) was given 3 h prior to ischemia by gavage. Kidneys were harvested at 1, 4, and 24 h after I/R and total RNA was subjected to quantitative real time RT-PCR. Tumor necrotic factor-α (TNF-α), is an early expressing cytokine with a peak expression at

one hour after I/R (12, 6.4, and 3.5 fold increase *versus*. sham control, after 1, 4, 24 h, respectively). Gene expression of Interleukin 6 (IL-6), KC and Monocyte chemoattractant protein-1 (MCP1) reached the peak at 4 h after ischemia (IL6: 348, 1187, and 117 fold; KC: 154, 266, and 19 fold; MCP1: 13, 86, and 9 fold; at 1, 4, and 24 h, respectively). SEW2781 treatment did not affect gene expression of cytokines at 1 h, but reduced TNF-α by 39% at 4 h and 47% at 24 h, and reduced IL6 by 65%, KC by 62%, and MCP1 by 42% at 24 (all $p < 0.05$). Immunofluorescence studies revealed that the expression of TNF-α and MCP1 after I/R was mainly in the tubular epithelial cells and was reduced by SEW2871 treatment. These findings were accompanied by 77% and 66% reduction in infiltrating neutrophils and macrophages in renal outer medulla, respectively. Taken together, our study demonstrated that gene expression of cytokines are rapidly and markedly upregulated by I/R injury in the kidney. The peak of TNF-α expression induced by I/R is earlier than other cytokines. The S1P1 agonist mediated reno-protection is at least partially modulated by reduction in neutrophil and macrophage infiltration, presumably by reducing the proinflammatory TNF-α, IL-6, KC and MCP-1.

10-43/P

BARTONELLA QUINTANA LPS IS A TLR4 ANTAGONIST

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Toll-like receptors (TLRs) are key detectors of the innate immune system involved in the recognition of invading microorganisms. Incubation of monocytes/macrophages with *Bartonella quintana,* a Gram-negative pathogen, induces the production of various cytokines. Lipopolysacharide (LPS) of Gram-negative bacteria binds to Toll-like receptor 4 (TLR4) and represents the main pathogen associated molecular pattern (PAMP) involved in the pathogenesis of Gram-negative septic shock. Despite the fact that *B. quintana* contains LPS, the infection is usually associated with the absence of septic shock. Giving these facts, we sought to investigate which are the particularities of interaction between TLR4 and *B. quintana*, which could explain this phenomenon. Firstly, human peripheral blood mononuclear cells were stimulated with heat-killed *B. quintana* and *E. coli* LPS in the presence and in the absence of human anti-TLR4 antibodies. Anti-TLR4 antibodies strongly inhibited *E. coli* LPS induced production of TNF-α (90%) and IL-10 (80%), while no such effect could be seen when cells were stimulated with heat-killed *B. quintana.* Secondly, peritoneal macrophages from TLR4 deficient mice (TLR4-/-) were challenged in cultures with heat-killed *B.quintana*, one-step purified *B. quintana* LPS and *E. coli* LPS. Incubation of these cells with *E.coli* LPS did not induce the production of IL-6 and IL-10 when compared to wild-type mice. Moreover, incubation with heat-killed *B. quintana* and one-step purified *B. quintana* LPS stimulated the production of these cytokines both in TLR4 -/- and wild type mice. Given these results, we further investigated the hypothesis that TLR2 holds a higher importance than TLR4 in the recognition of *B.quintana*. According to the literature, incubation of peritoneal macrophages from both wild-type mice and TLR2 deficient mice (TLR2 -/-) with *E.coli* LPS stimulated the production of IL-6 and TNF-α. In contrast, when TLR2-/- mice cells were challenged with heat-killed *B. quintana*, one-step purified *B.quintana* and peptidoglycan (PGN), the concentrations of TNF and IL-6 in the supernatants were significantly lower compared to wild-type mice. Remarkably, when incubated with purified *B. quintana* LPS, practically no cytokine release was observed, suggesting that the cytokine-stimulating capacity of the *B. quintana* LPS with only one-step purification, is due to contaminants with TLR2-stimulating capacity. Several studies have already demonstrated the existence of various LPS that bind TLR4 without having stimulatory effects. We therefore tested the effects of different concentrations of highly-purified *B. quintana* LPS compared to those of *E. coli* LPS on the production of TNF-α, IL-6 and IL-10 from PBMCs of 8 healthy volunteers. Highly-purified *B. quintana* LPS alone did not stimulate the cells, as indicated by the absence of TNF-α, IL-6 and IFN-β in the supernatants. Moreover, at a concentration of 100ng/ml, highly-purified *B. quintana* LPS was sufficient to completely block the response to 10ng/ml *E. coli* LPS. We conclude that TLR4 is scarcely involved in the recognition of *B. quintana* and additionally, TLR2 is likely to be the main PRR that triggers the innate immune response to *B. quintana*. Moreover, *B. quintana* LPS is a TLR4 antagonist and consecutively, this might represent one mechanism responsible for the absence of septic shock in infected individuals.

10-44/P

THE ABSENCE OF IL-1 RECEPTOR ANTAGONIST ATTENUATES ACETAMINOPHEN-INDUCED LIVER INJURY IN MICE

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We explored the pathophysiological roles of IL-1ra in acetaminophen (APAP)-induced acute liver injury by the use of mice lacking IL-1ra (KO). Pathogen-free, 8-week-old male BALB/c (WT) mice and IL-1ra-KO mice were intraperitoneally administered with 200mg/kg of APAP. Serum ALT levels, liver histology, and intrahepatic leukocyte recruitment were evaluated. Moreover, intrahepatic gene expression of cytokines, chemokines, and detoxification enzymes was examined by RT-PCR. When WT and KO mice were intraperitoneally injected with APAP of 200 mg/kg, there was no death in both WT and KO mice. However, WT mice exhibited a significant elevation of serum ALT level, and histopathological analyses demonstrated that massive centrilobular hepatocyte necrosis and hemorrhage with leukocyte accumulation. On the contrary, in KO mice, the elevation of serum ALT and histopathological alterations were significantly attenuated, compared with WT mice. The gene expression of TNF-α; IL-6, MIP-1α; and MIP-2 was up-regulated in the liver of WT after APAP challenge. However, the enhanced expression of those molecules was significantly inhibited in liver from IL-1ra-KO mice compared with WT mice. Moreover, we examined the intrahepatic gene expression of cytochrome P450 emzyme (CYP) 2E1 and CYP 3A11, which are mainly involved in the metabolism of APAP. KO mice exhibited attenuated gene expression of CYP 2E1 and CYP 3A11, compared with WT mice. These observations implied that the lack of IL-1ra could alleviate APAP-induced liver injury through the suppression of intrahepatic gene expression of CYP 2E1 and CYP 3A11.

10-46/P

BENZOXATHIOLONE LYR-64 INHIBITS LPS-INDUCED CYTOKINE PRODUCTION BY BLOCKING IKK IN NF-KB SIGNALING

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2-Cycylohexylimino-6-methyl-6,7-dihydro-5*H*-benzo[1,3]oxathiol-4-one (LYR-64) is a novel chemically synthetic compound. In this study, the LYR-64 was discovered to inhibit the transcriptional activity of nuclear factor (NF)-κB, a transcription factor, in lipopolysaccharide (LPS)-stimulated macrophages RAW 264.7. The compound reduced LPS-induced DNA binding activity and nuclear translocation of NF-κB complex as well as inhibited LPS-induced degradation and phosphorylation of inhibitory κBα (IκBα) protein. These results suggest that LYR-64 suppressed LPS signaling molecule, putatively IκB kinase (IKK) complex, upstream IκB degradation in NF-κB activating pathway in macrophages RAW 264.7. The possibility was further documented with transiently transfecting macrophages with pNF-κB-secretory alkaline phosphatase (SEAP)-NPT reporter

construct for NF-κB transcriptional activity in combination with expression vectors encoding IKKα, IKKβ, NF-κB p65 or p50 subunit. LYR-64 inhibited SEAP expressions elicited by expression of IKKα or IKKβ, but did not affect those by NF-κB p65 or p50 subunit. The compound inhibited SEAP expression elicited by expression of constitutively active IKKβ mutant (C/A, Cys-179 to Ala) on the activation loop of the enzyme, but did not affect SEAP expression by another constitutively active IKKβ mutant (SS/EE, Ser-177 and 181 to Glu). As well as, LYR-64 inhibited GST-IκBα phosphorylation of IKKβ mutant (C/A) but did not affect that of IKKβ mutant (SS/EE). Therefore, LYR-64 could inhibit LPS-induced NF-κB activating pathway by targeting the activation domain Ser-177 and/or 188 of IKKβ. As pharmacological actions, LYR-64 suppressed LPS-induced expression of iNOS, COX-2, IL-1, TNF or IL-6 at the transcription level. Further, the compound protected LPS-induced septic shock in mice. Finally, LYR-64 could provide an invaluable tool to investigate NF-κB-dependent inflammatory mediator production, in addition to its anti-inflammatory potential.

10-47/P

TO ELUCIDATE THE MECHANISM AND CONSEQUENCES OF MAL INTERACTION WITH TRAF6 IN TOLL-LIKE RECEPTOR SIGNALLING

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The innate immune system defends the body from microbial infection by initiating inflammation, the extreme form of which is sepsis or septic shock. Therefore, a greater understanding of the signalling pathways regulating the pro-inflammatory response to microbial infection is of crucial importance to developing new therapeutics to treat septic shock and chronic inflammation.

This study focuses on understanding Toll-like receptor (TLR) signalling which regulate the innate immune response to microbial pathogens, and the unique genes that are expressed upon their recognition, despite utilising a common signalling pathway to activate the prototypic inflammatory transcription factor, NF-κB. It is the involvement of adapter proteins such as Mal, and the emergence of recently described family members, that we believe dictate these unique gene subsets and which are responsible for the control of the pro-inflammatory response. We recently described the unique interaction between Mal and TRAF6, independently of the canonical TLR signalling pathway, suggesting a novel role for Mal in regulating NF-κB-dependent gene transcription *via* activation of the MAP kinase pathway and p65 transactivation. Mutation of this TRAF6 binding motif site within Mal has already shown to inhibit both TLR-2 and TLR-4 mediated NF-κB-dependent gene expression.

The project will use both *in vivo* and *in vitro* methods to fully describe and characterise the unique interaction between Mal/TRAF6 and to understand the mechanism that underpins the mediation of downstream signalling events emanating from association of these two proteins. The use of crystallography studies between Mal/TRAF6 will lead to an understanding of their interaction relationship. A greater mechanistic understanding of Mal/TRAF6 association will make a significant contribution to understanding innate immune responses to pathogen challenge, providing possible therapeutics for use in controlling chronic inflammatory responses.