02

Cytokines

02-02/P

IDENTIFICATION OF THE IL-10R2 BINDING EPITOPE ON IL-10 AND THE REQUIREMENT OF AN IL-10R1 INDUCED CONFORMATIONAL CHANGE

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IL-10 is a pleiotropic α-helical cytokine whose main biological function is to restrain inflammatory responses. IL-10 signals through an IL-10-specific receptor chain, IL-10R1, together with a promiscuous receptor chain, IL-10R2. IL-10R2 is also required for the biological activities of other cytokines including IL-22, IL-26, IL-28A, IL-28B and IL-29. Whereas the IL-10/IL-10R1 interface was elucidated in detail by structural studies, the IL-10R2 binding properties are poorly understood. Here, we define the IL-10R2 binding site on IL-10 by alanine scanning mutagenesis, and provide evidence that an IL-10R1 induced conformational change on IL-10 is required for optimal IL-10R2 binding. The IL-10R2 binding epitope is located, adjacent to the IL-10R1 binding site, on helices A and D. The site is partitioned into two distinct surfaces, IIa and IIb. While IL-22 also uses two separate surfaces on helices A and D for IL-10R2 interaction, the contribution of each residue to IL-10R2 binding is totally different. Thus, IL-10 residues critical in the IL-10R2 interaction are not conserved among IL-10R2 binding cytokines. These results provide a basis for the development of IL-10 antagonists by abolishing the IL-10R2 binding site on IL-10.

02-03/P

THE REGULATION OF B LYMPHOCYTE INDUCED MATURATION PROTEIN EXPRESSION IN T CELLS

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The transcriptional repressor, B-lymphocyte induced maturation protein (Blimp-1), is induced in B cells in response to BCR stimulation, TLR and cytokine signaling with the essential function of driving terminal differentiation of the B cell into an antibody-secreting plasma cell. However, Blimp-1 is not a B cell-specific protein. By quantitative RT-PCR we have characterized the expression pattern of Blimp-1 in the T cell lineage. Our studies have found that Blimp-1 is most highly expressed in effector T cells in vivo and is induced upon T cell activation in vitro in both primary cells and T cell lines. The level of Blimp-1 induction varies depending upon the strength of TCR activation, co-stimulation and signaling by cytokines IL2, IL4 and IFN-gamma. Blimp-1 mRNA levels are found to be differentially regulated upon CD4 T helper (Th) cell differentiation. Culture under polarizing conditions caused Blimp-1 levels to increase in Th2 cells and decrease in Th1 cells. Interestingly, the expression pattern of Blimp-1 in T cells during development and upon differentiation appears to be inversely correlated with that of BCL6, a known Blimp-1 repressor in B cells and a repressor of Th2 cytokine expression in T cells. These data indicate that similar mechanisms exist between B cells and T cells in the regulation of Blimp-1 expression, and suggest a novel role for Blimp-1 in CD4 T cell differentiation.

02-04/P

PATHOPHYSIOLOGICAL ROLE OF INTERLEUKIN 17 IN ACUTE PANCREATITIS

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Respiratory failure and hematologic changes are typical for acute necrotizing pancreatitis. Unfortunately, the pathogenesis of these changes is fully unknown. IL-17 is a relatively recently described T-cell derived cytokine. Many of its effects are similar to, although in isolation less potent than, those of IL-1beta and TNF-alpha.
Nineteen patients with severe acute pancreatitis with respiratory complications were studied. Quantity of neutrophils, levels of interleukins (IL) 1beta, 6, and 17, MCP-1, TNF-alpha in the plasma and bronchoalveolar fluid were measured.
The IL-17 levels clear correlated with the quantity of total leukocyte, polymorphonuclear leukocytes and hemoglobin level. The significant increase of neutrophils quantity and concentration of all cytokines in bronchoalveolar fluid were noted in all patients with pancreatitis-associated lung injury. The clear correlation between IL-17 levels and concentration of IL-1beta, IL-6, TNF-alpha, MCP-1, and neutrophils quantity was noted. IL-17 has prominent proinflammatory properties due to the induction numerous genes that associated with inflammation, such as IL-1beta, IL-6, TNF-alpha, and GM-CSF. Hematologic effects of IL-17 may connect with its ability induced the GM-CSF expression which also enhanced in severe pancreatitis.
These data pointed on the role of IL-17 in the pathogenesis of pancreatitis-associated lung injury and hematologic changes.

02-05/P

INTERLEUKIN-17 PLAYS ROLES IN SUCCESSFUL RESOLUTION OF *TRYPANOSOMA CRUZI* INFECTION.

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Interleukin-17 (IL-17) is a proinflammatory cytokine produced mainly by T lymphocytes. It has been known that the production of IL-17 is up-regulated by IL-23, an IL-12 cytokine family member, and the

over-production causes some inflammatory disorders including rheumatoid arthritis and inflammatory bowel disease. In contrast, our previous studies showed that signaling through IL-27, another IL-12-related cytokine, and its receptor (WSX-1) participated in suppression of excessive IL-17 production during *Trypanosoma cruzi* infection. Whereas IL-17 has been reported to be important for resolution of bacterial infection, the roles in host defense against infection of intracellular protozoan parasites remain to be elucidated. Therefore, we investigated here the effect of IL-17 deficiency on *T. cruzi* infection. C57BL/6 (wild-type; WT) and IL-17-deficient (IL-17$^{-/-}$) mice were intraperitoneally injected with the plasma containing $2x10^3$ of trypomastigotes, and the number of parasites in the blood was microscopically counted. IL-17$^{-/-}$ mice showed prolonged higher parasitemia over WT mice. As acute *T. cruzi* infection causes liver injury, we measured serum level of liver enzymes, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The level of ALT and AST increased along with the parasite expansion, and these were higher in IL-17$^{-/-}$ mice as compared with WT mice. Furthermore, mortality of the infected IL-17$^{-/-}$ mice was higher than that of WT mice. These results demonstrated that IL-17$^{-/-}$ mice were more susceptible to *T. cruzi* than were WT mice. Therefore, it is suggested that IL-17 plays an important role in resolution of *T. cruzi* infection. Detail functions of IL-17 in regulation of the protective immunity are currently under investigation.

02-06/P

INDIRECT EFFECTS OF LEPTIN RECEPTOR-DEFICIENCY ON LYMPHOCYTE POPULATIONS AND IMMUNE RESPONSE IN *DB/DB* MICE

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Leptin-deficient *ob/ob* and leptin receptor (Ob-Rb)-deficient *db/db* mice display a marked thymic atrophy and exhibit defective immune responses. Lymphocytes express leptin receptors and leptin exerts direct effects on T cells *in vitro*. In addition, *ob/ob* and *db/db* mice display multiple neuroendocrine and metabolic defects through which leptin-deficiency may indirectly affect the immune system *in vivo*. To study the relative contributions of direct and indirect effects of leptin on the immune system in a normal environment, we generated bone marrow chimeras (BMCs) by transplantation of leptin receptor-deficient *db/db*, or control *db/+* bone marrow cells into wild-type (WT) recipients. The size and cellularity of the thymus, as well as cellular and humoral immune responses were similar in *db/db* to WT and *db/+* to WT BMCs. The immune phenotype of *db/db* mice is thus not explained by a cell autonomous defect of *db/db* lymphocytes. Conversely, thymus weight and cell number were decreased in the reverse graft setting in WT to *db/db* BMCs, indicating that expression of the leptin receptor in the environment is important for T cell development. Finally, Ob-Rb was expressed on thymic epithelial cells, suggesting that leptin might exert indirect effects on thymocytes not only via systemic modifications, but also by acting locally on the thymic microenvironment. We thus examined thymocyte development in fetal *db/db* thymi transplanted into WT recipient mice. T cell differentiation was normal in *db/db* thymic grafts, indicating that T cell development does not depend on leptin signaling in the thymic stroma. In conclusion, direct effects of leptin on bone marrow-derived cells are not required for lymphocyte maturation and immune response in a normal environment. Similarly, direct effects of leptin on thymic stromal cells are dispensable for T cell maturation. In contrast, leptin receptor-deficiency affects the immune system indirectly via changes in the systemic environment.

02-07/P

THE NEW IL-1 FAMILY MEMBER IL-1F8 EXERTS PRO-INFLAMMATORY EFFECTS ON SYNOVIAL FIBROBLASTS AND ARTICULAR CHONDROCYTES

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Six novel members of the interleukin (IL)-1 family of cytokines were recently identified, primarily through use of DNA data base searches for IL-1 homologues, and termed IL-1F5 to IL-1F10. In the present study, we investigated the effect of IL-1F8 on primary human joint cells, and examined the expression of the new IL-1 family members in human and mouse joints. Human synovial fibroblasts (hSF) and human articular chondrocytes (hAC) expressed the IL-1F8 receptor IL-1Rrp2 and produced pro-inflammatory mediators in response to recombinant IL-1F8. IL-1F8 mRNA expression was increased in hSF upon stimulation with pro-inflammatory cytokines, while in hAC IL-1F8 mRNA expression was constitutive. However IL-1F8 protein was undetectable in hSF and hAC culture supernatants. Furthermore, while IL-1β protein levels were increased in inflamed human and mouse joint tissue, IL-1F8 protein levels were not. IL-1F8 levels in synovial fluids were comparable or lower than those in matched serum samples, suggesting that the joint itself is not a major source of IL-1F8. Serum levels of IL-1F8 were similar in healthy donors, RA, OA and septic shock patients, and did not correlate with inflammatory status. Interestingly however, we observed high IL-1F8 levels in several serum samples in all groups. In conclusion, IL-1F8 exerts pro-inflammatory effects in primary human joint cells. Joint and serum IL-1F8 protein levels did not correlate with inflammation, but were high in some human serum samples tested, including samples from RA patients. It remains to be determined whether circulating IL-1F8 can contribute to joint inflammation in RA.

02-08/P

INCREASED OSTEOCLASTOGENESIS IN 4-1BB-DEFICIENT MICE BY LOWER LEVEL OF INTERLEUKIN-10

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We have investigated that osteoclastogenesis is stimulated in bone marrow-derived monocytes and macrophage derived cells (BMMs) from 4-1BB-deficient mice, comparing with those from wild-type mice, suggesting a role of 4-1BB on bone metabolism. The expression level of interleukin-10 (IL-10) is higher in RANKL-stimulated BMM from wild-type mice, comparing that from 4-1BB-deficient mice. When 4-1BB ligand is stimulated with 4-1BB-Fc fusion protein, the expression level of IL-10 was increased in BMM, suggesting that interaction of 4-1BB and 4-1BB ligand produced higher level of IL-10. Neutralization of IL-10 did not suppress the inhibitory effect of IL-10 on osteoclast differentiation in BMM of 4-1BB-deficient mice as much as that of wild type mice. Exogenously added IL-10 inhibited OC formation more efficiently in BMM cells from 4-1BB-deficient mice than that in wild type mice. Those results suggest that IL-10 play a role in different level of OC formation involved in 4-1BB. Elevated level of osteoclastogenesis by BMMs from 4-1BB-deficient mice is, at least partly due to, decreased level of IL-10. This work was supported by SRC fund to IRC, University of Ulsan from KOSEF and Ministry of Korea Sciences and Technology.

02-09/P

THE EFFECTS OF COMBINED EXERCISE ON ADIPONECTIN AND ACYL-GHRELIN IN OVERWEIGHT AND OBESITY ELEMENTARY SCHOOL BOYS

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Backgrounds: The purpose of this study was to determine the effects of combined exercise program for 12 weeks on adiponectin, leptin and ghrelin in overweight and obesity elementary school boys.
Methods: Eighteen subjects were selected from one elementary school at Busan. They were divided into two groups: combined exercise group (EG, n=9) and control group (CG, n=9), respectively. The combined exercise group performed that the walking exercise was 55% to 75% HRmax, 50 minutes a day for 2 days a week and the band resistance exercise was 10-15 repetitions per set, 2 sets, and 50 minutes a day for 2 days a week.
Results: When compared with CG, the body weight and body mass index were significantly decreased after 1 week, 4 weeks and 12 weeks, % body fat (%BF) and fat mass were significantly decreased after 4 weeks and 12 weeks, in EG. HOMA indices were decreased after 1 week, 4 weeks and 12 weeks in EG. There were significant differences in glucose and insulin between groups. There were no significant differences of adiponectin levels in both groups. Adiponectin levels were significantly decreased after 4 weeks and tended to increase until 12 weeks in EG. Total ghrelin was significantly increased after 4 weeks and 12 weeks and acyl-ghrelin was significantly decreased after 1 week, 4 weeks and 12 weeks in EG. However, acyl-ghrelin was not changed in CG.
Conclusions: This combined exercise is favorable effective in %BF, insulin, ghrelin and acyl-ghrelin in overweight and obesity elementary school boys. Especially, the observed increase in total ghrelin and decrease in acyl-ghrelin after combined exercise-induced weight loss may be some evidence of improvement of energy metabolism by increase of fat use and energy expenditure. Exercise seems to effect the adiponectin concentration. Many studies may be necessary to draw that conclusion.

02-10/P

INTERLEUKIN-20: BIOLOGICAL FUNCTIONS AND CLINICAL IMPLICATIONS

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IL-20 belongs to the IL-10 family and plays a role in skin inflammation and the development of hematopoietic cells. This study was aimed to explore its other biological functions and clinical implications. Our studies show that IL-20 is a pleiotropic cytokine with potent inflammatory, angiogenic, and chemoattractive characteristics. Inflammation, chemotaxis and angiogenesis are essential for the pathogenesis of atherosclerosis. Both IL-20 and IL-20R1/IL-20R2 were up-regulated in human and mouse atherosclerotic plaques. The IL-20 transcript was induced in monocytes treated with oxidized low-density lipoprotein (OxLDL) and hypoxic condition. Incubation of IL-20 with HUVECs resulted in upregulation of CXCL9 and CXCL11 which are chemoattractant for T cells. *In vivo* administration of IL-20 expression vector using intramuscular electroporation promoted atherosclerosis in Apolipoprotein E-deficient mice. In addition, we demonstrated IL-20 was also involved in the pathogenesis of gastric ulcer. Both the clinical data and the *in vitro* assay on the gastric epithelial cells, MKN45 cells, confirmed that *Helicobacter pylori* infection up-regulated IL-20, but down-regulated IL-20R1 and IL-20R2. The patients with gastric ulcers had same level of IL-20, but had lower level of IL-20R1/IL-20R2, than the patients with duodenal ulcer (P<0.001). Down-regulation of IL-20R1 and IL-20R2 in *Helicobacter pylori* infections involved novel immune regulation of the intact mucosa and correlated with the severity of ulceration. Taken together, IL-20 plays crucial roles in the pathogenesis of atherosclerosis and gastric ulcer. An overview of the clinical implications of IL-20 in atherosclerosis and gastric ulcer based on our most recent novel discovery will be presented.

02-11/P

PRODUCTION OF PROINFLAMMATORY CYTOKINES BY SYNCYTIOTROPHOBLAST CELLS INFECTED WITH HUMAN CMV ISOLATES

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Forty per cent of women with primary cytomegalovirus (CMV) infection during gestation infect their fetuses, which may result in abnormalities for the newborn,varying from mild to severe. Factors determining the pathogenesis of this infection are widely unknown. Little is known about the mechanism of transplacental virus transmission.
One likely site of transmission is via the syncytiotrophoblasts (ST) layer that covers floating chorionic villi, which are also in direct contact with maternal blood. We can considere immunohistochemical analyses of CMV-infected placentas and recent data showing that CMV persistently or latently infects many of the cell types that trophoblast encounter in the uterus. The local and systemic cytokine environment may modulate the transplacental transmission of CMV.
Therefore we have been studying the posssible role of proimflammatory cytokines in this process. The production of TNF-α, IL-1β, IL-6 and IL-8 was studied in syncytiotrophoblast (ST) cultures infected with CMV strains. To resemble a natural infection, clinical CMV isolates and a low multiplicity of infection was used. The interrelationships between these cytokines and the number of nuclei of cells expressing CMV immediate-early (IE) gene were examined. TNF-α and IL-1β were not detected in the CMV-infected ST cultures. All the CMV strains except one induced a low basal secretion of IL-6. The IL-8-inducing capacities of the CMV strains differed in the ST cultures and the IE gene expression of the virus was IL-8 dose-dependent.
The observation indicates that IL-8 may be involved in the maternofetal transmission of CMV. Certain CMV strains induce high amounts of IL-8 and augment their replication in the placenta, while others can replicate if the IL-8 is provided by a coinfecting agent.

02-12/P

INTERLEUKIN 18 DEFICIENT MICE HAVE AN IMPAIRED HOST DEFENSE AGAINST NON-TYPEABLE HAEMO PHILUS INFLUENZAE

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Non-typeable *Haemophilus influenzae* (NTHi) is a Gram-negative respiratory pathogen and after *Streptococcus pneumoniae*, it is the most frequent organism causing community acquired pneumonia affecting mostly patients with underlying diseases like chronic obstructive pulmonary disease or asthma. Earlier studies revealed that in a mouse model for NTHi infection, appropriate recognition and inflammatory responses are important for clearance of NTHi. Herein, we set out to determine the role of the pro-inflammatory cytokine Interleukin (IL-)18. Therefore, we inoculated wild-type C57BL/6 (WT) and IL-18 knock-out (KO) mice with $1x10^7$ Colony Forming Units of NTHi via the intranasal route. After 6, 24, 44 h and 10 days, we sacrificed the animals and studied the inflammatory response and bacterial clearance from the pulmonary tract. After 6 h of infection, when bacterial loads in the lungs were similar, pulmonary cytokine (Tumor Necrosis Factor, IL-6, IL-1α and IL-1β) and chemokine (cytokine induced neutrophil chemoattractant/ KC but not Macrophage Inflammatory Protein 1α) levels were reduced in IL-18 KO animals when compared to WT animals. This attenuated early inflammatory response resulted in reduced bacterial clearance from the respiratory tract after 24 h. Nevertheless, at this and later time-points, lung inflammation was comparable in WT and IL-18 KO mice. Herein, we demonstrate that, IL-18 acts pro-inflammatory and participates in the antibacterial host defense against NTHi infection

02-13/P

SINGLE-NUCLEOTIDE POLYMORPHISMS OF THE INTERLEUKIN-18 GENE PROMOTER REGION IN HEALTHY DONORS OF SOUTHWEST SIBERIAN POPULATION.

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The IL-18 protein expression seems to be regulated by two single-nucleotide polymorphisms located at positions -607 and -137 in the promoter region of the gene. In various populations and at some immunopathology condition the frequency of these specific alleles of IL-18 gene promoter region genotypes changes. The aim of this work was the research of frequency of genotypes and gaplotypes of promoter region of a IL-18 gene in healthy donors of the Southwest Siberian Population. Methods: The polymorphisms of the promoter region were determined by a method an allele - specific amplification with fluorescent detection of results using a real-time polymerase chain reaction (PCR) using SYBR Green I chemistry. For realization of specific amplification were used 4 primers: 2 specific to polymorphic variants in - 607 positions and 2 specific for - 137 positions. The control of passage of amplification was carried out using pair of primers specific for intron of a human gene of IL18. The results were interpreted proceeding from the analysis of the diagrams of accumulation of fluorescence; the specificity was estimated with the help of the melting temperature analysis. Results: The frequency of the G allele of the IL-18/-137 gene polymorphism has made 80 %, of the allele A in –607 position 33%, of the C allele of the IL-18/-607 67%. The frequency of gaplotype CA (– 137 /– 607) has made 18 %, GA - 15 %, GC - 66 % and CC - 1 %. The work partially is maintained by RFBR (№ 05-04-48649-a) and FTSTP "Researches and development on priority directions of a science and technics" (№ 02.442.11.7493).

02-15/P

COMPLETION OF HSV-1 INFECTION OF HUMAN ENDOTHELIAL CELLS (ECS) DEPENDS ON CELL PRODUCED FACTORS

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Background. HSV-1 has been found in human vascular endothelium of organ' biopsies, furthermore HSV-1 participates in atherogenesis. **AIM.** To investigate the role of cell populations of blood and vascular wall in completion of HSV-1 infection of vascular endothelium. **Methods.** ECs were derived from human umbilical veins and smooth muscle cells (SMS) were derived from human aortas. ECs were seeded on the bottom of 24 wells plate and SMC were seeded on TransWell inserts. Leukocytes were derived from donor' blood. Cytokines were tested by ELISA. **Results.** HSV-1 infection of ECs was identified by CPE development and infectious virus accumulation in cultural medium. The results of experimental design were: 1. HSV-1 infection of EC resulted in IL-1β production; 2. HSV-1 infected and glutaraldehyde fixed EC induced in blood leukocytes IFNα, IFNγ, IL-1 β, IL-8 and TNFα production; 3. HSV-1 infection of leukocytes resulted in TNFα production; 4. HSV-1 infected and glutaraldehyde fixed leukocytes induced in blood leukocytes IFNα, IFNγ, IL-1 β, IL-8 and TNFα production; 5. HSV-1 induced in blood leukocytes IFNα, IFNγ, IL-1 β, IL-8 and TNFα production, 6. Non infected intact SMC produced active non identified factors. The completion of HSV-1 infection of cultured ECs depended on the effect of factors produced and resulted in the inflammatory events, delay or inhibition of HSV-1 infection of ECs or set up latent infection and furthermore depended on experimental design and MOI of EC. **Conclusions.** The issue of HSV-1 infection of ECs to viral latency, delayed virus reproduction or inflammatory process depends on the balance of the factors produced by SMC and blood leukocytes. Described cell culture systems can be considered as a model for the investigation of pathogenesis of herpetic and vascular diseases.

02-16/P

INTERLEUKIN-24 NDUCED SIGNALLING IN MELANOMA CELLS

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Interleukin (IL)-24, also named "melanoma differentiation-associated gene 7" (mda-7), is an IL-10-type cytokine that has been discovered by means of differentiation therapy and subtraction hybridization in melanoma cells. Via two receptor complexes (IL-20R1/IL-20R2 and IL-22R/IL-20R2), IL-24 can activate tyrosine kinases of the Janus family (Jaks) and STAT transcription factors in target cells. In addition to this „classical" signalling pathway, IL-24 has been attributed with the unique property to induce apoptosis in many different cancer cells, but not in normal cells, when applied via an adenoviral vector or as a GST fusion protein. Moreover, it promotes anti-oncogenic bystander activity, it inhibits angiogenesis, synergises with radiation, and modulates immune responses. Although the mechanisms of cancer cell selectivity and IL-24-induced apoptosis remain elusive, IL-24 is currently being evaluated in clinical trials. A panel of 20 melanoma cell lines as well as primary human melanocytes and normal human keratinocytes were analysed with respect to their Jak-STAT signalling capacity in response to IL-24 stimulation. Although almost all cells are RT-PCR-positive for the relevant cytokine receptor subunits, only keratinocytes respond to IL-24 stimulation by phosphorylation of STAT3; neither the primary melanocytes nor the 20 tested melanoma lines showed STAT3 activation after IL-24 stimulation. Furthermore, proliferation of these cells was also not altered in response to IL-24 treatment. To further investigate the cancer apoptosis-inducing function of IL-24, we have generated a GST-IL-24 fusion protein and variants thereof and. moreover, we aim at inducibly expressing IL-24 in stably transfected melanoma cell lines. The apoptosis-inducing capacities of these different IL-24s will be discussed.

02-17/P

THE TRANSCRIPTION FACTORS P53 IS REQUIRED FOR TGF-BETA INDUCED MATRIX METALLOPROTEINASE (MMP)-2 EXPRESSION IN MCF10A CELLS.

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Transforming growth factor (TGF)-beta has been reported that it induces matrix metallproteinase (MMP)-2 and MMP-9 upregulation, which involves transcriptional activation in MCF10A. The MMP-2 is p53 target gene and that its expression is subject to p53 regulation in HT1080 and Saos-2. The merging of p53 and TGF-beta signaling networks unveils a new way to link TGF-beta with other pathways within the cell. Integrating information on growth, stress and cellular signaling within TGF-beta gene responses may provide useful insights for cancer therapy. The role of p53 and TGF-beta has been studied only independently in the mouse skin model of chemical carcinogenesis. Little known about these functions of p53 interaction with TGF-beta in carcinoma. To address the functional importance of p53 in regulating constitutive expression of MMP-2 by TGF-beta treatment and interplay of p53 and TGF-beta, MCF10A (a spontaneously immortalized 'normal' breast epithelia cell) was used. MCF10A have a resistance to TGF-beta-induced growth inhibition and upregulate MMP-2 with treatment of TGF beta. Luciferase activity of MMP-2 construct with deletion of the transcription activity of p53 site is more decreased than wild type in promoter assay. These studies document the functional importance of p53 in regulating constitutive expression of MMP-2 by TGF-beta treatment. In further study, we are going to confirm these results through EMSA.

02-18/P

A NOVEL LINEAGE OF INFLAMMATORY HELPER T CELLS

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Upon activation, CD4+ helper T (TH) cells differentiate into effector subsets with different cytokine expression profiles and immune regulatory function. Effector TH cells have been classified into TH1 and TH2 lineages: TH1 cells express IFNg, and TH2 cells produce IL-4, -5 and -13. Although IL-17 has been associated with autoimmune diseases, the lineage definition and function of IL-17-expressing T cells have been unclear. By dissecting the molecular mechanisms whereby IL-17-producing cells were generated in vitro and in vivo, we found that naïve TH cell differentiation to this novel linage required CD28/ICOS costimulation, but was independent of the cytokines or transcription factors crucial for TH1/TH2 differentiation. Furthermore, IL-4 and IFN negatively regulated IL-17 production in the effector phase. In vivo, IL-17 functions to regulate chemokine expression. Anti-IL-17 inhibited chemokine upregulation in the brain during experimental autoimmune encephalomyelitis disease. Transgenic overexpression of IL-17 in lung resulted in chemokine production and leukocyte infiltration. Therefore, IL-17 signatures a distinct TH subset, which regulates tissue inflammation.

02-20/P

SIMULTANEOUS MEASUREMENT OF MULTIPLE CYTOKINES IN SERUM OF PATIENTS WITH SUBARACHNOID HEMORRHAGE

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Previous studies demonstrated neurological injury correlates with the extent of cardiac damage caused by subarachnoid hemorrhage (SAH). The cardiac and neurological variable and outcomes after SAH include: regional wall motion abnormality (RWMA), ejection fraction (EF) <50%, death, cerebral vasospasm and cerebral infarction. In addition, two cardiovascular biomarkers predicting cardiac injury were: B-type natriuretic peptide (BNP) and cardiac troponin I (cTnI). It has been reported that early after SAH, the increased BNP concentrations are associated with myocardial necrosis, pulmonary edema and dysfunction of the left ventricles; and the cTnI release after SAH was associated with a neurogenic form of myocardial injury. In this study, we established the association of abnormal inflammatory cytokines with an increase risk of developing cardiac injury and dysfunction after SAH. For this, blood samples were collected from patients immediately after SAH, and during the clinical follow up period. We measured five cytokines (IL-1a, IL-1b, IL-6, IL-10 and TNF-a) using multiplex Bio-Plex assays. The relationship of these cytokines with each of the variables was quantified. We found that increased IL-6 concentrations associated with EF<50% (OR 4.1, P=0.017, 95% CI 1.3 to 13.2), with cTnI>1.0 (OR 4.1, P=0.017, 95% CI 1.3 to 13.2) and with BNP>275 (OR 2.6, P=0.034, 95% CI 1.1 to 6.4); whereas increased IL-10 concentrations were associated with cerebral infarction (OR 2.9, P=0.046, 95% CI 1.0 to 8.2) and death (OR 10.4, P=0.032, 95% CI 1.2 to 88.0). The Bio-Plex cytokine assays allow testing a large quantity (>200) of samples with limited sample volume (12.5 µL/well). Moreover, the capability of testing simultaneously for different cytokines in multiplex not only conserves the precious samples, but also dramatically decreases the labor and assay time while maintaining high reliability and sensitivity. Thus, the Bio-Plex suspension array system presents a potential platform for developing diagnostic and/or prognostic biomarkers.

02-21/P

IFNγ REGULATION OF TNFα-ENHANCED GLIOMA CELL MIGRATION THROUGH INDUCTION OF MATRIX METALLOPROTEINASE EXPRESSION

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Induction of pro-inflammatory cytokines in response to malignant cells is believed to be an effective immune response to control tumor development. However, growing evidences have suggested an indispensable role of inflammation in the growth of cancer. During metastasis, tumor cells disrupt interstitial tissue barrier by secreting matrix metalloproteinases (MMPs), and their expression is regulated by specific cytokines including TNFα. Glioblastoma is highly invasive and its growth is associated with TNFα expression. In view of the tumor's resistance to TNFα-induced cytotoxicity, we postulate that the tumor takes advantage of TNFα overexpression to enhance its invasiveness. Here, we examined the role of proinflammatory cytokines on MMP expression and its consequent effects on the invasiveness of a malignant human glioma cell-line, T98G, in attempts to delineate the intricate role of inflammation in tumor migration. Following seeding of T98G cells on matri-gel, the cells invaded the basement membrane spontaneously and this transmembrane migration was significantly enhanced by TNFα. In contrast, IFNγ reduced both basal and TNFα-enhanced cell migration. To test the role of TNFα in enhancing glioma invasion, we demonstrated that TNFα upregulated MMP-3 expression as measured by QPCR, whereas IFNγ downregulated its expression. These observations were verified by ELISA of MMP-3 from the culture supernatants. To delineate the mechanisms involved, we showed that IFNγ exerts an inhibitory effect on the binding of TNFα-activated Ets-1 and NFκB to their respective responsive nucleotide elements found in the MMP3 promoter. In summary, our results indicated that TNFα enhances the invasiveness of glioma cells through MMP-3 induction, and such enhanced trans-gel migration can be inhibited by IFNγ via its effects on blocking the activity of Ets-1 and NFκB resulting in transcriptional inhibition of MMP3 gene. Thus, understanding the complex role of cytokines in MMP activation and tumor migration may provide insights to biological behavior of tumor metastasis.

02-22/P

EXPRESSION ANALYSIS OF HUMAN IL-21

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IL-21 is a newly identified cytokine that regulates the functions of T cells, B cells, natural killer (NK) cells and dendritic cells. However it was reported that IL-21 was produced from activated $CD4^+$ T cells, there are conflicting data on IL-21 producing cells. Mouse IL-21 message was detected in T_H2 cells, whereas that of human IL-21 was detected in T_H1 cells and follicular helper T cells (T_{FH}). To identify the IL-21 secreting cell populations, we established a hybridoma cell line producing an anti-human IL-21 monoclonal antibody. Human peripheral lymphocytes were isolated from healthy donors. First of all, we confirmed that human IL-21 was expressed in activated $CD4^+$ T cells but not either in resting $CD4^+$ T cell or activated $CD8^+$ T cells by intracellular cytokine staining method. Next we examined which $CD4^+$ T cell populations produce IL-21. Purified $CD4^+$ T cells were stained with anti-IL-21 antibody in combination with some other surface markers. Human IL-21 was expressed in activated $CD4^+CD45RO^+$ T cells but not in activated $CD4^+CD45RA^+$ T cells. Furthermore we demonstrated that the IL-21 producing $CD4^+CD45RO^+$ T cells were $CD45RA^-CCR7^+$ central memory T cells (T_{CM}). Then we investigated which type of helper T cell expresses IL-21. When purified $CD4^+CD45RA^+$ naïve T cells were activated and cultured in T_H1 or T_H2 skewing condition, only a small population of IFN-gamma producing T_H1 cells, but not IL-4 producing T_H2 cells, could express human IL-21. We are now proceeding with further study to assess the functional role of human IL-21 on $CD4^+$ naïve and memory T cell subsets.

02-23/P

ENHANCED BIOLOGICAL PROPERTIES RESULTING FROM HUMAN CELL EXPRESSION OF RECOMBINANT HUMAN INTERLEUKIN-4.

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Many cytokines and growth factors are heavily glycosylated, with up to 75% of their mass consisting of carbohydrate moieties. Traditionally, human cytokines have been expressed in non-human cells, including bacteria, yeast and murine expression systems. However, the biological importance of species-specific post-translational modifications, in particular glycosylation, is increasingly being recognised as pivotal to protein function. It has been proposed that glycosylation is important for secretion, solubility, resistance to proteolysis, immunogenicity, biological recognition, biological activity, *in vivo* stability and clearance of glycoproteins including cytokines and growth factors. We have purified recombinant human interleukin-4 (IL-4) expressed in modified human HEK 293 cells. *In vitro* comparisons of the biological activity of human cell expressed IL-4 with IL-4 expressed in other species including *E. coli*, CHO and *Pichia pastoris* revealed that glycosylated forms of IL-4 were more stable. Non-glycosylated IL-4 produced either by treating cells with tunicamycin or removing carbohydrate moieties with PNGase F demonstrated reduced activity and stability compared to glycosylated IL-4. The enhanced biological stability of cytokines such as IL-4, using our expression system, may prove useful in deriving cultures of immune cells *ex vivo* such as dendritic cells for cancer therapy. More broadly, therapeutic efficacy of human proteins expressed using human cell systems may be improved relative to existing biologics from non-human sources.

02-24/P

POSTTRANSLATIONAL MODIFICATIONS ON HUMAN CELL EXPRESSED (HCX™) HUMAN RECOMBINANT PROTEINS.

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Most proteins undergo posttranslational modification (PTM), which can alter their physical and chemical properties (e.g., MW, pI, folding, stability, activity, antigenicity, and function). The presence or absence of PTMs may be significant to both the activity and longevity of the protein in a biological system. Recombinant proteins are dependent on the machinery of the cell line in which they are made to determine the presence and type of PTM. Hence the PTMs of human proteins made recombinantly in a human cell line may differ significantly from the same protein made in NS0, CHO, *E. coli* or any other non-human cell line. *E. coli*, for example does not possess the type of cellular machinery used for glycosylation in higher organisms, hence human proteins produced in an *E. coli* cell line are non-glycosylated. Consequently, the function of this protein may vary significantly from the glycosylated version. In this study various methods were used to determine the PTMs, in particular glycosylation, that occurs on proteins produced recombinantly in modified human 293 cells. For example, one-dimensional electrophoresis combined with enzymatic de-glycosylation was used to determine the relative mass of the glycosylated versus the de-glycosylated protein. Enzymatic and chemical de-glycosylation methods combined with MALDI-MS and LC-MS were used to determine the glycosylation sites as well as the *N*- and *O*-linked glycan structures present on the protein. These methods determine not only the differences in the glycosylation but may also give some insight into the possible differences in function of the protein.

02-25/P

IL-8 IS ELEVATED IN NEONATAL DRIED BLOOD SPOT SAMPLES FROM PRETERM NEWBORNS

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Foetal-maternal inflammation is an accepted cause of preterm birth, and many reports have demonstrated elevated concentrations of inflammatory markers in maternal serum and amniotic fluid. In addition, IL-8 has been reported elevated in cord blood and serum from preterm infants drawn shortly after birth.
The aim of this study is to investigate if inflammatory conditions are still present in preterm infants several days after birth. Dried blood spots samples (DBSS) collected routinely for the Danish neonatal screening program 4-11 days after birth, were analysed for IL-8 together with IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, TNF-α, TNF-β, leptin, adiponectin, IGF-BP1, IGF-BP3, CRP and NT-4 with the Luminex xMAP technology using an in-house method as previously described (Skogstrand et.al. Clin Chem 2005). 123 newborns, 62 born preterm and 61 born term, were included in the study. The gestational age was between 24-42 weeks. IL-8 was found significantly higher in infants born before 32 weeks gestation, mean concentration 1154 pg/ml and 338 pg/ml, respectively, $p<0.0001$, indicating an ongoing inflammatory condition in the child. It is possible that the elevated IL-8 levels are caused by increased production in the infants, because IL-8 transferred from mother to fetus is considered to be degraded a few days after birth.
Congenital brain damages like cerebral palsy and autism are more often seen in preterm children, and inflammatory conditions have been proposed as a possible cause. This study suggests that inflammatory markers in newborns in relation to preterm birth and brain damage should be more thoroughly examined.

02-27/P

ATORVASTATIN INDUCES ATHEROSCLEROTIC PLAQUE STABILITY IN HUMANS BY ELICITING A TH2 RESPONSE.

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Background. Atherosclerosis is an inflammatory disease of the arterial wall where and adaptive Th1-driven immunoinflammatory responses contributes to atherosclerotic plaque progression towards instability by mediating inflammatory cell recruitment and activation and extracellular matrix degradation. Statins exert actions beyond that of simply lowering cholesterol levels, and some effects on immune function have been reported. In particular, recent data in vitro demonstrated that atorvastatin may orient immunity from a Th1 towards a Th2-prevalent profile. However, whether this effect is also present in human in vivo, and therefore could contribute to plaque stabilization after statin therapy is still unknown. Thus, in this study we investigated the effect of atorvastatin on Th1/Th2 cytokine release in atherosclerotic plaques derived from patients underwent carotid endarterectomy.
Methods and results. Seventy patients with severe (>70%) stenosis of the extracranial tract of the internal carotid artery were randomized to the American Heart Association Step 1 diet plus atorvastatin (20 mg/d) or the American Heart Association Step 1 diet alone for 3 months before endarterectomy. ELISA was performed on plaque omogenates to assess the release of IL-4, IL-10, IL-12 and INFgamma. Plaques were also subjected to analysis of IFNgamma, MMP-2 and MMP-9, type 1 procollagen content by immunohistochemistry.
Atorvastatin treatment resulted in a significant ($P<0.0001$) reduction in plaque concentrations of IL-12 and IF, Th1-associated cytokines. By contrast, production of IL-4 and IL-10, Th2-associated cytokines was highly increased ($P<0.0001$) by atorvastatin therapy. Plaques from the atorvastatin group had fewer ($P<0.0001$) immunoreactivity for INFgamma, MMP-2 and MMP-9; reduced ($P<0.0001$) gelatinolytic activity; increased ($P<0.0001$) collagen content; reduced ($P<0.0001$) macrophages, T-lymphocytes, and HLA-DR+ cell infiltration.
Conclusions. This is the first demonstration in humans, to the best of our knowledge, that statins may produce a shift from a Th-1 to a Th-2 profile in inflammatory cells infiltrating atherosclerotic plaques. This effect could contribute to plaque stabilization by inhibiting cell recruitment and collagenolytic activity, and in contrast promoting collagen synthesis and deposition.

02-28/P

INCREASED PRO-INFLAMMATORY CYTOKINE PRODUCTION IN DOWN SYNDROME CHILDREN UPON STIMULATION WITH LIVE INFLUENZA VIRUS.

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Children with Down syndrome (DS) have an increased susceptibility to infections, due to altered humoral and/or cellular immunity. Aim of the study was to determine the cytokine production in whole blood of children with DS upon stimulation with lipopolysaccharide (LPS), heat-killed *S. pneumoniae* and live influenza A virus.
Material and methods. Whole blood of 61 children with DS and 57 of their healthy siblings was stimulated with 200 ng/ml LPS, 4 x 10E7 CFU/ml *S.pneumoniae* and 2,5 x 10E4 TCID50/ml influenza A during 6, 24 and 48 hours.Concentrations of IL-1β, IL-6, IL12p70, TNFα and IL-10 were determined with Cytometric Bead Array at all time-points.
Results. At most of the time-points TNFα, IL-1β, and IL-6 concentrations were significantly higher in children with DS following stimulation with influenza A virus, but not following stimulation with LPS nor *S. pneumoniae.*
In detail: (mean ± SEM)
TNFα: 150 ± 24 pg/ml in DS *versus* 101 ± 1 pg/ml in controls at 6 h (p=0.001),382 ± 78 pg/ml in DS *versus* 294 ± 96 pg/ml in controls at 48 h (p=0.003).IL-1β: 1193 ± 94 pg/ml in DS *versus* 1016 ± 16 pg/ml in controls at 24 h (p=0.033), 1262 ± 118 pg/ml in DS *versus* 1022 ± 22 pg/ml in controls at 48 h (p=0.002).IL-6: 2274 ± 268 pg/ml in DS *versus* 1205 ± 81 pg/ml in controls at 6 h (p=0.000), 4722 ± 852 pg/ml in DS *versus* 1689 ± 266 pg/ml in controls at 24 h (p=0.000) and 6446 ± 1145 pg/ml in DS *versus* 1685 ± 204 pg/ml in controls at 48 h (p=0.000).
Conclusion. Compared to their siblings, children with DS have an altered immune response to a viral stimulus, which includes the production of higher levels of pro-inflammatory cytokines.

02-29/P

INTERLEUKIN 18 BINDING PROTEIN IS ASSOCIATED WITH SYSTEMIC JUVENILE IDIOPATHIC ARTHRITIS

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Juvenile Idiopathic Arthritis (JIA) occurs before the age of 16 and is the most common childhood rheumatic disease. The systemic subtype of JIA (sJIA) is potentially fatal and has the characteristic feature of daily spiking fevers, with elevated levels of inflammatory cytokines. Interleukin-18 (IL-18) is an inflammatory cytokine and promotes the production of interferon-γ (IFNγ). It has a naturally occurring soluble antagonist, IL-18 binding protein (IL-18BP). Increased levels of IL-18 have been shown in patients with various auto-inflammatory diseases including sJIA, and treatment with IL-18BP significantly decreases the severity of collagen-induced arthritis. These observations suggest the possible involvement of these proteins in sJIA. Genotyping data for all single nucleotide polymorphisms (SNPs), with a minor allele frequency >0.05, in the two genes was obtained from publicly available databases. HAPLOVIEW was used to examine the pairwise linkage disequilibrium (LD) between the SNPs and TAGGER to select tagging SNPs (tSNPs). These tSNPs, 13 for IL-18 and 3 for IL-18BP, were genotyped in 130 sJIA patients and 146 healthy controls. The SNPs were examined for association by single marker and haplotype analysis using UNPHASED. The IL-18 SNPs showed no significant difference in frequencies between the two cohorts, either individually or as haplotypes. The IL-18BP SNPs showed no significant association individually (marker 2 was marginally significant with p=0.07), however haplotype analysis with markers 1-2 showed a global p value of 0.007, with a lower frequency of the common haplotype in the case cohort (p=0.02). In conclusion, this study has found no association between IL-18 and sJIA but has found an association with a two marker IL-18BP haplotype. As the markers in this associated haplotype are located within the promoter region and the 5'UTR they could affect gene transcription or mRNA stability, altering the levels of IL-18BP and so in turn the levels of active IL-18.

02-30/O

TRANSITION FROM INNATE TO ACQUIRED IMMUNITY IS BALANCED BY GP130-MEDIATED STAT3 ACTIVATION DURING ACUTE INFLAMMATION

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During a successfully resolving episode of acute inflammation, leukocyte recruitment is defined by an initial influx of neutrophils, which are rapidly cleared and replace by a more sustained population of mononuclear cells. This profile of leukocyte trafficking predicts a transition from innate to acquired immunity and is governed by a coordinated regulation of leukocyte recruitment and clearance. Using a peritoneal model of acute inflammation, we have previously identified a central role for interleukin-6 in the control of this immunological switch. However the signal transduction events that bring about these changes remain unknown. IL-6 responses are transmitted through gp130, which serves as the universal signal-transducing receptor subunit for all IL-6-related cytokines. Consequently, to dissect the gp130-mediated events controlling transition from innate to acquired immunity, peritoneal inflammation was established in gp130 knock-in mice that display altered signalling capacity. In mice ($gp130^{Y757F/Y757F}$) deficient in SHP2 and SOCS3 binding, but exhibiting hyperactivation of STAT1 and STAT3, chemokine-mediated neutrophil recruitment was impaired. In contrast, T-cell infiltration and CCL5 expression was enhanced. This phenotype was however completely inversed in mice lacking gp130-mediated STAT1/STAT3 activity ($gp130^{\Delta STAT/\Delta STAT}$). Control of this process was related to STAT3 signalling, because monoallelic deletion of *STAT3* in $gp130^{Y757F/Y757F}$ mice ($gp130^{Y757F/Y757F:STAT3+/-}$) corrected the exaggerated phenotype displayed by $gp130^{Y757F/Y757F}$ mice. These processes were reliant on IL-6 activity since $gp130^{Y757F/Y757F}$ mice crossed onto an IL-6-deficient background displayed no such changes in neutrophil and lymphocyte migration. Consequently, gp130-mediated STAT3 activation represents a '*master switch*' for the transition from innate to acquired immunity.

02-31/P

IL-21 INHIBITS LPS-INDUCED CYTOKINE PRODUCTION OF HUMAN MONOCYTE-DERIVED DENDRITIC CELLS

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Dendritic cells (DCs) play an important role in innate and adaptive immune responses. In addition to their phagocytic activity, dendritic cells present foreign antigens to naïve T cells and regulate the development of adaptive immune responses. Upon contact with DCs activated T cells produce large quantities of cytokines such as IFN-γ and IL-21. T cell-derived IL-21 regulates both innate and adaptive immune responses. It enhances maturation and cytotoxicity of NK cells and promotes IFN-γ production in these cells. IL-21 enhances the expression of Th1 associated genes, such as IL-12R and IFN-γ, in T cells and regulates immunoglobulin production in B cells. Here we have analyzed the effect of IL-21 and IFN-γ on LPS-induced maturation and cytokine production of human monocyte-derived DCs. Both IL-21 and IFN-γ receptor genes were expressed in high levels in immature DCs. Pretreatment of immature DCs with IL-21 inhibited LPS-stimulated DC maturation and expression of CD86 and HLA class II molecules. IL-21 pretreatment also dramatically reduced LPS-stimulated production of TNF-α, IL-12, CCL5 and CXCL10 but not that of CXCL8. In contrast, IFN-γ had a positive feed-back effect on immature DCs and it enhanced LPS-induced DC maturation and the production of cytokines. IL-21 weakly induced the

expression Toll-like receptor 4 (TLR4) and TIRAP genes, whereas the expression of TRIF, MyD88 or TRAM genes remained unchanged. However, IL-21 strongly stimulated the expression of suppressor of cytokine signalling (SOCS)-1 and SOCS-3 genes. SOCS are known to suppress DC functions and interfere with TLR4 signaling. Our results demonstrate that IL-21, a cytokine produced by activated T cells, can directly inhibit the activation and cytokine production of myeloid DCs providing a negative feed-back loop between DCs and T lymphocytes.

02-32/P

INTERLEUKIN-20 FUNCTIONS AS A PROINFLAMMATORY MOLECULE IN RHEUMATOID AND EXPERIMENTAL ARTHRITIS

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The pathogenesis of rheumatoid arthritis (RA) reflects an ongoing imbalance between pro- and anti-inflammatory cytokines. Interleukin-20 (IL-20) has pro-inflammatory properties for keratinocytes. We wanted to determine whether IL-20 was involved in RA. Using ELISA, we demonstrated that RA patients expressed significantly higher levels of IL-20 in synovial fluid than did gout or osteoarthritis patients. IL-20 and its receptors were consistently expressed in the synovial membranes and synovial fibroblasts of RA patients (RASFs) and it acted on RASFs through the ERK 1/2 signal transduction pathway. The effect of IL-20 on endothelial cells, RASFs, and neutrophils was investigated using MTT and the migration assay. The result showed that IL-20 induced RASFs to secrete monocyte chemoattractant protein-1 (MCP-1), IL-6, and IL-8, and it promoted neutrophil chemotaxis and the proliferation of endothelial cells *in vitro*. The expression of rat IL-20 and its receptors in healthy and collagen-induced arthritis (CIA) rats was analyzed and compared. Both IL-20 and IL-20R1 were upregulated in the rat CIA model. We used intramuscular electroporation to deliver soluble (s)IL-20R1 or sIL-20R2 into CIA rats and monitored the severity of arthritis *in vivo*. Electroporated sIL-20R1 plasmid DNA significantly decreased the severity of arthritis in CIA rats. These data indicated that IL-20 is upregulated in the synovial fluid of RA patients and acts as a chemokine that attracts migration of neutrophils, SFs, and promotes proliferation of endothelial cells. The rat CIA model further demonstrated that IL-20 is involved in the pathogenesis of arthritis because sIL-20R1 effectively reduced arthritis in CIA rats. Thus, IL-20 regulated several crucial molecules in RA and might play multiple roles in the pathogenesis of RA.

02-33/P

SYSTEMIC LIPOPOLYSACCHARIDE CHALLENGE OF HUMAN VOLUNTEERS INDUCES TOLERANCE TO MULTIPLE TOLL-LIKE RECEPTOR LIGANDS

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Exposure of cells to lipopolysaccharide (LPS) induces tolerance towards a second exposure to LPS and induces cross tolerance to certain other bacterial components (and vice versa). LPS tolerance is observed in blood leukocytes of patients with sepsis and during human endotoxemia. In the present study we investigated whether *in vivo* systemic LPS challenge of human volunteers induced tolerance to the Toll-like receptor 4 (TLR4) ligand LPS and tolerance to other TLR ligands. Healthy human volunteers were injected intravenously with LPS (4 ng/kg) and blood was collected before (t=0) and at t=3, 6, 8 and 24 hours after LPS challenge. Blood was stimulated *ex vivo* with LPS or ligands for TLR2 (peptidoglycan, lipoteichoic acid, zymosan), TLR3 (polyinosinic-polycytidylic acid), TLR5 (flagellin), TLR7 (imidazoquinoline) or TLR9 (bacterial DNA). Plasma was collected after 24 hours of stimulation and analysed for cytokines by CBA. *Ex vivo* stimulation of whole blood obtained before i.v. challenge (t=0) with ligands for TLR2, TLR3, TLR4, TLR5 or TLR7, but not TLR9, induced secretion of TNF, IL-1β, IL-6, IL-8 and IL-10 in plasma. In LPS-stimulated whole blood obtained at t=3 and 6 hours, low or undetectable plasma levels of these cytokines were found. Except for IL-10, plasma cytokine levels returned to baseline level in whole blood obtained at t=8 or 24 hours. Stimulation of whole blood obtained at t=3 and 6 hours with ligands for TLR2, TLR5 or TLR7 in general also resulted in reduced cytokine release into plasma. Strikingly, blood leukocytes were also hyporesponsive to the TLR3 ligand, which in contrast to the other TLR ligands signals solely through a MyD88-independent pathway. Taken together, these data indicate that systemic LPS challenge of human volunteers induces cross tolerance to multiple TLR ligands, and suggest that LPS exposure of human blood leukocytes may hamper the cytokine response to various microbial components.

02-34/P

EFFECTS OF IL-23 IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS AND SPLENOCYTES

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IL-23 is a cytokine secreted by dendritic cells (DC) and macrophages, which is composed of the p40 subunit of IL-12 and a specific p19 subunit. It binds to the IL-23 receptor (IL-23R), a dimer of the IL-12Rβ1 chain and the IL-23p19 receptor on T cells, macrophages and DC. In the murine system, IL-23 induces a specific subset of T cells, termed Th17, which is responsible for the maintenance of autoimmune inflammation in animal models of allergic encephalomyelitis, arthritis and skin inflammation. In humans, IL-23 is overexpressed in psoriasis. Its role in the secretion of cytokines, such as IFN-γ and IL-17, in human cells is less well characterized. In human peripheral blood mononuclear cells (PBMC), PHA blasts and splenocytes, IL-23 together with either IL-18 or IL-1 induced IFN-γ, but only to ~ 30% of the amount induced by IL-12 + IL-18 or IL-12 + IL-1. This IFN-γ production could be inhibited by an antibody directed against the common IL-12Rβ1 chain, as well as by a p38 MAPK inhibitor. For the secretion of IL-17, T cell stimulation was required. When PBMC or PHA blasts were stimulated with IL-23 in the presence of anti-CD3 + anti-CD28 monoclonal antibodies, IL-17 secretion was enhanced by 30 – 60% in 10/13 donors and by 100 – 400% in 3/13 donors. IL-12 had no influence on anti-CD3 + anti-CD28-induced IL-17 secretion. While IFN-γ had no effect on IL-23-induced IL-17 secretion, this process could be inhibited by IL-4 and IL-10, which also markedly reduced IL-23 induction in LPS-stimulated PBMC, DC and macrophages. This indicates that the anti-inflammatory cytokine IL-10 and the Th2 cytokine IL-4 antagonize both the secretion and action of IL-23.

02-35/P

ENHANCED NUCLEAR TRANSLOCATION OF THE INTERLEUKIN-1 HOMOLOGUE IL-1F7B AFTER LPS-STIMULATION.

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IL-1F7b like IL-1β, IL-18 and IL-33 requires caspase-1 cleavage to generate the mature cytokine. This cleavage allows active secretion of IL-1β and IL-18. However, IL-1F7b is rarely observed in the extracellular compartment. It remains possible that caspase-1 cleavage renders intracellular functionality to IL-1F7b. Following LPS stimulation, 80% of IL-1β is diffusely present in the cytoplasm of macrophages whereas 20% is secreted together with caspase-1 by secretory lysosomes. In the same cell, IL-1α translocates to the nucleus via its N-terminal nuclear localization sequence. To analyze whether IL-1F7b shares similarity to IL-1α, we studied the intracellular distribution of this cytokine before and after LPS stimulation. Two expression vectors encoding human IL-1F7b tagged to YFP and CFP at

the N- and C-terminus, respectively, were constructed and transfected into RAW264.7 cells. Digital confocal microscopy shows low basal expression of IL-1F7b-fusion proteins despite a constitutively active CMV promoter. However, LPS stimulation significantly increased the expression of both fusion proteins as confirmed by western blot. These results are in concordance to our previous study showing that LPS stabilizes mRNA instability elements within the coding region of IL-1F7b and thus induces upregulation of IL-1F7b protein. Importantly, only IL-1F7b-YFP fusion protein with IL-1F7b at the N-terminus shows nuclear translocation after LPS-stimulation. We therefore propose that LPS-stimulation induces caspase-1 cleavage and nuclear translocation of mature IL-1F7b. In order to address this hypothesis two vectors encoding mature IL-1F7b and propiece IL-1F7b tagged to the N-terminus of YFP were constructed. Following transfection into RAW264.7 cells the propiece IL-1F7b showed no change in expression and intracellular distribution pattern before and after LPS stimulation. However, mature IL-1F7b shows significantly increased total cell and particularly nuclear expression after LPS-stimulation. These results indicate that IL-1F7b like IL-1α undergoes nuclear translocation and might also act as a transcriptional modulator.

02-36/P

THE PROTECTIVE EFFECT OF IL- 1α AND β ON THE ACETAMINOPHEN INDUCED LIVER TOXICITY RELIES ON THEIR EARLY INDUCTION BY ACETAMINOPHEN ITSELF

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We have previously shown that both IL-1α and IL-6 exhibit hepatoprotective effect if given before administration of acetaminophen (APAP) that is partially mediated by PGE_2. When the effect of IL-1β was investigated using the same model, IL-1β (1500 IU/mouse) or anti-IL-1β antibody (0.5 ml of rabbit to mouse IL-1, obtained by three consecutive injection of IL-1 β, first in complete FCA) were given i.p. 3 hours before APAP. The survival of mice has been followed for 72 hours and the serum concentration of aminotransferases (AST and ALT) were determined 18-24 hours after APAP administration. IL-1β significantly increased the survival of mice and decreased serum level of AST and ALT ($p < 0.05$). As expected, when anti-IL-1Rα antibody was administered, significant increase in the serum concentrations of AST and ALT ($p < 0.05$ or better) were observed. The survival of animals has not been followed in this experiment. The finding that anti-IL-1Rα antibody treatment causes increased AST and ALT serum level, the same effect as anti- IL-1β antibody therapy does, prompted us to analyze the endogenous synthesis of IL-1 cytokine in liver samples from IL-1 non-exposed but APAP intoxicated animals. RT-PCR analysis of the IL-1α and IL-1β expression level in liver samples from APAP intoxicated mice has revealed that acetaminophen induces the expression of both cytokines, though somewhat different, already 1 hour after its intragastric administration. This expression was even higher at 6 hours following APAP administration and could be blocked by intraperitoneal injection of aspirin at the dose which inhibits NF-κB activity. Based on this we hypothesized that organism intoxicated with APAP synthesizes its own IL-1 early on following APAP administration which may represent a host defense reaction that could be helped by applying appropriate dose of exogenous cytokines.

02-37/P

CYTOKINE SNPS ASSOCIATED WITH PRETERM BIRTH

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Preterm Birth (PTB), delivery prior to 37' weeks of gestation, complicates ~6.3% of all pregnancies in Denmark increasing the risk of neonatal morbidity and mortality. Some of the long-term complications seen in relation to PTB are cerebral palsy, respiratory disease, blindness and deafness. Well-established observations support a genetic influence on the risk for PTB: the leading risk factor for PTB is a previous delivery preterm; mothers themselves born preterm have an increased risk of PTB; and the association between ethnicity/race and PTB persists in some instances even if social status and economic factors are changed. Several studies indicate evidence for upper reproductive tract infection involving the maternal/fetal unit for being a part of the etiologic pathways leading to PTB. Our hypothesis was that SNPs within the three cytokine genes, TNF, IL1B and IL6 contribute to the pathogenesis and complications of PTB. To investigate this, a pilot study of 117 women (Danish, Caucasians) consisting of 62 cases (labor < 37 weeks of gestation) and 55 controls (labor ≥ 37 weeks of gestation) were genotyped at 19 known SNP sites. The genotyping was done by direct sequencing or by the TaqMan method. Our results showed that mothers carrying the TNF -857 rare allele and mothers homozygous for the IL1B -31/-511 rare allele had increased risks of PTB (P=0.048 and P=0.018 respectively). Two estimated TNF haplotypes were also found to be associated to PTB (P =0.037 and P=0.045). Because of the complexity and racial differences in PTB the results presented here need to be confirmed in a larger scale and in different ethnic groups. We hope that the results from this pilot study inspire other groups to include some of these SNPs into their SNP panels when looking for associations in larger populations.

02-38/P

TUMOR NECROSIS FACTOR ALPHA PROMOTER POLYMORPHISM IN SECONDARY HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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Secondary hemophagocytic lymphohistiocytosis (HLH) can be associated with various diseases including infections and lymphoma. The clinical findings of HLH can be explained by an increased production of cytokines such as tumor necrosis factor alpha (TNF-α). As not all the patients with infection or lymphoma have secondary HLH, we investigated the relationship between susceptibility to secondary HLH and TNF-α promoter polymorphisms to identify genetic factors of secondary HLH. We determined the alleles of four promoter sites (-1031/-857/-308/-238) of TNF-α gene by using Taqman-based allelic discrimination assays in the 66 Korean patients with secondary HLH and 100 healthy Korean controls. We found that the frequency of the TNF-α -1031C allele, which is associated with higher-plasma TNF-α levels, was enriched in patients with secondary HLH compared with healthy controls (OR = 2.00, 95% CI 1.20-3.30, P = 0.007). In haplotype analysis of TNF-α polymorphisms, the haplotype CTGG was detected only in the patient group, and the haplotype group (CCGG or CCGA or CTGG) including TNF-α -1031C allele was overexpressed in secondary HLH patients (OR = 2.52, 95% CI 1.33-4.77, P = 0.004). These results suggested that TNF-α -1031C allele and its associated haplotypes in Koreans may enhance susceptibility to secondary HLH.

02-40/P

THE BDNF PRODUCTION IN HTLV-1 INFECTED CELL LINES, MT-2 AND HUT102

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Adult T cell leukemia (ATL), which is caused by the human T-cell leukemia virus type I (HTLV-I) infection, is an aggressive and frequently fatal malignancy. While the exact mechanisms in the spontaneous growth of T cell leukemia are not known, studies have shown constitutive expressions of several growth factors and active growth-signaling pathways in T cell leukemia. Although earlier reports have indicated the secretion of IL-2, more recent studies have shown that IL-2 mRNA expression in ATL cells was not detected, suggesting an another mediator involved in the growth of ATL cells. BDNF is one of the neurotrophic factors, which was reported the relation with the disease of Alzheimer's disease, however, neither appearance nor the role in parts other than the brain have been much clarified yet. In this study, we show the BDNF production in HTLV-1 infected cell lines, MT-2 and HUT102. Furthermore, mRNA of TrkB, which is receptors of BDNF, was detected by the RT-PCR method. These suggest that BDNF may take part in the proliferation of ATL cells and could be a new target for T-cell leukemia research.

02-41/O

ROLE OF IL-13 IN THE ASTHMA-LIKE PHENOTYPE INDUCED BY IL-9 IN VIVO.

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IL-9 is a typical TH2 cytokine, whose overexpression, either systemically or under the control of a lung specific promoter, induces an asthma-like phenotype, including mucus overproduction, sub-epithelial fibrosis, mastocytosis, lung eosinophilia, increased mucus production and airway hyperresponsiveness. These activities correlate with increased production of other TH2 cytokines such as IL-4, IL-5 and IL-13 in IL-9 transgenic mice. In order to determine the exact role of IL-13 in this phenotype, mice overexpressing IL-9 were crossed with IL-13-deficient mice. In these animals, IL-9 could still induce eosinophilia, mastocytosis and B lymphocyte infiltration of the lungs. By contrast, mucus production and upregulation of epithelial genes in the lungs upon IL-9 overexpression were completely abolished in the absence of IL-13. Using bone marrow or spleen cell transfer experiments with mice that overexpressed IL-9 but were deficient in the IL-9R, we could demonstrate that the effect of IL-9 on lung epithelial cells is indirect, and could be fully restored by transfer of hematopoietic cells expressing IL-9R. However, IL-9 responsive hematopoietic cells needed a functional IL-13 gene to restore mucus production, indicating that IL-13 is a direct mediator of the effect of IL-9 on lung epithelial cells. Taken together, these data indicate that IL-9 can promote asthma through IL-13-independent pathways via mast cells, eosinophils and B cells, and through IL-13 induction by hematopoietic cells for mucus production by lung epithelial cells.

02-42/P

SIMVASTATIN REGULATES MYOCARDIAL INTERLEUKIN-1β MRNA EXPRESSION AND LIVER CONGESTION IN RATS WITH CHRONIC HEART FAILURE

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Proinflammatory cytokines such as interleukin 1β (IL-1β) were recently identified as important contributors to the syndrome of chronic heart failure (CHF) and to the underlying cardimyopathic processes of adverse left ventricular remodeling, pulmonary edema and liver congestion. Hydroxymethylglutaryl coenzyme A reductase inhibition by Statins may affect the expression of pro-inflammatory cytokines, as well as having being found to be beneficial in left ventricle remodeling, immediately after myocardial infarction. However, studies regarding Simvastatin treatment after CHF establishment are few.
The left anterior descending coronary artery in male Wistar rats was ligated. Control (sham-operated) and CHF animals were assessed for transthoracic echocardiogram 4 weeks after operation and were assigned to the following groups: Sham-operated (Sham, n=7); CHF-Gavage (CHF-G, for 8 weeks, n=10) and CHF-Sinvastatin (20mg/kg body weight/ per day, for 8 weeks, CHF-S, n=5). After 12 weeks, the hearts were isolated and the proximal portion of the left ventricle was sectioned by visual inspections to study the relative expression of mRNA for interleukin (IL)-1β and plasma lipids as well lung and liver weights.
CHF at 12 weeks in infarcted rats was indicated by an increase in the left ventricular end-systolic diameter, and wet/dry weights lung and liver ratios compared with sham-operated rats. Simvastatin markedly attenuated the wet/dry weights liver ratios without affecting lung ratio. The mRNA expression of IL-1β in the left ventricle which was significantly elevated compared with the control was also attenuated by Simvastatin. Plasma lipids were significantly reduced in CHF-Simvastatin group.
Treatment with Simvastatin after 4 weeks of myocardial infarction showed to be effective in reducing the signs of liver congestion after CHF development. Simultaneously, the treatment decreased pro-inflammatory cytokine gene expression and showed plasma-lipid lowering effects, suggesting an important role of Statin treatment after CHF development.

02-43/P

INTERLEUKIN-1β MRNA EXPRESSION IN THE MESENTERIC ADIPOSE TISSUE OF RATS WITH CHRONIC HEART FAILURE

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Chronic heart failure (CHF) is associated with metabolic abnormalities that include several anabolic and catabolic systems and results in a progressive catabolic state leading to cardiac cachexia in the advanced stages of the disease. Impairment in the function of multiple systems such as the pulmonary tract, the liver, the gastrointestinal tract and the skeletal muscle is found in CHF. Recently, white adipose tissue (WAT) has been recognized as an important source of production and site of action of pro-inflammatory cytokines. However, few studies have addressed the role of WAT in CHF.
To assess the importance of WAT in the production of pro-inflammatory cytokines, the local expression of interleukin-1 beta (IL-1β) was evaluated in an animal model of CHF. Control (sham-operated) and CHF animals were assessed for transthoracic echocardiogram 4 weeks after operation and were assigned to the following groups: Sham-operated (Sham, n=7) and CHF (CHF, n=9). After 12 weeks, the mesenteric adipose tissue (MAT), was removed and the relative expression of mRNA for interleukin (IL)-1β by RT-PCR and quantitative immunocytochemistry for RT1B (indicating the presence of

MHC II) and with antibody against mononuclear phagocytes, showed the infiltrating cells in the adipose tissue to be macrophages.
CHF at 12 weeks in infarcted rats was indicated by an increase in the left ventricular end-systolic diameter, and wet/dry weights lung and liver ratios compared with sham-operated rats. The mRNA expression of l-1β in the left ventricle increased significantly in animals with CHF (0.12 ± 0.04 versus 0.61 ± 0.08 arbitrary units; $p < 0.01$). No immunoreactivity for mononuclear cells in MAT was found for any of the studied groups.
In CHF, local IL-1β gene expression was found, despite the lack of mononuclear cell infiltration. These observations suggest that the mesenteric adipocytes are expressing IL-1β mRNA and that these cells may play an important role in the synthesis and release of the cytokine with local (autocrine) and systemic (endocrine) consequences under CHF.

02-44/P

EFFECTS OF INTERLEUKIN-2 ON IMMUNE FUNCTIONS OF UNDERNOURISHED RATS SUBMITTED TO CHRONIC MODERATED-INTENSITY EXERCISE

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Regular moderate exercise modulates the response to stressing stimuli and thus improves immune function in conditions commonly associated with immunodepression. Considering that IL-2 is a very important cytokine for the stimulation of lymphocyte-T proliferation, we have sought to study immunodepression induced by undernourishment on the proliferative response under the stimulus of interleukin-2 (IL-2); concanavalinA (ConA); lipopolysaccharide (LPS) of cells from the spleen and lymph nodes.
Forty male Wistar rats were randomly assigned to the following groups: sedentary animals fed ad libitum (SF, N = 10), or submitted to energy restriction (SER, N = 10, receiving 50% of the mean amount of chow consumed by SF); and trained animals fed ad libitum (TF, N = 10) or submitted to energy restriction (TER, N = 10), who exercised on a treadmill (at 60-65%VO_{2max} 5 $d.wk^{-1}$ for 10 wk^{-1}), after 30 d under the caloric restriction protocol. The incorporation of [2-(14)C]-thymidine by lymphocytes obtained from the mesenteric lymph nodes and spleen was measured.
Undernourishment provoked a marked reduction in the response to ConA, LPS, and IL-2 by the lymphocytes obtained from mesenteric lymph nodes and from the spleen in comparison with SF. When results were expressed as proliferative index, SER showed a reduction in ConA and IL-2 in both tissues. TER induced an increase in the proliferative response under the stimulus of all mitogens in cells from the lymph nodes and spleen. When results were expressed as proliferative index, TER showed an increased in response to IL-2 and ConA and decreased in response to LPS in the cell from lymph nodes and from the spleen, in comparison with SER.
Chronic moderate-intensity exercise was effective in recovering the proliferative function of cells from the spleen and lymph nodes, as well as, in restoring IL-2 sensibility.

02-47/P

ANTI-PROLIFERATIVE AND ANTI-VASCULAR EFFECTS OF RAPAMYCIN IN EXPERIMENTAL LOCALIZED ACUTE MYELOGENOUS LEUKEMIA

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Rapamycin interferes with the PI3K[1]/AKT[2]/mTOR[3]/ribosomal proteins activation pathway and, in addition, suppresses VEGF[4] mediated vasculogenesis. The drug has been used as an anti-fungal, immunosuppressive, and anti-endothelial agent. Its anti-vascular properties have prompted therapeutic trials in solid tumors and leukemias. We haveassessed the anti-vascular and anti-proliferative effects of Rapamycin, +/-Ara-C, in acute myelogeneous leukemia (AML) using a murine model of experimentally localized leukemia (Reichert, Eur J Haematol 2005; 75: 41). The unique advantage of this technique is that it permits identification, quantitation, and distinction of "localized" growth from dissemination of AML and may, thus, provide detailed insight into the very early, initiating stages of leukemic development.
Murine AML cells of the line C-1498 were embedded in 1% semi-solid agar in 0.8 cm diameter moulded discs and inserted subcutaneously into the back region of C57BL mice. Post-implantation, animals received injections of Rapamycin, followed in one group by Ara-C, or saline, over a period of 14 days. Local AML growth inside the discs and the anti-proliferative as well as anti-vascular effects of Rapamycin were assessed by macro- and microscopic evaluation of the degree of vascularization of its solid mode of leukemic growth. To assess bone marrow involvement of leukemia, we carried out total and differential marrow cell counts. Our results show a high leukemic load in untreated animals which decreased with Rapamycin and Ara-C administration (30% ± 4 *versus* 8% ± 2 $p < 0.05$). Furthermore, the results indicate pronounced anti-proliferative and anti-vascular effects of Rapamycin on localized intra-pseudocapsular AML growth. Survival times of animals were prolonged, with an additional effect of Ara-C. However, none of the animals were cured of their leukemia. In fact, Rapamycin alone, or in combination with Ara-C, did not prevent leukemia cells to transgress from their local site of implantation through the fibrous pseudocapsule and disseminate throughout the body. Leukemia involvement rebounded sometime after completion of the treatment. Causes of death were distant metastases and/or large local tumors. We conclude that Rapamycin suppresses experimentally localized leukemic growth and temporarily prolongs survival times, especially in conjunction with other cytotoxic drugs such as Ara-C.

02-48/P

IL-21 ACTIVATES KEY TRANSCRIPTIONAL FACTORS INCLUDING STAT3/5 AND SIGNAL TRANSDUCERS SMAD1/2/3 IN U937 LEUKEMIA CELLS.

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IL-21 is a cytokine produced by activated CD4+ T cells and expressed in lymphoid tissues. IL-21 has an effect on the growth, survival, and activation of B, T, and NK cells.
IL-21 has been shown to induce apoptosis in B cells. Since IL-21 affects the responses of several immune cells, it may be a beneficial treatment for the regulation of the immune system in asthma and cancer patients. Though Jak kinases and MAPK have been suggested to be involved in IL-21 signaling their down stream effector molecules remain unknown. Likewise, the effects and mechanisms of IL-21 remain unclear. In this study, human U937 leukemia cells that express IL-21 receptor were examined to determine whether the JAK/STAT and MAPK pathways play key roles in the signal mechanisms of IL-21. Using DNA/protein transcriptional factor arrays we observed marked (2 to 5-fold) activation of STAT3 and STAT5 respectively by IL-21. Furthermore, DNA binding ELISA assay confirmed that IL-21 activates STAT3 DNA binding activity by about 6-fold. Also, luciferase activity assays in STAT3 reporter HeLa stable cells showed that STAT3 transcription increased by 4 to 7-fold by IL-21. These results suggest that the mechanisms of IL-21 perhaps involve transcriptional regulation of key transcriptional factors including STAT3. Through Western Blotting techniques, IL-21 was found to stimulate ERK and

[1] phosphatidylinositol 3- kinase
[2] AKT serine/threonine kinase
[3] mammalian target of Rapamycin
[4] vascular endothelial growth factor

Raf activation. Also, intact cell phosphorylation assays showed that IL-21 stimulates significant increases in protein phosphorylation. To further identify signaling molecules that are regulated by IL-21, we performed signal transduction arrays. The results indicate that IL-17 upregulates key signal transduction molecules, MMP-9, Smad1/2/3, and STAT3. Lastly, IL-17 promotes proliferation in leukemia cells. Further studies are in progress to elucidate the roles of these molecules in the mechanisms by which IL-21 regulates leukemia cell growth and survival as well as immune response. Supported by NCI/NIGMS SPORE. T32HL007735-11.

02-50/P

IMPAIRED SKIN WOUND HEALING IN IL-1 RECEPTOR ANTAGONIST-DEFICIENT MICE WITH SUPPRESSED TGF-BETA SIGNALING BY NF-KAPPA B ACTIVATION

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To clarify the roles of IL-1 receptor antagonist (IL-1ra) in skin wound healing, we prepared skin excisions in wild-type (WT) and IL-1ra-deficient mice. Compared with WT mice, IL-1ra-deficient mice exhibited impaired wound healing as evidenced by delayed wound closure and attenuated collagen deposition. In contrast, the recruitment of leukocytes such as neutrophils and macrophages was significantly exaggerated with the augmented expression of IL-1alpha and IL-1beta in IL-1ra-deficient mice compared with WT mice, implying that lack of IL-1ra enhanced local inflammatory reaction at the wound sites. We also found that nuclear translocation of NF-kappaB p65 was significantly enhanced and prolonged in mainly fibroblasts in IL-1ra-deficient mice, compared with that in WT mice. Because the crosstalk between NF-kappaB and TGF-beta-mediated signaling has been proposed based on in vitro observations, the aberrant NF-kappaB activation in the wound sites of IL-1ra-deficient mice prompted us to investigate TGF-beta/Smad pathways which has crucial roles for collagen deposition in wound healing processes. In IL-1ra-deficient mice, the TGF-beta-mediated signaling pathway was suppressed as evidenced by decreases in the level of total and phosphorylated Smad2 and Smad3, and a reciprocal increases in the levels of Smad7 at the wound sites, compared with WT mice. Theses results demonstrated that the absence of IL-1ra negatively regulates TGF-beta/Smad signaling, and eventually attenuate collagen deposition during wound healing in vivo.

02-51/O

DISCOVERY OF IL-33 AND THE IDENTIFICATION OF ITS SIGNALING RECEPTOR COMPLEX

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Interleukin (IL)-33 is a newly identified member in the IL-1 family of cytokines, and shares homology with IL-1β and IL-18. IL-33 is involved in TH2 responses. In vivo administration of IL-33 induces the expression of TH2 cytokines IL-4, IL-5, and IL-13, and leads to blood eosinophilia and severe pathological changes in mucosal organs. ST2, a former orphan receptor in the IL-1 receptor family that is expressed on both TH2 cells and mast cells, has been identified as a receptor component for IL-33. We report here the identification of the second member of the IL-33 receptor complex which is essential to mediate the biological effects of IL-33. We explore the biological consequences of this receptor make-up.

02-52/P

COMPARISON OF GENE EXPRESSION OF TH1/TH2 CYTOKINES AND THEIR RECEPTORS IN HUMAN AND NON-HUMAN PRIMATES

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Non-human primate is an invaluable model for biomedical studies on human diseases because of its genetic, physiologic and metabolic similarities with human. Up to date little information about biomedical characteristic, especially cytokine networks in non-human primate has been accumulated. In this study we examined gene expression profiles of cytokines (IL-4, IL-12 and IFN-γ) and their receptors (IL-4Rα, IFN-γR1 and IFN-γR2) among human, chimpanzee and several monkeys (cynomolgus, rhesus and Japanese macaques, green monkey and baboon) using real time RT-PCR. In human and chimpanzee, the gene expression levels of a Th2 cytokine, IL-4, were significantly higher than those in monkeys, whereas the gene expression levels of a Th1 cytokine, IL-12, were apparently higher in monkey. Thus, these gene expression profiles were anti-parallel in primates. The gene expression levels of IFN-γ and IL-4Rα were almost same among human, chimpanzee and monkeys. Interestingly, the gene expression levels of IFN-γR1 and IFN-γR2 in cynomolgus macaque were the highest among primates examined here. The expression levels of IFN-γR1 and IFN-γR2 genes in cynomolgus macaque were also markedly higher than those of close-related macaques, rhesus and Japanese macaques. These results indicate that human and chimpanzee have similar characteristics in Th1/Th2 immune responses, but distinguishable from monkeys. Among macaques, cynomolgus macaque appears to have a unique immunological characteristic in IFN–γ/IFN-γR pathway participating in Th1 response, which could affect susceptibilities to Th1-associated diseases such as tuberculosis.

02-53/P

QUANTITATIVE ANALYSIS OF 12 CYTOKINES IN THE SERUM SAMPLES FROM 92 NORMAL HEALTHY KOREAN SUBJECTS USING A PROTEIN CHIP ANALYZER

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Cytokines are physiologically active mediators of immune system. These include mediators transmitting signals between cells and local tissues. They control the immune responses, growth and differentiation, suppressing a cancer growth and defeat viral infections. We attempted to establish a quantitative baseline levels of 12 cytokines with healthy normal Korean subjects. These data could be a useful references to correlate various diseases with cytokine profiles. Twelve cytokines including Interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, vascular endothelial growth factor (VEGF), interferon (IFN)-γ, tumor necrosis factor (TNF)-α, monocyte chemotactic protein (MCP)-1, epidermal growth factor (EGF) were analyzed in the serum samples from 92 healthy normal subjects who were chosen randomly with age groups from healthy physical examinees (30s, 24 persons; 40s, 31 persons; 50s, 37 persons) by a protein microarray analyzer, Evidence® (Randox, Antrium, UK). The cytokine values were as below, IL-1α: 0.06±0.4 pg/mL (F: 0.11±8.06, M: 0.01±0.05), IL-1β: 1.6±4.9 pg/mL (2.21±4.39, 0.82±3.02), IL-2: 2.54±9.83 pg/mL (3.69±16.6, 1.19±2.32), IL-4: 0.48±1.31 pg/mL (0.68±0.79, 0.26±0.93), IL-6: 3.1±9.6 pg/mL (4.37±29.19, 1.49±5.19), IL-8: 160.8±362.7 pg/mL (190.91±392.49, 14 0.2±319.92), IL-10: 0.06±0.2 pg/mL (0.05±0.21, 0.08±0.24), VEGF: 183.5±180.5 pg/mL (169.22±174.21, 200.59±190.63), IFN-γ: 0.2±0.7 pg/mL (0.17±0.78, 0.28±0.87), TNF-α: 5.2±14.5 pg/mL (6.17±7.03, 4.02±1.6), MCP-1: 292.3±133.4 pg/mL (266.9±127.21, 322.48±134.28), EGF: 111.8±92.7 pg/mL (91.76±77.65, 135.74±103.12). IL-2 was decreased with ages (30s: 5.9±18.3 pg/mL, 40s: 1.9±4.4 pg/mL, 50s: 0.8±1.8 pg/mL). In contrast, IL-8 and MCP-1 were increased with ages (IL-8; 30s: 62.5±13.2 pg/mL, 40s: 62.7±99.9 pg/mL, 50s: 324.6±526.19 pg/mL, MCP-1; 30s: 206.1±4.2 pg/mL, 40s: 298.2±102.2 pg/mL, 50s: 338.3±139.9 pg/mL) The VEGF and TNF-α values were the highest in their forties (VEGF; 30s: 149.1±117.3 pg/mL, 40s: 225.3±223.4 pg/mL, 50s: 170.8±171.9pg/mL, TNF-α; 30s: 3.2±1.5 pg/mL, 40s: 8.2±24.8 pg/mL, 50s: 3.9±1.9 pg/mL). We obtained reference values of 12 cy-

tokines from 92 normal healthy volunteers, which can be used as a baseline data for the diagnosis and monitoring diseases. IL-2 was decreased with ages, and IL-8 and MCP-1 were increased with ages. VEGF and TNF-α levels showed the highest in their forties.

02-55/O

DEFICIENCY OF INTERLEUKIN-18 IN MICE LEADS TO OBESITY, INSULIN RESISTANCE AND HYPERGLYCEMIA

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Interleukin-18 (IL-18) is a proinflammatory cytokine with a large constitutively-expressed intracellular pool, especially in liver cells, suggesting additional roles than those played in innate immunity. We report the presence of obesity, insulin resistance, and hyperglycemia - pathological conditions associated with the metabolic syndrome - in knock-out mice deficient in IL-18 or IL-18 receptor, as well as in transgenic mice for IL-18 binding protein. Obesity of IL-18-/- mice was due to accumulation of fat tissue based on increased food intake. IL-18-/- mice also displayed hyperinsulinemia, consistent with insulin-resistance and hyperglycemia. Further analysis of the glucose metabolism in IL-18 -/- mice showed insulin resistance at the hepatic level causing hyperglycemia in these mice, with enhanced expression of gluconeogenesis genes in the liver of IL-18-/- mice. In addition, the molecular mechanisms responsible for the hepatic insulin resistance in the IL-18-/- mice likely involved defective phosphorylation of STAT3, one of the intracellular pathways activated by IL-18. In contrast, MyD88, which mediates a second pathway of activation by IL-18 receptor, was not involved in this process. Recombinant IL-18 reversed hyperglycemia in IL-18-/- mice through activation of STAT3 phosphorylation. These findings demonstrate a new role of the cytokine IL-18 in the homeostasis of energy intake and insulin sensitivity.