Abstract - 1

ROLE OF SLY1 IN T AND B CELL FUNCTION

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Adaptive immunity is crucial for protective host defense and the development of immunological disorders. An increasing number of molecules, especially adapter proteins with SH2 or SH3 domains, involved in the signal transduction pathway downstream of T or B cell receptors have been discovered in the last years. SLy1 (SH3 lymphocyte protein) was recently identified as a X-chromosomal SH3 protein that is PI3 kinase and/or PKC-dependent serine-phosphorylated (Ser27) upon B and T cell receptor engagement. These studies therefore defined SLy1 as a previously unrecognized target for antigen receptor signal transduction and suggested that it may play a role in adaptive immunity. We generated mice expressing a mutant version of SLy1 lacking the Ser27 phosphorylation site and parts of a functional nuclear localization signal (SLy1Δaa20-100). Sly1d/d mice exhibit reduced lymphoid organ sizes, diminished marginal zone B cell numbers and severely impaired antibody responses against T-dependent and -independent antigens. B and T cell proliferation is attenuated and T cell cytokine production is severely reduced. Moreover, survival of semi-identical cardiac allografts was substantially prolonged in Sly1d/d mice. However, global tyrosine and MAP kinase phosphorylation, Ca2+ influx and transcription factor activation are normal. A new family of proteins playing a nonredundant role needed for full activation of lymphocytes has been identified.

Abstract - 2

A DOMINANT SUPPRESSIVE MHC HAPLOTYPE (H-2D) REGULATES DEVELOPMENT OF AUTOIMMUNITY AND CHRONICITY OF COLLAGEN INDUCED ARTHRITIS

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To investigate the genetic control of chronic development of arthritis, we used a variant of collagen-induced arthritis induced after immunization of type II collagen (CII) lacking mycobacteria adjuvant. F1 mice of BALB/c and B10.Q are highly prone to develop a chronic relapsing arthritis (66%) whereas both parentals are relatively resistant; BALB/c (H-2d; 0%) and B10.Q (H-2q; 4.5%). As this suggest the involvement of dominant regulatory loci, we performed a CIA experiment on 684 N2 backcross mice, backcrossed to B10.Q; 38% of the backcrossed mice developed arthritis and more than half of these mice developed a chronic form of arthritis that persisted for a minimum period of 4 months. For genome-wide genotyping, 190 animals with the most severe phenotype were selected. Despite the high statistical power, only the MHC locus had independently a significant dominant influence on the chronicity. Interestingly the H2d allele had a dominant suppressive effect. This effect overrided the role of other loci as interaction analysis, conditioning MHC, revealed additional loci on chromosomes 5,8,9,11,13 and 15. Importantly the observed genetic regulation is operating through autoimmunity as we also found several loci linked to anti-CII autoantibody response.

Abstract - 3

DEVELOPMENT OF HUMANIZED MICE FOR THE STUDY OF HUMAN VASCULAR INFLAMMATORY PROCESSES

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We developed a new xenograft mouse model, to study the interaction of human leukocyte fractions within a human microvascular environment. The capability of Pfp/Rag2 -/- mice for haematopoietic reconstitution and to host human cells and skin were investigated. Mice were transplanted with human skin. The animals received human peripheral blood mononuclear cells (HuMNC) i.p and human polymorphonuclear leukocytes (huPMN) by i.v. injection. Haematopoietic reconstitution and infiltrations of human cells were estimated in skin grafts and organs by histology and immunohistochemistry (CD 45). Different entities of infiltrating and circulating human cells were evaluated. After 21 days, 70% (19/27) of the mice showed signs of haematopoietic engraftment. No signs of graft versus host disease appeared. CD45 positive human cells in the mouse circulation varied between 1,8 – 8,24% (mean 5,02%). Dense infiltrates were found in lung, liver kidneys and skin grafts. The subsets of infiltrating cells mainly consisted of PMNs and T cells.. B-cells were rare. In contrast to existing models of human haematopoiesis , a stable haematopoietic reconstitution in the Pfp/Rag2 -/- mouse strain carrying human skin could be established without specific pre-treatment (radiation, NK cell depletion). The model seems to be suitable to study e.g. the pathogenetic mechanisms of ANCA-associated vasculitis.

Abstract - 4

VELOCIMMUNE: HUMANIZATION OF IMMUNOGLOBULIN LOCI USING VELOCIGENE TECHNOLOGY

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There is a growing need for more and better mouse models of human disease for discovery and validation of therapeutics. Many humanized mouse models exists, however, these are commonly human transgenes combined with a knockout of the endogenous locus. While these models are useful, they can be limited by lack of appropriate gene expression or inability to replace complex loci. We precisely replaced the mouse immunoglobulin variable loci with the equivalent human variable sequences, which we call 'VelocImmune'. By replacing only the variable segments at the heavy and kappa light chain loci, endogenous gene control mechanisms are retained. By leaving the mouse constant regions intact, antibody effector functions, such as Fc receptor and complement binding, are conserved. VelocImmune mice exhibit wild type immune systems, with normal populations at all stages of B cell development, and normal rates of variable segment usage, somatic hypermutation and class switching. Antigen responses of VelocImmune mice are equivalent to wild type mice, generating monoclonal antibodies to a variety of antigens. Therefore, VelocImmune demonstrates that it is possible to humanize complex genomic loci in mice and faithfully reproduce locus expression and function and, more importantly, represents a novel platform for the generation of human therapeutic antibodies.

Abstract - 5

HUMORAL AMPLIFICATION OF THE INFLAMMATORY RESPONSE TO OUTER MEMBRANE PROTEIN A (OMPA) BY THE LONG PENTRAXIN PTX3

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PTX3 is a prototypic long pentraxin playing a non-redundant role in innate immunity against selected pathogens. PTX3 binds with high affinity the complement component C1q, selected microorganisms including Klebsiella pneumoniae (Kp) and microbial moieties such as KpOmpA. We investigated the in vivo relevance of the interaction between PTX3 and KpOmpA. Footpad swelling induced by KpOmpA injection was significantly reduced in PTX3 -/- mice compared to wild type mice. Administration of exogenous PTX3 reverted the phenotype. Moreover, in the air pouch model, co-injection of KpOmpA and PTX3 significantly augmented cell recruitment in the pouch compared to administration of KpOmpA alone. In contrast the combination of LPS with PTX3 did not lead to enhanced inflammatory cell recruitment. Pro-inflammatory molecules, IL-6 and JE, were significantly higher in the air pouch exudates of mice treated with the combination of PTX3 and KpOmpA, compared to KpOmpA alone. Experiments carried out using Cobra Venum Factor and C5 deficient mice (DBA/2) indicate that PTX3 amplifies the inflammatory response to KpOmpA through the activation of the classical complement pathway. Thus, PTX3 represents a non-redundant humoral amplification loop of the local inflammatory response induced by KpOmpA probably mediated by complement activation.

Abstract - 6

BIOLUMINESCENCE IMAGING OF TUMORS IN LIVE ANIMALS WITH BACTERIA ENCODING LUCIFERASE AND THEIR USAGE IN TUMOR THERAPY

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Several Bacterial strains have been shown to specifically target and survive within tumors while they are cleared in the other tissues after intravenous injection. Vibrio cholerae.lux (generously provided by A.Szalay, Genelux, San Diego) expressing a bacterial luminescent gene (luxCDABE) was visualized by in vivo bioluminescence imaging in nude mice bearing subcutaneous tumors. We have investigated whether or not V.cholerae.lux would also target tumors in immunocompetent mice. Indeed, in wild type mice Vibrio cholerae.lux efficiently targeted various subcutaneous transplanted tumors. We also tested transgenic Albumin-Tag mice which develop spontaneous hepatocarcinoma. In contrast to subcutaneous tumors the hepatoma are not directly visible by optical inspection. Again, Vibrio cholerae.lux targeted the tumors and allowed bioluminescence imaging of hepatoma nodules. In addition to imaging we are using these bacteria as delivery vectors for GMCSF in order to improve elimination of tumors by the immune system. Usually recombinant proteins are expressed intracellularly. To allow secretion of GM-CSF, we have combined it with the Hemolysin A secretory system which will allow its secretion into the tumor environement. The engineered bacteria will be injected in tumor bearing mice either alone or together with tumor immunization for assessment of their effect on tumor growth.

Abstract - 7

CHARACTERIZATION OF SUBCHRONIC VS. ACUTE MOUSE MODELS OF DNFB-INDUCED SKIN INFLAMMATION

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We evaluated whether an elaborated 2,4-dinitrofluorobenzene (DNFB) dosing schedule in comparison to an acute standard schedule i) is suitable for a subchronic mouse model of contact hypersensitivity (CHS), ii) exhibits improved chronic characteristics of skin inflammation, and iii) closer reflects the therapeutic response pattern of chronic T cell-dependent skin diseases like psoriasis. In subchronic model ear edema formation and treatment response could be followed over time courses of 3-4 days pursuing ear thickness. Strong cutaneous T cell infiltration as well as disability of athymic nude mice to respond to subchronic DNFB treatment demonstrated the pivotal role of T cells. Moreover, only in subchronic setting characteristics of psoriasis as epidermal thickening, hypervascularization and a strongly enhanced cytokine expression were observed. Systemic or topical treatment with standard glucocorticoids over the elicitation phase was dose-dependently efficacious. Remarkably, antagonization of cytokines was anti-inflammatory active in psoriasis and in subchronic model but not in acute CHS. Taken together, repetitive hapten challenges facilitate the development of hallmarks of chronic skin inflammation in DNFB-induced CHS model in mice and increase the similarity to chronic inflammatory skin diseases like psoriasis.

Abstract - 8

ANIMAL MODELS OF TMA-INDUCED CONTACT HYPERSENSITIVITY WITH IMPROVED CHRONIC CHARACTER AND A PRONOUNCED ROLE OF SECONDARY MEMORY RESPONSE AIMING AT IMPROVED CLINICAL RELEVANCE

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TMA (trimellitic anhydride) is routinely used to trigger T cell dependent contact hypersensitivity (CHS) in mice. TMA-induced skin inflammation is characterized by a Th2 cytokine profile and serves as a model exhibiting characteristics of atopic dermatitis. In this study we compared a standard acute model with new prolonged models of TMA-induced skin inflammation. We investigated the influence of repeated challenges on chronic character of the induced inflammation and the role of a prime boost setting for the outcome. We measured ear thickness over the time course and endpoint parameters such as cutaneous immune cell infiltration, weight of corresponding lymph nodes and proliferation of lymph node cells, cytokine production at mRNA and protein level in skin and lymph nodes as well as IgE levels. In altogether 5 models we found excellent treatment response to the clinical gold standard prednisolone. So far, this treatment response has not been shown for any model which exhibited increased chronic character in TMA-induced CHS. Taken together, we were able to show a way to improve similarity of mouse skin inflammation to chronic skin inflammation in atopic dermatitis. Hopefully, this will help to increase the predictive value of animal models for drug discovery projects in the future.

Abstract - 9

IMMUNOLOGICAL DEFICIENCIES OF *Hfe* KNOCK-OUT MICE

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Hereditary hemochromatosis (HH) is a common iron overload disorder that is most frequently caused by mutations in the HFE gene. In this disease iron is overloaded thorough the body, mostly in the parenchyma of the tissues leading to malfunction of the iron accumulating organs. Hemochromatosis patients are also characterized by an imbalance in their immune functions. A bigger susceptibility to certain bacterial infections as well as inappropriate number of circulating cytotoxic T lymphocytes has been observed.

During inflammatory reaction the regulation of iron absorption, storage and release is regulated by the liver-expressed iron hormone and anti-microbial peptide hepcidin. This process results in a low level of available iron for the microbes (hypoferremia) and anemia of inflammation (AI). Work from our lab and others demonstrate that in HH the inflammatory stimulus is no longer able to induce hypoferremia and AI. Also, hepcidin expression is also not inducible in case of *Hfe*-deficiency. Some cytokines have also been shown to modulate iron metabolism in the condition of inflammation. Interleukin-6, one of the inflammatory cytokines has been shown to stimulate hepcidin production not only in vitro but in vivo. However, it is not clear, whether the production of inflammatory cytokines is how much influenced by *Hfe* mutation. We assume, that HFE, an MHC Class I like protein, has an impact on the immune functions either directly or indirectly via influencing the expression of hepcidin. Therefore we started to investigate the immune system of constitutive *Hfe*-knock out mice. Experiments are ongoing to assess the effect of inflammatory stimuli on targeted cells obtained from the C57BL/6 wild-type and constitutive *Hfe*-knock-out strain. At the different time-point LPS-treated bone-marrow derived macrophages can elucidate the role of *Hfe* in the immune system.

Abstract - 10

OBESITY, TYPE 2 DIABETES, STEATOHEPATITS AND STERILITY – LESSONS FROM ALMS1-DEFICIENT FAT AUSSIE MICE

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Fat aussie is a newly identified spontaneous obese mouse variant which inherits a mutation in the primary cilium component Alms1, a gene responsible for the human Alström syndrome. Mutant mice are hyperphagic and develop obesity, hyperinsulinemia, hyperglycemia and hypercholesterolemia, which is accompanied by a moderate to severe fatty liver disease. When fed high fat diet the benign hepatic steatosis progresses to steatohepatitis, with inflammatory infiltration, 10-fold increased ALT levels and low adiponectin levels. Male fat aussie mice are sterile due to a spermatogenesis failure which results in complete azoospermia. Fat aussie mice become hyperphagic coincidently with the early signs of obesity (60 days of age). Pair feeding experiments established a clear cause-effect relationship between the hyperphagia and obesity, and also showed that the metabolic syndrome in fat aussie mice is at least partially a consequence of the obesity rather than primary effect of the Alms1 loss of function. Further elucidation of the primary cilia involvement in the complex and obscure mechanisms that govern body energy homeostasis, glucose and lipid metabolism and spermatogenesis could pinpoint to new targets for development of drugs for treatment of some of the most outstanding medical problems of our time– obesity, type 2 diabetes, hypercholesterolemia and sterility.

Abstract - 11

THE ACTIVATING FcgRI AND FcgRIII ARE DISPENSABLE FOR CARTILAGE- AND BONE DESTRUCTION IN THE COLLAGEN INDUCED ARTHRITIS (CIA)

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Studies in mice with CIA in DBA/1 background and KRN serum induced arthritis, suggest that FcgRIII is the only FcR involved. We took advantage of the fact that FcgRII KO mice on C57Bl6 background are susceptible for the induction of CIA. We established I/II-, II/III- and I/II/III KO mice on C57Bl6 background in which we analyzed the development of CIA during 165 days. FcgRI/II KO mice showed similar disease as FcgRII KO mice. In FcgRII/III KO mice CIA developed with later onset, but eventually reached the same incidence (65-90%) and severity as in FcgRII KO mice (58-88%). FcgRI/II/III triple KO mice showed markedly lower incidence (35-40 %) and severity. Remarkably, the FcgRI/II/III KO mice showed cartilage- and bone destruction, indicating that FcgRI and FcgRIII are not absolutely required for these pathogenic processes. In the KRN model FcgRII KO mice showed increased severity of the disease as compared to WT animals. Disease, albeit with a low severity, still developed in FcgRI/II/III KO mice, whereas FcRg-chain KO mice were completely protected suggesting a (secondary) role for FcgRIV. Taken together, these results indicate that the respective contributions of the three activating FcgRs, in the development of arthritis are FcgRIII > FcgRI > FcgRIV.

Abstract - 12

THE MITOCHONDRION AS A PRIMARY SITE OF ACTION OF REGULATORY AGENTS INVOLVED IN IMMUNOMODULATION

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Glucocorticoids and their intracellular signalling elements, exert both stimulatory and inhibitory effects on the immune reaction. In some cells and/or in extreme stress conditions, apoptosis is evoked. In some processes related to immunomodulation a prominent role is emerging for mitochondria. Evidence has accumulated for a primary action of steroid and thyroid hormones by way of cognate receptors on mitochondrial genes (reviewed in Scheller et al., (2003) Int. Rev. Cytol. 222, 1-61). Such evidence is a) the detection of receptors for glucocorticoids, thyroid hormones, estrogens, androgens and retinoic acid in mitochondria, b) the presence of sequences in the mitochondrial genome similar to hormone responsive elements to which the steroid receptors bind and which confer hormone susceptibility to reporter genes in transfection experiments and c) the effects of thyroid hormones on mitochondrial transcription in an in organello system. Recently, additional nuclear transcription factors involved in immunomodulation have been detected in mitochondria (NF-κB, AP-1, p-53, CREB), binding sites for these in the mitochondrial genome have been identified and, in same cases, their effect on modulation of mitochondrial transcription and on energy yield has been shown (reviewed in Psarra et al., Ann. N. Y. Acad Sci., in press). The mitochondria store a host of critical apoptotic activators and inhibitors in their intermembrane space and the release of these factors could be modulated by the mitochondrially localized regulatory agents.

Abstract - 13

EXACERBATION OF INFLAMMATORY ARTHRITIS IN IFN-g-DEFICIENT MICE BY INCREASED IL 17 PRODUCTION.

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In rheumatoid arthritis (RA), Th cells are supposed to be involved in induction and perpetuation of autoimmune disease with a dominance of the pro-inflammatory Th1 response. We investigated antigen-induced arthritis, an animal model of RA, in IFN-g-deficient and wild type C57Bl/6. Inflammatory response in the acute stage was strikingly increased in IFN-g-deficient mice, demonstrated by exacerbated joint swelling, delayed-type hypersensitivity reaction and histopathological assessment of arthritis. Increased production of IL-2, IL-4, IL-5, IL-6 and in particular IL-17 upon stimulation of lymph node and spleen cells in knockout mice was associated with a decreased humoral immune response with low serum levels of total and antigen specific immunoglobulins (IgG, IgG1, IgG2a, IgG2b, IgG3). The lack of endogenous IFN-g resulted in large numbers of neutrophil granulocytes infiltrating acute inflamed knee joints. Intraarticular administration of exogenous IFN-g significantly suppressed inflammation in IFN-g-deficient as well as in wild type mice. In vitro, we found that Th cell expansion and production of IL-17 upon re-stimulation was effectively inhibited by IFN-g, suggesting disease promoting effects of IFN-g-deficiency acting via IL-17 modulated pathways. These results clearly show in vivo a dominance of anti-inflammatory properties of IFN-g during acute phase of arthritis.

Abstract - 14

GENOME-WIDE MUTAGENESIS FOR ULTRA-FAST GENERATION OF GENE-DRIVEN MOUSE MODELS FORM A PRE-MADE SPERM ARCHIVE COMPRISING OVER 300.000 NOVEL MURINE ALLELES

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Genetic mouse models are and will continue to be essential for the improvement of our knowledge of health and disease. To fully exploit the power of murine genetics it is mandatory to have access to multiple alleles of a disease gene. In order to make mouse models with multiple genetic alterations readily available to the scientific community, we have developed a F1 genotyping technology platform based on an archive of over 300.000 murine alleles. Gene driven mouse models can be generated upon customer demand within four months on a simple fee for service basis.

Abstract - 15

OSTEOPONTIN INVOLVEMENT IN T HELPER TYPE 2 ALLERGIC AIRWAY DISEASE: REGULATION OF DENDRITIC CELL SUBSETS

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Osteopontin (Opn) is important for T helper type 1 (Th1) immunity and autoimmunity. However, involvement of this cytokine in Th2-mediated allergic disease as well as its role during primary versus secondary antigenic encounters, have not been investigated. Here we demonstrate that Opn is expressed in the lungs of asthmatic patients and exerts opposing effects on murine Th2 effector responses and subsequent allergic airway disease: pro-inflammatory at primary systemic sensitization, and anti-inflammatory during secondary pulmonary antigenic challenge. Blockade of secreted Opn during systemic chicken ovalbumin (OVA) sensitization significantly reduced allergic responses, including decreased pulmonary eosinophilia, Th2 cytokines, IgE levels and airway hyperresponsiveness (AHR). This effect was mediated by increased recruitment of tolerogenic plasmacytoid DCs (pDCs) in draining lymph nodes (DLNs). In contrast, Opn blockade during pulmonary OVA challenge, significantly enhanced allergic inflammation, as shown by increased pulmonary eosinophilia, Th2 cytokines, AHR, Th2 cell recruitment, CCL22 and CCL17 production. This was mediated by increased recruitment of immunogenic conventional DCs (cDCs) in DLNs. In support, Opn-/- mice showed enhanced allergic responses. The novel effect of secreted Opn on DC subset regulation during Th2 allergic responses places it as an important therapeutic target and provides new insight into its role in immunity.

Abstract - 16

WHAT IS THE UNDERLYING MECHANISM OF THE ANTITUMOUR ACTIVITY OF TNF

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TNF has strong antitumour effects, both in patients and animals. Unfortunately TNF also has shock-inducing properties, which limits its use as antitumour drug to locoregional treatment. To expand the use of this cancer treatment it is necessary to elucidate the downstream pathways. Using transformed tumour cells and knockout mice we could prove that the antitumour effect of TNF solely depends on host-mediated effects via TNF-RI. Endothelial cells and immune cells are the most likely host target cells. Bone marrow transplantation experiments showed that TNF-RI on immune cells is not sufficient, nor necessary for efficient tumour regression by TNF. To investigate whether endothelial cells are the relevant target cells, we generated mice expressing the TNF-RI only on the vasculature. Tumours could still be destroyed by TNF in these mice, indicating that endothelial cells are the relevant target cells in TNF-induced tumour regression. We also investigated several pathways, like NO production, MAPK pathway and arachidonic acid metabolism, using different inhibitors and knockout mice. Our results prove for the first time that endothelial cells are the only relevant target cells of TNF in its antitumour effect. Further analysis of downstream effects will enable us to create an equally efficient, but non-toxic antitumour therapy.

Abstract - 17

GENERATION OF A MOUSE MODEL FOR MASTOCYTOSIS

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Mastocytosis is a rare disease characterized by infiltration of mast cells into skin and internal organs. Mastocytosis was associated with activating somatic point mutations in the receptor tyrosine kinase Kit. However, these activating mutations do not explain the broad clinical heterogeneity of mastocytosis. Our transgenic mouse model (Mut-kit) is based on a Bacterial Artifical Chromosome (BAC) containing the entire kit gene and, most likely, all its regulatory elements. Three modifications were introduced: 1) the activating point mutation D814V, 2) a floxed transcriptional / translational “stop” cassette, allowing Cre/LoxP-mediated control of transgene expression and 3) an IRES-fluorescent protein cassette to visualize transgene expression in vivo. The construct was pronucleus-injected and founder animals are viable and fertile. Mating of Mut-kit transgenic mice to the hCMV-Cre-line will lead to the deletion of “stop” in all cell types resulting in expression of mutated kit under control of its endogenous promoter and regulatory elements. Phenotypic analysis will focus on mast cell infiltration of various organs and hematological abnormalities. Breeding of Mut-kit animals to cell type-specific or inducible transgenic Cre-lines (e.g. mast cell-, hematopoetic stem cell specific and mosaic-Cre mice) may show the effect of constitutively active kit by different cell types and at different time points during hematopoesis.

Abstract - 18

EPIGENETIC CONTROL OF TH1 CELL DIFFERENTIATION

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IL-12 is a key cytokine for the development of Th1 responses, however na?ve CD4+ T cells do not express IL-12Rß2 and are therefore unresponsive to IL-12. IL-12Rß2 is rapidly induced during Th1 cell differentiation, but the mechanisms accounting for Th1-specific expression of this gene are unknown. We have analyzed changes in the chromatin structure at the IL-12Rß2 locus in Th1 and Th2 cells. We identified a DNAseI hypersensitive site (HS) that appeared very early in Th1 lineage-committed cells but was not present in Th2 cells. This HS defined an enhancer element that is recognized by STAT4 in vivo in developing Th1 cells. To define the molecules that induce epigenetic changes during T helper cell differentiation, we have analyzed the role of ATP-dependent chromatin remodeling complexes in this process. We found that TCR triggering rapidly induced recruitment of BRG1, the ATPase subunit of the BAF remodeling complex, to the IL-12Rß2 regulatory regions. BRG1 recruitment was associated with a rapid increase of H3 acetylation and low-level IL-12Rß2 expression. TCR triggering in the presence of IL-12 induced high-level IL-12Rß2 gene expression. Our results indicate a synergistic role of TCR-induced chromatin remodeling and cytokine-induced STAT4 activation to direct IL-12Rß2 expression during Th1 cell development.

Abstract - 19

NONREDUNDANT ROLE OF THE ORPHAN CHEMOKINE RECEPTOR CCRL2 IN THE DEVELOPMENT OF ALLERGEN-INDUCED AIRWAY INFLAMMATION IN VIVO

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CCRL2 is a putative orphan chemotactic receptor of unknown function. Monoclonal antibodies against mouse CCRL2 were generated. CCRL2 was poorly expressed by circulating leukocytes and in vitro by resting cells. However, CCRL2 expression was rapidly induced in neutrophils, macrophages and dendritic cells following in vitro/in vivo stimulation. CCRL2-deficient mice were tested in an established model of OVA-induced allergic airway inflammation. Deletion of the CCRL2 gene results in markedly reduced leukocyte recruitment to the airway lumen. Th2 cytokine levels in both airway lumen and lung tissue were reduced, as well as CCL11 and CCL17 chemokine levels in airway lumen in CCRL2 deficient mice. CCRL2-/- mice showed no differences in airway hyperreactivity or IgE levels. Production of Th2 cytokines was almost abolished in peribronchial lymph node cell cultures from treated CCRL2-/- mice. This effect was correlated with a defect in antigen-loaded pulmonary dendritic cell accumulation to the regional lymph nodes. This study provides the first evidence of a functional role for CCRL2. CCRL2 appears to be critical for leukocyte trafficking between the lung parenchyma and the airway lumen, as well as for appropriate local Th2 response that depends, at least in part, on defective dendritic cell accumulation to the regional lymph nodes.

Abstract - 20

STUDY THE EFFECTS OF PULSED DENDRITIC CELLS IN INDUCTION OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOPATHY (EAE) IN C57BL/6 MICE

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Dendritic cells (DCs) could initiate autoimmunity in animal models and human disease. In this study we used pulsed DCs with myelin oligodendroglial glycoprotein (MOG) peptide to establish of EAE in C57BL/6. To generate DCs, bone marrow precursors of C57BL/6 mice were cultured with mrGM-CSF and mrIL-4 for 7 days and after pulsed with MOG peptide, cultured for 2 days with TNF-alpha. Then DCs were harvested and injected (5*10(5) cells/mouse) into female C57BL/6 in three different rout (i.v, i.p. and s.c.). After three injections every other 4-day, the animals were followed for 4 weeks. In some experiences pertusis toxin (pt) injected zero and 24 hour after DCs injection. s.c injected of DCs plus pt induced mild clinical signs of EAE in these mice, where as we didn't find any signs of EAE in i.v or i.p injection with or without pt after 4 weeks. Culture, spleen cells of treated mice by MOG peptide, showed more proliferation in i.v and s.c group than i.p injected group. Induction of EAE in treated mice developed more severs EAE in i.v or s.c groups than i.p-injected group. So TNF-alpha matured DCs is not suitable either to induction or prevention of clinical EAE in C57BL/6 mice

Abstract - 21

VELOCIGENE TECHNOLOGY EXTENDED TO HUMANIZATION OF SEVERAL MEGABASES OF COMPLEX GENE LOCI

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Genetic humanization, the process of replacing mouse genes with their human orthologues, is useful for the study of human genes and human disease processes in laboratory animals. We describe methods for large-scale humanization utilizing bacterial artificial chromosome (BAC) recombineering and BAC-based targeting of mouse embryonic stem (ES) cells. Novel methods for creation of human/mouse chimeric BACS were employed to precisely replace a 3 megabase segment of the mouse heavy chain immunoglobulin locus, encompassing all of the mouse V, D and J gene segments, with the equivalent 1 megabase segment of the human genome, containing all human heavy chain V, D and J gene segments. Likewise, a 3 megabase segment of the immunoglobulin kappa chain locus containing all of the mouse kappa V and J segment genes was replaced with a 0.4 megabase segment of the human genome containing one of two repeats encoding all human kappa chain V and J gene segments. Mice homozygous at both humanized heavy and light chain loci were derived from the targeted ES cells by blastocyst injection and breeding, and were found to be viable and healthy. Humanization of any loci could be implemented using this technology to create novel human disease models in mice.

Abstract - 22

STABLE LENTIVIRAL AND RETROVIRAL TRANSDUCTION OF HUMAN SYNOVIAL FIBROBLAST AND MURINE BONE MARROW MSC AND ITS APPLICATION TO CELL THERAPY IN THE RHEUMATOID ARTHRITIS TG197 MICE MODEL

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Efficient gene targeting Fibroblasts like synoviocyte (FLS) are in the basis of current therapy approaches as well as experimental studies on RA. We have investigated the effect of experimental conditions as well as of intrinsic properties of the cells in the efficiency of Retroviral and 3rd generation Lentiviral-derived vectors transduction of human FLS and murine bone marrow-derived mesenchimal stem cells (MSC). We have also focussed on the stability of the transgen and the expression over the time and cell passaging. Optimising factors affecting the early interaction of the virus with the cell as well as those related to cellular and virus properties, led us to establish a procedure that render consistent and reproducible efficiency of transduction close to 70% with retrovirus and reproducibly, almost 99% with lentiviral-derived vectors. Similar protocols have been followed to stably transduce murine MSC to further study the feasibility of using transduced cells as a vehicle to deliver genes into the arthtitic joint. Experiments using the transgenic Tg197 mice are underway to evaluate the effect that the onset of the inflammatory process has on the homing of the transplanted cells and the potential use of MSC based future therapies.

Abstract - 23

KNOCKOUT AND TRANSGENIC MODELS TO STUDY THE PHYSIOLOGICAL ROLE OF TNF

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We want to determine how the balance between beneficial and deleterious effects of TNF is maintained in vivo. To this end we generated mice with TNF ablation in distinct types of leukocytes, such as macrophages/neutrophils, T cells, and B cells. These mice were evaluated in several pathophysiological models. Macrophages and neutrophils are the main source of systemic TNF in response to LPS. However, TNF from macrophages and neutrophils, as well as TNF from T cells, was critically involved in both toxic and infectious models in a nonredundant fashion. We also generated mice which carry human TNF gene locus instead of its murine counterpart. In this model both types of clinically applied TNF blockers, Enbrel and Remicade, can be used for TNF ablation in vivo. TNF can also be inducibly ablated in mice by using Mx1-Cre system. We hope that these models will help to define critical thresholds for protective and detrimental effects of TNF in vivo. Additionally, we compared phenotypes of pairs of TNF and LT-alpha knockout mice generated by conventional and LoxP-Cre mediated technologies, and in both cases found the evidence for collateral damage to the regulation of the neighbouring gene, presumably due to the action of the neo-cassette.

Abstract - 24

ELAVL1/HuR AS A MODULATOR OF T-CELL RESPONSES

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HuR (HuA) is a ubiquitously expressed RNA binding protein involved in turnover and translation of immune mRNAs. Here, we use a conditional gene targeted strategy in ES cells and Cre/loxP mediated recombination to ablate HuR in mouse thymocytes. Consistant deletion of HuR was achieved in thymocytes and past the DN2 stage of thymocyte development, whereas the deletion extends to both CD4+ and CD8+ peripheral T-lymphocytic subpopulations and lymphoid rich tissues. Mice with HuR deficiency in T-lymphocytes show age dependant increases in thymic cellularity and perturbed number of peripheral lymphocytes. Most importantly HuR deficient thymocytes appear resistant to TCR induced apoptosis indicating possible defects in negative selection. In aggreement with this finding, mice with HuR deficient T-lymphocytes show exacerbated autoimmune responses in the periphery. Finally, both thymic and peripheral T-lymphocytes show altered thresholds of TCR induced or mitogenic proliferation. Overall, our current data indicate a pleiotropic role for HuR in modulating T-cell activation and apoptosis through the T-cell receptor with important consequences to T-cell maturation and autoimmunity.

Abstract - 25

THE PLEIOTROPIC ROLE OF HuR IN THE MODULATION OF INNATE RESPONSES

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HuR (HuA) is an RNA binding protein with relevance to the modulation of immune effector mRNAs bearing AU-rich elements. Aberrant HuR functions in myeloid cells have been considered contributory to the development of inflammatory disease. To address this issue we have generated transgenic mice that overexpress or are devoid of HuR in myeloid compartments. In TLR-4 stimulated macrophages, HuR overexpression induced the translational silencing of specific cytokine mRNAs despite positive or nominal effects in their corresponding turnover. In compliance to the reduction in inflammatory mediators, HuR overexpression suppressed acute inflammatory reactions in vivo. Conversly, HuR deficient macrophages display exacerbated cytokine production profiles in response to TLR ligands and appear more sensitive to endotoxemia. However, the representation of HuR deficient myeloid cells in the periphery was significantly reduced, attributed to a partial reduction of mature myeloid cells exiting the bone marrow due to defects in growth factor functions. Overall our data suggests that HuR acts in a complex and pleiotropic fashion to modulate myelopoiesis and inflammation.

Abstract - 26

MACROPHAGE-SPECIFIC LITAF-DEFICIENT MICE EXPRESS REDUCED LPS-INDUCED CYTOKINE PROFILING: FURTHER EVIDENCE FOR LITAF-DEPENDENT LPS SIGNALING PATHWAYS

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Previously we identified a new transcription factor, LPS-Induced TNF-Alpha Factor (LITAF), mediating inflammatory cytokine expression in LPS-induced processes. To characterize the role of LITAF in vivo we generated a macrophage-specific LITAF-deficient mouse (macLITAF$^{-/-}$). Our data demonstrate that in macrophages 1) several cytokines (such as TNF-α, IL-6, sTNF-RII, CXCL16) are induced at lower levels in macLITAF$^{-/-}$ compared to LITAF$^{+/+}$ control macrophages; 2) macLITAF$^{-/-}$ mice are more resistant to LPS-induced lethality. To further identify LITAF signaling pathways, we tested mouse TLR2$^{-/-}$, 4$^{-/-}$, 9$^{-/-}$ and wild-type (WT) peritoneal macrophages exposed to LPS. Using these cells, we now show that LITAF expression can be induced after challenge either with LPS from *Porphyromonas gingivalis (P.g.)* via agonism at TLR2, or with LPS from *E. coli* via agonism at TLR4, both requiring functional MyD88. We also show that in response to LPS the MyD88-dependent LITAF pathway differs from the NF-κB pathway. Furthermore, using a kinase array, p38α was found to mediate LITAF phosphorylation and the inhibition of p38α with a p38 specific inhibitor (SB203580) blocked LITAF nuclear translocation and reduced LPS-induced TNF-α protein levels. Finally macLITAF$^{-/-}$ macrophages rescued by LITAF cDNA transfection restored levels of TNF-α similar to those observed in WT cells. We conclude that LITAF is an important mediator of the LPS-induced inflammatory response that can be distinguished from NF-κB pathway and that p38α is the specific kinase involved in the pathway linking LPS/MyD88/LITAF to TNF.

Abstract - 27

TRANSMEMBRANE TNF PROTECTS MUTANT MICE AGAINST INTRACELLULAR BACTERIAL INFECTIONS, CHRONIC INFLAMMATION AND AUTOIMMUNITY.

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Using targeted mutagenesis in mice we have blocked the shedding of the endogenous murine TNF by deleting its cleavage site. Mutant mice produce physiologically regulated levels of transmembrane TNF which suffice to support thymocyte proliferation, but cannot substitute for the hepatotoxic activities of wild-type TNF following LPS/D-galactosamine challenge *in vivo* and are not sufficient to support secondary lymphoid organ structure and function. Notably however, transmembrane TNF is capable of exerting anti-Listerial host defenses while remaining inadequate to mediate arthritogenic functions as tested in the tristetraprolin-deficient model of TNF-dependent arthritis. Most interestingly, in the EAE model of autoimmune demyelination, transmembrane TNF suppresses disease onset and progression and retains the autoimmune suppressive properties of WT TNF. Together, these results indicate that transmembrane TNF preserves a subset of TNF's beneficial activities while lacking detrimental effects. These data support the hypothesis that selective targeting of soluble TNF may offer several advantages over complete blockade of TNF in the treatment of chronic inflammation and autoimmunity.

Abstract - 28

T REGULATORY CELLS (Tregs) IN SJÖGREN'S SYNDROME (SS): CORRELATION WITH THE PROGRESSION OF THE AUTOIMMUNE LESION.

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Tregs are implicated in the development of autoimmunity. SS is an autoimmune exocrinopathy that is associated with dense lymphocytic infiltration of the affected organs (mainly salivary and lacrimal glands). Herein, we aimed to investigate the occurrence of Tregs in the minor salivary gland (MSG) inflammatory lesions of SS patients.
Tregs were identified by the immunohistochemical detection of the specific marker FOXP3 in MSG biopsies obtained from 23 SS patients and 7 controls (3 that did not fulfill the SS American-European classification criteria, 2 with sarcoidosis- and 2 with HCV-related sialadenitis). SS specimens were classified in three groups. The first group included specimens with 2+ or 3+ Tarpley biopsy score without germinal center (GC) formation (n=7), the second samples with 3+ score and concurrent GC formation (n=8) and the third biopsies of 4+ score (n=8).
FOXP3+ cells were detected in the areas of infiltrating CD3+ T cells in all SS samples, but not in controls. The mean of the FOXP3+/CD3+ cell percentage+/-SE was found 2.36%+/-0.15, 19.21%+/-0.78 and 7.52%+/-0.25 for the first, second and third group, respectively. In sarcoidosis and HCV specimens FOXP3+/CD3+ cell percentage (4.66%+/-0.94 and 12.86%+/-0.63, respectively) seems independent from the lesion progression. FOXP3+ cells were not detected in the MSG tissues from the 3 controls that did not fulfill the SS classification criteria.
Tregs are present in the autoimmune MSG lesions of SS patients. Their frequency is associated with the SS lesion progression, with the highest frequency detected in the intermediate and the lowest in the early lesions.

Abstract - 29

EVIDENCE FOR INTERACTION OF B2-GLYCOPROTEIN I (B2GPI) WITH CD40 LIGAND (CD40L): IMPLICATIONS FOR THE PATHOGENESIS OF ANTIPHOSPHOLIPID SYNDROME (APS).

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Antiphospholipid syndrome (APS) a cause of vascular thrombosis is an autoimmune disease characterized by anti-β2GPI antibodies, which stimulate platelets to express tissue factor (TF) and to secret proinflammatory cytokines. .
To study the molecular mechanism of interaction between anti-β2GPI/ β2GPI complex and the CD40-CD40L pathway in platelet protein extracts.
Platelets from healthy donors preincubated with β2GPI were immunoprecipitated (IP) by anti-CD40 and anti-β2GPI antibodies. The precipitated proteins were electrophoresed in a 10% polyacrilamide gel under non reducing conditions. Western Blot (WB) analysis with anti-β2GPI and anti-CD40 antibodies respectively, was subsequently performed. In addition, IP with anti-CD40L followed by WB with anti-β2GPI and IP with anti-β2GPI followed by WB with anti-CD40L was performed.
IP with anti-CD40L precipitates a protein band of 47kDa which is recognized in WB by anti-β2GPI and IP with anti-β2GPI precipitates another protein band of 50KDa which is recognized in WB by anti-CD40L. These protein bands did not appear in negative controls (IP with normal IgG) nor the represented IgG heavy chains
Anti-β2GPI precipitate CD40L while anti-CD40L precipitate β2GPI from whole platelet protein extract. This interaction may explain platelet activation in APS

Abstract - 30

PREFERENTIAL RECOGNITION OF THE PHOSPHORYLATED MAJOR LINEAR B-CELL EPITOPE OF La/SSB 349-368aa BY ANTI-La/SSB AUTOANTIBODIES FROM PATIENTS WITH SYSTEMIC AUTOIMMUNE DISEASES

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Sera from patients with primary Sjögren Syndrome (pSS) or Systemic Lupus Erythematosus (SLE) often contain autoantibodies directed against La/SSB. The major B-cell epitope, spanning the sequence 349-368aa, contains at the position 366 a serine aminoacid residue (Ser366), which constitutes the main phosphorylation site of La/SSB. The aim of this study was to investigate the differential recognition of the 349-368aa epitope and its phosphorylated form by autoantibodies. Peptides corresponding to the sequence of the unphosphorylated (pep349-368aa) and the phosphorylated form (pep349-368aaPh) of the La/SSB epitope, as well as to a truncated form of the epitope (lacking the phosphorylation site, pep349-364aa), were synthesized. Sera from patients with pSS (n=40) and SLE (n=13) with anti-La/SSB specificity, 9 patients with pSS and SLE without anti-La/SSB antibodies, 25 patients with rheumatoid arthritis (disease controls), as well as sera 32 from healthy individuals were investigated by ELISA experiments. Autoantibodies to pep349-368aaPh were detected in sera of anti-La/SSB positive patients with statistically significant higher prevalence compared to the pep349-368aa (66% versus 45%, p=0.03). Pep349-368aaPh inhibited the antibody binding almost completely (92%), while pep349-368aa inhibited the binding only partially. Anti-La/SSB antibodies presented higher relative avidity for the phosphorylated than for the unphosphorylated peptide. Immunoadsorption experiments using the truncated peptide pep349-364aa suggested the existence of two distinct groups of antibodies against the major epitope of La/SSB; one group against the region of the 349-364aa and the other against the rest of the epitope, which contains the phosphorylated of Ser366.These data suggest that sera from pSS and SLE patients with anti-La/SSB reactivity possess autoantibodies that bind more frequently and with higher avidity onto the phosphorylated major B-cell epitope of the molecule.

Abstract - 31

POSTTRANSLATIONAL MODIFICATIONS OF THE MAJOR LINEAR EPITOPE 169-190aa OF Ro60kD AUTOANTIGEN ALTER THE AUTOANTIBODY BINDING

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Ro60kD is a member of the Ro/LaRNP ribonucleoprotein complex and its major linear B-cell epitope, corresponding to the region 169-190aa, has been found to be the initial target of the autoimmune response in patients with Systemic Lupus Erythematosus. This sequence contains one serine and two arginine aminoacid residues, which can potentially be posttranslationally modified, by phosphorylation or citrullination, respectively.
The aim of this study was to develop an immunoassay for anti-Ro60kD epitope antibody detection and to investigate the changes in the antigenicity of the Ro60kD epitope, when it is posttranslationally modified, by either citrullination or phosphorylation. Peptide analogues corresponding to the unmodified form of the epitope, its phosphorylated form, and a form with both arginine residues citrullinated were synthesized.
The peptide coating conditions were investigated and it was found that the use of highly hydrophilic surfaces increases the efficiency of the coating, as well as the sensitivity of the method for anti-peptide antibody detection. All peptides were tested by the optimized ELISA against 119 sera from patients with primary Sjögren Syndrome, Systemic Lupus Erythematosus, and Rheumatoid Arthritis with anti-Ro/SSA reactivity, 20 sera from patients with systemic diseases without anti-Ro/SSA immune reactivity, as well as against 65 sera from normal individuals. A large proportion of the tested sera reacted against all three peptide analogues, with a preference though for the unmodified form of the epitope.
In conclusion, posttranslational modifications of the major Ro60kD B-cell epitope can alter the autoantibody binding.

Abstract - 32

ANTI-La/SSB ANTIIDIOTYPIC ANTIBODIES IN MATERNAL SERUM: A MARKER OF LOW RISK FOR NEONATAL LUPUS IN AN OFFSPRING

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The anti-La/SSB response to major B cell epitopes of La/SSB can be blocked by an active idiotypic/antiidiotypic network, which can be identified using synthetic complementary epitopes deduced from the sequence of the major B cell epitopes of the molecule. This study evaluated the role of this network in pregnant women with anti-Ro/SSA and/or anti-La/SSB antibodies in the development of neonatal lupus syndrome (NLS).
Sixty-three serum samples collected from anti-Ro/anti-La–positive women during pregnancy or within 6 months after delivery were obtained from the Research Registry for Neonatal Lupus and the PR Interval Dexamethasone Evaluation study. These samples, as well as 30 sera from healthy individuals, were tested in a blinded manner by enzyme-linked immunosorbent assay against synthetic peptides corresponding to major B cell epitopes and complementary epitopes of La/SSB.
Sera from mothers giving birth to a healthy child and having no history of a child with NLS exhibited higher antiidiotypic antibody activity compared with mothers carrying a child with NLS ($P < 0.0001$) or mothers giving birth to a healthy child but who previously gave birth to a child with NLS ($P = 0.0151$). Sera from mothers of healthy children, which exhibited no apparent epitope activity against amino acids 349–364, revealed a significantly greater frequency of hidden anti–349–364aa epitope responses, blocked by antiidiotypic antibodies, as compared with sera from women pregnant with an affected child ($P = 0.0094$).
The presence of antiidiotypic antibodies to autoantibodies against La/SSB may protect the fetus by blocking pathogenic maternal autoantibodies. Testing for these antiidiotypic responses may be useful in predicting a decreased risk of NLS.

Abstract - 33

ANALYSIS OF PAROTID GLANDS OF PRIMARY SJÖGREN'S SYNDROME PATIENTS USING PROTEOMIC TECHNOLOGY REVEALS ALTERED AUTOANTIGEN COMPOSITION AND NOVEL ANTIGENIC TARGETS

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Sjögren's syndrome (SS) is an autoimmune disease characterized by lymphocytic infiltration, destruction of the salivary and lacrimal glands and production of autoantibodies against a variety of cellular proteins. The aberrant immune response against these autoantigens may begin or extend to other proteins that are not yet defined. Several studies have pointed that the autoantibody production is taking place in the affected salivary glands.
In the present study using proteomic approaches we aimed to: A) Identify new autoantigens in the salivary glands of pSS patients, B) Evaluate the epigenetic changes of known autoantigens.
Total parotid gland extracts of pSS patients were analyzed using two-dimensional gel electrophoresis, SDS-PAGE and immunoblot with pSS patients' sera or purified autoantibodies and immunoprecipitation using homologous IgG. Identification of the unknown proteins was performed using mass-spectroscopy.
Immunoblot analysis on 2-D gels using purified anti-La/SSB antibodies revealed that pSS salivary glands contain high levels of post-translationally modified La/SSB autoantigen, while the native form of the protein is faintly recognized, by contrast to normal controls. Moreover, salivary glands of pSS patients contain post-translationally modified actin that becomes immunogenic in the microenviroment of the affected tissue.
The alteration of the physicochemical properties of self-proteins could thus contribute to the break of immune tolerance against them.

Abstract - 34

PREDICTIVE CLASSIFICATION OF PRIMARY SJÖGREN'S SYNDROME: IDENTIFICATION OF PATIENTS AT RISK USING AUTOANTIBODY TITRATION.

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Disease outcome studies in primary Sjögren's Syndrome (pSS), a chronic autoimmune exocrinopathy, have identified palpable purpura and hypocomplementemia as the major predictors of poor prognosis. On the basis of these findings a predictive classification of pSS, that designates patients with highly likely adverse outcome as "Type I" and the remainder as "Type II", has been developed. We sought to assess whether autoantibody titers can serve as predictors of disease outcome in the context of the above classification.
144 consecutive patients with pSS were included in the study. Demographical, clinical and serological data were recorded from the medical files of the patients. Serum sample from each patient was available for further testing. Autoantibody titers were measured with specific ELISA assays developed in our laboratory, using as antigens either human recombinant Ro52 or La/SS-B.
The study population comprised of patients with mean age at the time of diagnosis 50.2 years and mean disease duration of 7.36 years. Nonparametric analyses revealed that high autoantibody titers are strongly associated with both hypocomplementemia and purpura ($p<0.005$) as well as with poor prognosis overall (type I, $p<0.0001$). ROC curve analysis revealed a threshold value of 224.81 BU for anti-Ro52 (Area Under the Curve, AUC, 76.7%, $p<0.0001$) and 49.89 BU for anti-La (AUC 71.5%, $P<0.0001$) that discriminate "type I" from "type II" with the highest sensitivity and specificty. A multivariate logistic binary regression analysis revealed that the titers of anti-Ro52 in combination with anti-La can correctly classify 74.3 % of the patients.
In conclusion, serum titers of anti-Ro52 and anti-La can be used as laboratory indices to classify the majority of the patients according to their prognosis.

Abstract - 35

TLR-4 CONSTITUTIVE AND LPS- INDUCED LEVELS ARE TNF-MEDIATED

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Toll-like receptors (TLRs) are members of a conserved family of type I transmembrane receptors. Expression of TLRs is induced, by cytokines (IFNγ, TNF, IL-1β, IL-2, -5) and besides various microbial elements, self molecules including heat shock proteins, DNA/RNA from dying cells, stress-released fibronectin and fibrinogen fragments have been shown to act as TLR ligands. TLR-4 triggering has been linked to excess programmed cell death either through the production of various cytokines or directly through a Fas-associated death domain protein-dependent pathway. We examined TNF implication in TLR-4 constitutive or LPS-induced expression and apoptosis. Furthermore, through transient depletion of the Interferon Regulatory Factor -1 (IRF-1) transcription factor mRNAs by RNA interference we sought to investigate IRF-1 participation in the TLR-4 cascade.
Herein we report that, TNF up-regulated cell surface TLR-4 by 170±30% in THP-1 neoplastic monocytic cell line. Interestingly, constitutive TLR-4 levels were also found to be TNF-dependent as the addition of infliximab, an anti-TNF monoclonal antibody inhibited TLR-4 levels (100% reduction). LPS stimulation with simultaneous anti-TNFtreatment, demonstrated a decrease in TLR-4 levels of 91±5%. This suggests that TNF is the main regulator of TLR-4, constitutive or LPS-induced expression.
Apoptosis was monitored by Annexin-V binding assay. TNF addition resulted in a statistically significant increase of apoptosis to [12±1.6% vs. 18±2.0% ($p<0.001$)]. After anti-TNF treatment the levels of apoptosis decreased in comparison to the constitutive levels [8±0.4% vs.12±1.6%]. Finally, concurrent LPS induction and TNF blockade impelled the apoptotic levels to 35.0±2.7% ($p<0.001$) revealing statistically significant reductions when compared to the LPS driven apoptotic levels (42±2.9%).
Our study is the first to suggest that TLR-4 is up-regulated in a TNF-dependent mechanism and that IRF-1 could negatively regulates TLR-4 expression.

Abstract - 36

ERYTHROID TRANSCRIPTION FACTOR GATA-1 mRNA EXPRESSION IS UP-REGULATED IN MYELODYSPLASIA: POSITIVE CORRELATION OF GATA-1 LEVELS WITH ERYTHROBLAST APOPTOSIS AND PERIPHERAL ANEMIA.

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The expression of the transcription factor GATA-1, in hematopoietic stem cells commits them to the myeloid lineage. If GATA-1 expression remains unobstructed then the myeloid precursors are committed towards erythropoiesis and magakaryopoiesis. Myelodysplastic Syndromes (MDS), a clonal disorder of the progenitor CD34+ cell, predominately present dyspoiesis in these two lineages. We reasoned that measurement of GATA-1 expression levels as well as search for genetic alternations in progenitor CD34+ and erythroid CD71+ cells derived from MDS patients could illuminate any GATA-1 involvement in the disease pathogenesis. Herein, we report significantly elevated GATA-1 mRNA expression levels, in MDS CD34+ and CD71+ cells in contrast to the iron deficiency controls. Furthermore GATA-1 expression levels were found to correlate with disease progression since High/INT-2 MDS patients expressed significantly higher GATA-1 mRNA levels than Low/INT-1 patients. GATA-1 levels were found to correlate with disease severity, as High/INT-2 patients displayed increased apoptosis in CD71+ cells and significantly lower levels of neutrophils, platelets and hemoglobin than Low/INT-1 patients. None genetic alternation in either the full length or the spliced GATA-1 mRNA was detected.
These observations suggest that GATA-1 is significantly up-regulated in MDS and monitoring its levels may prove useful in following disease progression towards leukemic transformation.

Abstract - 37

EVIDENCE OF DETACHMENT-INDUCED CELL DEATH (ANOIKIA) IN CULTURED NON-NEOPLASTIC SALIVARY GLAND EPITHELIAL CELLS (SGEC) UPON TRIGGERING OF TOLL-LIKE RECEPTOR-3

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Sjögren's syndrome (SS) is characterized by destructive lesions of salivary epithelium associated with apoptosis. Previous evidence from this laboratory had indicated that cultured non-neoplastic SGEC are susceptible to apoptosis after treatment with IFN¤, largely due to the loss of epithelial cell anchorage to cell matrix and of survival signals, a phenomenon termed anoikia. Preliminary experiments showed significant detachment of SGEC upon triggering of TLR-3, whereas, in cells pre-treated with an RNA-synthesis inhibitor, TLR-3 triggering induced significant apoptosis.
To investigate whether triggering of TLR3 molecules on SGEC is capable to induce apoptosis via anoikia, without pre-treatment with an RNA-synthesis inhibitor.
Cultured SGEC lines from patients with non-specific sialadenitis (n=5) were stimulated by poly-inosinic:cytidylic acid (polyI:C; TLR3-ligand; 5μg/ml) or peptidoglycan (PGN; TLR2-ligand; 100μg/ml, as control), with or without 1-hr pre-treatment with actinomycin D (ActD, RNA synthesis inhibitor, 0.5μg/ml), for 24-96 hours. Anoikia was assessed by cell morphology and by the survival MTT assay. Apoptosis was monitored by Annexin V-binding assays.
SGEC stimulation by polyI:C (but not PGN) induced significant apoptosis at 48-hrs to attached ActD-pretreated cells (30.6% ± 3.0), but not to ActD-untreated cells (6.6% ± 1.1, $p<0.005$), without though involving cell detachment. SGEC stimulation by polyI:C for 96-hrs resulted in significant cell detachment and anoikia (mean cell loss ±SE: 50.2% ± 4.0), but not by PGN (2.3% ± 0.5, $p<0.001$), compared to untreated cells. The remaining attached cells appeared viable.
TLR-3 triggering on cultured SGEC was shown to result in the disruption of cell anchorage and apoptosis. Thus, pathogen-related or endogenous-derived TLR3-ligands released locally in the salivary glands of SS patients appear able to induce epithelial apoptosis via anoikia, contributing further to the glandular dysfunction of the patients.

Abstract - 38

FUNCTIONAL TOLL-LIKE RECEPTORS ARE EXPRESSED BY SALIVARY GLAND EPITHELIAL CELLS: INCREASED mRNA EXPRESSION IN CELLS DERIVED FROM PATIENTS WITH PRIMARY SJÖGREN'S SYNDROME

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Innate immune responses are induced via the stimulation of Toll-like receptors (TLR) by foreign pathogens. TLR expression by salivary gland epithelial cells (SGEC) may participate in the development of glandular inflammatory reactions that characterize primary Sjögren's syndrome (pSS). In this study we sought to assess the expression and function of several TLR molecules in cultured non-neoplastic SGEC obtained from pSS patients and disease controls.
Long-term cultured non-neoplastic SGEC derived from pSS patients (SS-SGEC) and disease controls (control-SGEC), normal peripheral blood monocytes and neoplastic monocytic THP-1 cell line were examined by RT-PCR analysis and quantitative real-time PCR for mRNA expression of TLR-1, -2, -3 and -4 molecules. TLR function was assessed by the induction of the adhesion molecule CD54/ICAM.1 expression (flow cytometry) following treatment with the TLR-ligands: S. aureus peptidoglycan (TLR-2), the synthetic dsRNA analogue poly-inosinic:cytidylic acid (TLR-3) and E. coli lipopolysaccharide (TLR-4).
SGEC were found to express functional TLR-2, -3 and -4 molecules, as attested by dose-dependent up-regulation of surface ICAM.1 expression following treatment with the respective TLR-ligands. Most pronounced SGEC responses were observed following TLR-3 triggering, that were significantly higher than normal monocytes and THP-1 cells. SS-SGEC were found to display significantly higher expression of TLR-1 ($p=0.0027$), TLR-2 ($p=0.01$) and TLR-4 ($p=0.03$) mRNA compared to control-SGEC lines, as well as compared to normal monocytes (all for $p=0.0001$).
Cultured SGEC express functional TLR molecules. The high constitutive TLR expression by SS-SGEC further supports the intrinsic activation and the role of epithelial cells in pSS pathogenesis.

Abstract - 39

TNFR2 AND THE EFFICIENT REMYELINATION OF THE CENTRAL NERVOUS SYSTEM

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Experimental Autoimmune Encephalomyelitis (EAE) develops through the immunologic attack of the myelin sheath of the central nervous system (CNS). The adult nervous system maintains the ability to self-repair (remyelination) by recruiting neuronal stem cells and precursors (NPCs). Tumor Necrosis Factor (TNF) is a multipotent cytokine that signals through two receptors: TNFR1, which mediates tissue damage; and TNFR2 implicated in reparative processes. In the absence of TNFR2, EAE exhibits exacerbated clinical course with sustained motor deficits. Our detailed histological examination of the CNS of EAE-affected wild type animals revealed the formation of characteristic foci where astrocytes encircle inflammatory and NPCs. In the absence of TNFR2, these foci are less numerous and overwhelmed by aggressive inflammatory infiltrates and dispersed NPCs. Furthermore, using the cuprizone model of neurotoxicant demyelination we show that in the absence of TNFR2, there is delayed remyelination and exclusion of astrocytes and microglia from the site of demyelination. We found TNFR2 to be dispensable for the ability of NPCs to proliferate, form neurospheres and differentiate, it may therefore be the lack of organized cellular microarchitecture that is responsible for the defective remyelination observed in TNFR2-/- animals. We are currently examining the role of TNFR2 on the function and migration of cellular components necessary for the formation of remyelination foci.

Abstract - 40

SALIVARY GLAND EPITHELIAL CELLS (SGEC) EXPRESS A NOVEL B7.2 (CD86) SPLICE VARIANT WITH A PUTATIVE NEGATIVE T CELL REGULATORY ROLE

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SGEC express three distinct alternate transcripts of the B7.2 costimulatory molecule. These included the two previously described transcripts (encoding the full-length and the soluble form, respectively), as well as a third novel form. In this study we aimed to study the properties of this novel splice variant, designated as B7.2C.

B7.2C is characterized by exon 4 deletion, which encodes the IgV-like CD28/CTLA4-binding domain of the B7.2 protein. Besides SGEC, this B7.2C mRNA variant was also detected in peripheral blood monocytes, but not T and B cells. B7.2C molecule is expressed on the cell-surface as verified by transfection of CHO cells with this transcript (CHO-B7.2C). CHO-B7.2C cell lines were found unable to provide T cell costimulation. On the other hand, compared to CHO-B7.2A/mock transfectants with similar surface expression of the full-length B7.2 (B7.2A), double-transfected CHO cell lines that expressed both B7.2A and B7.2C forms (CHO-B7.2A/C, 5 distinct cell lines) exhibited decreased costimulatory activity, as indicated by median reduction (range) of 69% (58-82%), 34% (31-41%), 39% (24-44%), 32% (23-33%) and 23% (21-27%), respectively (3 independent experiments for each cell line).

Our findings suggest that B7.2C transcript has a putative negative T cell regulatory role, possibly owing to its interference with the formation of B7.2 clusters and network, which is considered necessary for the effective interaction between B7.2 and CD28/CTLA4 receptors.

Abstract - 41

ROLE OF ACTIVIN A ON DENDRITIC CELL MIGRATION

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Activin A is a dimeric protein member of the TGF-beta family. This cytokine exerts both pro- and anti-inflammatory effects and was shown to play a crucial role in wound repair, in inflammatory diseases and in foetal tolerance. In vitro experiments showed that Activin A is chemotactic for monocytes, immature monocyte-derived dendritic cells, and monocytes-derived Langerhans cells. The migration was concentration-dependent, pertussis toxin sensitive and inhibited in the presence of blocking antibodies directed against Activin A receptors. In addition, a brief exposure to Activin A induced a CCR7-dependent migration of monocytes and immature dendritic cells to CCL19, a classical chemotactic factor for mature dendritic cells. These results suggest that Activin A may play a role in the migration of dendritic cells and their precursors to peripheral tissues and to secondary lymphoid organs. Furthermore, these findings may provide the molecular basis for the strong decrease in the number of Langerhans cells observed in the skin on the transgenic mice overespressing follistatin, a natural Activin A inhibitor.

Abstract - 42

DECREASED TLR4 EXPRESSION IN CRH-NULL MICE: A REASON FOR THEIR INCREASED SUSCEPTIBILITY TO COLITIS?

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Immunomodulatory neuropeptides expressed in the intestine play an important role in the pathogenesis of inflammatory bowel disease (IBD). We and others have previously described the proinflammatory effects of peripheral Corticotropin-Releasing Hormone (CRH) in mice, including models of intestinal inflammation. Inappropriate innate immune responses to commensal flora play a major role in the pathogenesis of IBD. Our preliminary studies show altered expression of TLR4 in several tissues of Crh-/- mice with unclear yet significance in their inflammatory responses. In this study we evaluated the effect of Crh-deficiency in intestinal TLR4 expression and in the DSS-induced colitis, which severity is correlated to TLR4 activity. TLR4 expression was assessed in the Crh+/+ and Crh-/- colon. Crh-/- and Crh+/+ mice administered DSS for 7 days. Scid mice implanted with intestinal Crh+/+and Crh-/- isografts administered LPS and isograft Tnfa expression was determined 4hrs later. We found similar DSS-induced intestinal inflammation in Crh+/+ and Crh-/- mice, decreased TLR4 expression in the Crh-/- colon, and increased levels of Tnfa in the Crh-/- intestinal isografts. Lack of protection of the Crh-/- mice from DSS colitis suggests that they provide a relevant model to dissect the contribution of proinflammatory neuropeptides in intestinal innate responses and the development of colitis.

Abstract - 43

GENETIC DISSECTION OF THE ROLE OF THE CHEMOKINE/CHEMOKINE RECEPTOR PAIR CCL25/CCR9 IN THE PATHOGENESIS OF THE MURINE TNFΔARE MODEL OF CROHN'S DISEASE

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TNFΔARE mice bearing a deletion in the 3´AU-rich elements (AREs) of TNF mRNA overproduce TNF and develop Crohn's-like IBD pathology that requires CD8+ T-cell effector function. The chemokine/receptor pair CCL25/CCR9 has been strongly associated with CD8+ T-cell migration to the small intestine. Furthermore Crohn's disease patients have an increased frequency of peripheral blood CCR9+ T-lymphocytes implicating CCL25/CCR9 in the pathogenesis of the disease. In the present work we employ genetically deficient animals in order to determine the role of intestinal-specific homing molecules, both the chemokine/chemokine receptor pair CCL25/CCR9 and beta7-integrin, in the TNFΔARE mouse model of Crohn's disease. Genetic ablation of the chemokine CCL25 or its receptor CCR9 failed to attenuate disease. In contrast, genetic ablation of beta7-integrin resulted in amelioration of IBD pathology in the TNFΔARE mice in accordance with the requirement for beta7-integrin for T-cell localization to the intestinal mucosa. In addition we show that intestinal inflammation in the TNFΔARE mice is associated with the marked reduction of the CD8αα+ intraepithelial lymphocyte compartment. We conclude that the chemokine CCL25 and its receptor CCR9 are dispensable for the generation of a pathogenic CD8+ T-lymphocyte compartment in the murine small intestine.

Abstract - 44

A SENSITIZED ENU MUTAGENESIS SCREEN FOR GENETIC MODIFIERS OF RHEUMATOID ARTHRITIS AND INFLAMMATORY BOWEL DISEASE

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Genome-wide, random mutagenesis with the ethylating mutagen N-ethyl-N-nitrosourea (ENU) on animal models of human diseases offers unique opportunities to discover gene functions directly associated with prevention or therapy of diseases. We have thus initiated a programme of sensitized ENU mutagenesis screen applied on our established TNFΔARE model of arthritis and Crohn's-like inflammatory bowel disease (IBD), to identify modifier gene candidates associated with development of these diseases. By using simple and accurate phenotypic screens, clinical score for arthritis and macroscopic or histological analysis for IBD, we are selecting the individual progeny that display disease attenuation. By screening 6318 G3 offspring we have identified 3 families which show significant delay on the onset and progression of arthritis and 6 families with dramatic attenuation of IBD. In parallel to the sensitized screens we have also identified novel recessive phenotypes ie. a mouse mutant model which shows severe osteopetrosis, defect in tooth eruption and complete lack of osteoclasts. Initial mapping efforts have already identified candidate chromosomal regions for specific mutants, whereas fine mapping using SNPs analysis is currently underway. Once identified these novel gene functions may constitute validated pharmaceutical targets for the treatment of chronic inflammatory disease.

Abstract - 45

QUANTITATIVE TRAIT LOCI (QTL) MAPPING OF RESISTANCE AND SUSCEPTIBILITY TO TRICHURIS MURIS INFECTION IN THE MOUSE; IDENTIFICATION OF LOCI IMPORTANT IN CHRONIC GUT INFLAMMATION

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Chronic intestinal helminth infection is a global public health problem. Chemotherapeutic intervention is likely to lead to drug resistance therefore information about these diseases is of paramount importance for understanding host susceptibility. Trichuris muris, a natural gastrointestinal nematode of mice, induces chronic inflammation and cell hyperplasia in the large intestine, pathologically similar to inflammatory bowel diseases of the western world. The genetic factors which predispose to susceptibility are still not known. Using an F2 intercross of resistant and susceptible mice, we have identified four significant QTL on autosomal regions which predispose to Trichuris muris infection. The major susceptibility locus lies within the MHC class III region, and we suggest that TNFalpha is a strong candidate gene at this QTL. Three of the defined QTL are highly homologous with loci associated with susceptibility to Crohns disease in humans. We suggest therefore that chronic mucosal inflammation of the large bowel is controlled by definite genetic factors which promote the disease endpoint common to both T. muris infection and Crohns disease.

Abstract - 46

DYNAMIC CHANGES IN THE BMP SIGNALING NETWORK DURING PULMONARY FIBROSIS

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Bone Morphogenetic Proteins are involved in the development and homeostatic regulation of many adult tissues and are implicated in several forms of inflammatory disorders including airway inflammation. This study addresses the potential involvement of the BMP signaling network in lung homeostasis and pulmonary fibrosis. We used LacZ reporter mice for BMP-4, Gremlin Noggin, and Twisted Gastrulation in combination with immunohistochemistry to visualize and identify lacZ positive cells in healthy lung. To assess for changes in Pulmonary Fibrosis, we administered Bleomycin intratracheally and sacrificed the mice at 3, 7, 14 and 21 days after insult. A significant increase in the expression levels of all molecules was observed. Interestingly, the molecules were expressed in cell types different than seen in healthy lungs and mainly co-localized with inflammatory-fibrotic clusters. Our findings indicate that BMP signaling network must be involved in the homeostasis of the healthy lung. The dramatic changes to the BMP signaling network observed during Bleomycin induced Pulmonary Fibrosis suggest that these molecules are important in lung tissue repair. Disturbances to the network possibly induced by the inflammatory condition could thus be a factor contributing to chronic tissue damage, and pharmaceutical manipulation of BMP signals might have therapeutic potential for lung pathologies.

Abstract - 47

MESENCHYMAL CELLS ARE SUFFICIENT TARGETS FOR TNF IN MODELED JOINT AND GUT PATHOLOGIES.

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TNF plays an essential role in the pathogenesis of Rheumatoid Arthritis and Crohn's Inflammatory Bowel Disease. Anti-TNF therapies have proved successful in their clinical treatment. The cellular mechanisms of TNF/TNF-R function in these diseases have remained poorly characterized. In general it is thought that TNF delivers mostly innate activation and pro-inflammatory signals through its action on myeloid / monocytic cells or other haemopoietic cell types. Using reciprocal bone marrow grafting experiments in previously established TNF transgenic animal models of arthritis and Crohn's-like IBD (Tg197 and TNFΔARE mice), we show that development of arthritis requires the expression of TNFRI in cells of the radiation-resistant compartment, which are also sufficient targets for TNF in the development of the Crohn's-like IBD as previously demonstrated. Moreover, we have generated a Cre-expressing mouse line (Cre expression driven by Collagen VI (a1) promoter). As expected, Cre activity could be detected in mesenchymal cells such as synovial fibroblasts, articular chondrocytes, skeletal myocytes, keratinocytes, dermal fibroblasts and intestinal myofibroblasts. Mesenchymal cell-specific reactivation of a mutant floxed TNFRI allele in Col(VI)-Cre/TNFΔARE/TNFRIcneo mice led to a full blown arthritic and intestinal phenotype indicating that cells of mesenchymal origin are sufficient targets of pathogenic TNF function. Our results offer a novel mechanistic perspective for TNF function in gut and joint pathologies and indicate a common TNF target that may explain the often observed synovial /gut axis in human disease.

Abstract - 48

Development of haemato-lymphoid progenitors: dissection of foetal lymphopoiesis reveals asynchronous lineage decision of T and B cells.

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All lymphocytes derive from haematopoietic stem cells (HSCs), which are in the bone marrow sequestrated by a specialized microenvironment. In contrast to their relative immobilization during adult life, fetal haematopoietic progenitors are mobilised in distinct waves in the embryo. The key objectives of the ongoing work in our laboratory are:

- To identify where and when lymphopoiesis is initiated during embryogenesis and what is the role of compartmentalization/migration for this process.
- To determine what is the particular role of adhesion molecules for the development and the function of blood cells under normal and pathological conditions.

During embryogenesis HSCs need to migrate to an appropriate microenvironment provided by the foetal liver where their number is expanded and lineage commitment to the major blood lineages is induced. Both the colonization of the foetal liver and the expansion of haematopoietic progenitors are critically dependent on the expression of adhesion molecules. We have previously shown that beta1 and alpha4 integrins are relevant for the homing of haematopoietic cells into the foetal liver and their subsequent expansion at this site. The analysis of progenitor compartments in mice deficient for integrins therefore provides a basis to generate a "high-resolution" map of the migration and compartmentalization of haemato-lymphoid progenitors.

The absence of beta1 integrin completely blocks the colonization of the foetal liver, foetal thymus and bone marrow, but does not interfere with the primitive haematopoiesis in the yolk sac nor the generation of definitive HSCs in the aorto-gonad-mesonephros (AGM) region. Both AGM- and foetal blood-derived beta1-null haematopoietic progenitors could successfully differentiate *in vitro* into lymphoid cells. Since transmigration into primary haematopoietic organs is completely blocked we analysed the fetal blood for the presence of lymphoid committed cells. In Rag-2 complementation assays, none of the beta1-null derived haematopoietic cells in AGM or foetal blood expressed genes indicative for the B cell lineage (VpreB, lambda5, Pax5, E2A), whereas some cells expressed transcripts for preT-alpha, components of the CD3 complex, Notch, and TCF-1. Both their genetic profile, as well as their prospective capacity in differentiation assays, clearly defines them as pro-T cells. These data indicate that lymphoid commitment during embryogenesis dissociates *in vivo*: B cell development strictly requires the foetal liver environment whereas T cell commitment could already be initiated before seeding the thymus.

In summary, using hematopoietic cells deficient for adhesion molecule we have the unique possibility to study naturally mobilized stem cells and early blood cells. These cells exhibit, in contrast to adult HSCs, an impressive potential to expand both *in vitro* and *in vivo*. Hence, the availability of increased numbers of such cells offers an ideal situation for the *in vitro* manipulation of stem cells.

Abstract - 49

DIFFERENTIATION OF THE TH17 SUBSET ANS ITS ROLE IN EAE

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We have recently described de novo generation of a new Thelper subset (Th17) of IL-17 producing T cells from naïve CD4 T cells. TGFβ1, together with the pro-inflammatory cytokine IL-6 supports the differentiation of IL-17 producing T cells, a process that is amplified by IL-1β and TNFα. We could not detect a role for IL-23 in the differentiation of IL-17 producing T cells, but confirmed its importance for their survival and expansion. The Th17 CD4 T cell plays a crucial role in the pathogenesis of autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE) and arthritis. Here we show that CD4 T cells from mice whose T cells cannot respond to TGFß cannot differentiate into Th17 cells and these mice do not develop EAE. Furthermore, local, but not systemic antibody blockade of TGFß prevents Th17 differentiation and the onset of EAE. EAE can also be induced with zymosan, which strongly skews T cell differentiation to Th17. However, zymosan induced EAE is transient and the failure of EAE progression may be linked to reduced levels of IL-23.

Abstract - 50

AUTOTAXIN, A SECRETED LYSOPHOSPHOLIPASE D, IS ESSENTIAL FOR NEURONAL DEVELOPMENT

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Autotaxin (ATX), originally isolated from melanoma cells as a potent cell motility-stimulating factor, is a secreted lysophospholipase D that converts lysophosphatidyl choline (LPC) to lysophosphatidyl Acid (LPA). LPA, an important lipid mediator, plays a crucial role in various neuronal developmental processes, including neurogenesis, neuronal migration, neuritogenesis and myelination. ATX mRNA expression is first detected at the floor plate of the neural tube at embryonic day 9.5 (E9.5), to reach widespread expression postnatally, with highest mRNA levels detected in brain, ovary and intestine. Enhanced ATX expression has been repeatedly demonstrated in a variety of malignant tumor tissues, including tumors of the central nervous system such as glioblastoma and neuroblastoma. To uncover the physiological role of ATX and LPA signalling in vivo we generated a conditionally inactivated allele by homologous recombination in embryonic stem cells (ES) using the Cre-LoxP system. To induce germ line, complete inactivation of ATX, ATXfl/fl mice were mated with transgenic mice overexpressing the Cre recombinase under the control of the human CMV minimal promoter. ATX-/- mice die at E9.5 with vascular defects in the yolk sac and severe malformations of the nervous system (open neural tube, asymmetric head folds, swollen alantois) most likely due to aberrant dorsal ventral patterning. Therefore, our results indicate an essential role of ATX and LPA signalling in the development of the nervous system.

Abstract - 51

INVESTIGATING THE ROLE OF AUTOTAXIN AND LPA SIGNALLING IN THE PATHOGENESIS OF RHEUMATOID ARTHRITIS

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Rheumatoid Arthritis (RA) is a chronic inflammatory disease, characterized by synovial hyperplasia, bone erosion and cartilage destruction. Activated hyperplastic RA Synovial Fibroblasts (SFs) exhibit reorganization of their actin cytoskeleton and deregulated ECM adhesion that could explain the tumor-like behavior of the arthritic synovium. Lysophosphatidic acid (LPA), the enzymatic product of Autotaxin (ATX) - a lysophospholipase D highly expressed in a variety of tumors, is an important lipid mediator that has been found to induce cytoskeletal reorganization, proliferation and migration in many cell types. Here we show that the expression of ATX is upregulated in SFs from an animal model of RA, and ex vivo cultured RA SFs secreted significantly more ATX protein than their wt counterparts. Immunocytochemistry with an a-ATX Ab on joints from arthritic mice and human RA patients indicated massive ATX protein upregulation, despite the observed downregulation of ATX mRNA in the same samples, possibly suggesting direct or indirect (through LPA) negative feed back autoregulation in other cell types. These findings suggest a pathophysiological role of ATX and LPA signalling in RA, which is currently explored by conditional ablation of ATX expression in mouse joints, as well as with human cartilage invasion assays in vivo.

Abstract - 52

TNF-DRIVEN ANIMAL MODELS OF INFLAMMATORY DISEASE: EFFECTIVE TOOLS FOR PRE-CLINICAL IN-VIVO EVALUATION OF PHARMACEUTICALS.

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Target validation involves proving that a molecule is directly implicated in a disease process and can be a suitable target for drug development. Predictive animal models are crucial for this stage of Research and Development. We are offering several murine models that reflect particular human diseases with a causative relation to human or murine TNF activity. These models develop spontaneous disease with 100% phenotypic penetrance and homogeneous and provide reliable in vivo tools for the evaluation of the preventive or therapeutic efficacy of new pharmaceuticals.

BioMedCode Hellas offers services for the set up and execution of pharmaceutical evaluation protocols, accompanied by full histopathological analysis and/or other tests as deemed necessary, currently for the following mouse models of human disease:

Chronic Polyarthritis / Rheumatoid Arthritis:
- **Tg197**, deregulated expression of a human TNF transgene
- **Tg5453**, deregulated expression of a transmembrane human TNF transgene
- **TgA86**, deregulated overexpression of a murine transmembrane TNF transgene
- **TNF$^{\Delta ARE}$**, deregulated overexpression of TNF by knock-in of a murine TNF$^{\Delta ARE}$ gene

Inflammatory Bowel Syndrome / Cronh's disease:
- **TNF$^{\Delta ARE}$**, deregulated overexpression of TNF by knock-in of a murine TNF$^{\Delta ARE}$ gene

Systemic / Multi Organ Inflammation/Cachexia
- **Tg211,** T-cell specific over-expression of a humanTNF transgene
- **TgE1335,** over-expression of human p75 TNF receptor transgene

Inflammatory Demyelinating Diseases/Multiple Sclerosis
- **Tg6074,** CNS deregulated expression of CD2-murineTNF-globin transgene construct
- **TgK21,** astrocyte deregulated expression of transmembrane humanTNF transgene construct