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GEBIN Abstracts

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Neuroimmunology of the CNS

Acute effects of interferon-beta administration on the hypothalamic-pituitary-adrenal axis and leukocyte distribution: transient effects in healthy subjects, long-term consequences for MS patients

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Objective: Interferon-beta (IFNb) beneficially influences the disease course in multiple sclerosis (MS) patients. We have demonstrated that IFN-b leads to extensive effects on the psycho-neuroendocrine-immune network. Eight hours after IFN-b injection healthy subjects showed increased cortisol, prolactin, growth hormone levels, and increased granulocytes, but reduced monocytes and lymphocytes in circulation. These changes were accompanied by flu-like symptoms, increased body temperature and mood changes. Since MS patients under IFN-b treatment undergo these changes every other day it is important to evaluate the neuroendocrine response to IFN-b in these patients. We questioned whether these changes in the psycho-neuroendocrine-immune network are still present after one year of treatment.

Methods: Thirteen MS patients were analyzed for blood leukocyte subsets, cortisol, prolactin, growth hormone, epinephrine and norepinephrine concentrations, body temperature and mood states at the beginning and after one year of continuous IFN-b treatment. Blood samples were taken before, 1, 2, 4 and 8 h following drug administration.

Results: The initial IFN-b administration significantly increased granulocyte numbers, ACTH and cortisol, while monocyte and lymphocyte numbers decreased.

After one year the secretory activity of the HPA-axis and changes in leukocyte subsets distribution became attenuated. Baseline ACTH and cortisol concentrations were significantly elevated during IFN-b treatment.

Conclusions: Focusing on the immediate post-injection period we demonstrated that an initial IFN-b injection extensively affects the psycho-neuroendocrine-immune network in healthy subjects and MS patients. In contrast, after long-term therapy MS patients show altered responsiveness to the injection. These effects provide a possible clue to the mechanism of action of IFN-b.

Endothelial expression of adhesion molecules in the CNS of mice with chronic graft-versus-host disease

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Objective: Graft-versus-host disease (GvHD) is a major complication after allogeneic bone marrow transplantation (BMT). GvHD can cause peripheral neuromuscular disorders, and there is growing evidence that it may also affect the central nervous system. Therefore we have studied the expression of cerebral vascular adhesion molecules in a murine BMT model.

Methods: We investigated endothelial expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) on brain sections of mice after allogeneic BMT by immunofluorescence. Controls were untransplanted mice and mice after syngeneic transplantation without GvHD.

Results: We could demonstrate upregulation of VCAM-1 and ICAM-1 on cerebral endothelium after allogeneic BMT compared to controls or syngeneic transplanted animals. Endothelial expression of cellular adhesion molecules correlated well with earlier findings of cellular infiltrations in brain parenchyma in the same animals.

Conclusions: Our results suggest that in this model of experimental chronic GvHD cerebral endothelial VCAM-1 and ICAM-1 expression play an important role in the recruitment of lymphocytes to the CNS.

Interferon-γ modulation of cyto- and chemokine production by microglia and macrophages in models of bacterial infection

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Objective: Considering their macrophage nature and immune competence microglial cells likely have a critical impact on the clinical course of bacterial infections, e. g. by supporting the chemoattractive recruitment of leukocytes to the brain through the synthesis of cyto- and chemokines. In turn, invading leukocytes may feedback on microglia to influence the release pattern. We analyzed the capacity of interferon-γ (IFNγ) to serve as such a leukocyte-to-microglia signal.

Methods: Production of cyto/chemokines was stimulated in mouse microglia cultures by treatment with either lipopolysaccharide (LPS, *E. coli*) or purified pneumococcal cell walls (PCW).

Results: IFNγ (0.1–100 ng/ml) modulated the patterns of LPS- and PCW-induced cyto/chemokine release in a dose-dependent, potent and complex manner. While amounts of TNFα and IL-6 remained nearly unchanged, IFNγ enhanced the production of IL-12, MCP-1 and RANTES, but attenuated that of KC, MIP-1α and MIP-2. Release modulation was obtained with IFNγ pre-incubation (treatment before LPS/PCW administration), co-incubation and even delayed addition to an ongoing stimulation. Changes in the release pattern upon IFNγ presence would shift the chemoattractive profile from favoring neutrophils to a preferential attraction of monocytes and T lymphocyte populations as actually seen in bacterial meningitis.

Conclusions: Activated microglia may represent a major CNS-resident source for an instant and substantial production of a variety of chemoattractive factors. Further our results suggest that leukocyte-produced IFNγ could massively alter the microglial release profile to adjust the chemoattractive signals. Supported by the DFG (SFB507).

IL-1-beta induces the expression of the prostaglandin E2 receptor EP3 in astrocytes

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PGE2 is a potent modulator of several brain functions e. g. sleep/wake cycle, generation of fever, perception of pain. The diverse effects of PGE2 are mediated by four G-protein-coupled receptors (EP1–4). In neurodegenerative disorders like Alzheimer's Disease (AD) elevated levels of PGE2 have been reported and cyclooxygenase-inhibitors seem to have a protective effect in AD.

Objectives: We investigated whether cytokines such as interleukin-1 regulate EP-receptors in astrocytic cells.

Methods: U373-MG human astrocytoma cells and primary astrocytes were exposed to IL-1-beta. Expression of the EP3 receptor was analyzed by RT-PCR and Western blots.

Results: We found IL-1beta to induce the expression and synthesis of the prostaglandin E2 receptor EP3 in astrocytes. Other EP receptors were not affected. Studying the signal transduction pathways involved in IL-1beta induced EP3 receptor we found protein kinase C to be the key signal transducer in astrocytic EP3 expression.

Conclusion: EP3 receptors might play an important role in neuroinflammatory events and PGE2 effects in the CNS.

Valproic acids inhibits interleukin-1-induced expression of cyclooxigenase-2 and prostaglandin release in human neuroblastoma cells

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Despite the longstanding clinical use of lithium or valproic acid as mood stabilisers in bipolar disorder, their mechanisms of action are not fully elucidated so far. Recent data from animal experiments have suggested that lithium and valproic acid may interfere with phospholipase A2 activity and synthesis of prostanoids.

Objectives: We investigated whether valproic acid has an influence on prostanoid synthesis in neuronal cell cultures.

Methods: We used the human neuroblastoma cell line SH-N-SK in which the expression of cyclooxigenase-2 (COX-2) and synthesis of prostaglandine E2 (PGE2) is inducible by interleukin-1 (IL-1). We investigated the effects of valproic acid on the expression of COX-2 and PGE2 by PCR, western blot and ELISA assays.

Results: Valproic acid dose dependently inhibited IL-1-induced COX-2 expression and protein synthesis as well as PGE2 release. Valproic acid did not inhibit enzymatic activity of COX-2.

Conclusion: Our results support the hypothesis that valproic acid interferes with the synthesis of prostanoids in neurons. This finding suggests that the inhibition of COX-2 may be a potential target for mood stabilizing substances.

RAGE mediated S100B secretion regulates GFAP assembly

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Objective: Increased levels of the astrocytic, calcium-binding protein S100B have been detected in neurodegenerative, inflammatory and psychiatric disorders. Secreted S100B promotes cell growth, survival and proliferation by binding to the cell surface receptor RAGE expressed on astrocytes and neurons. Protein binding studies reveal that S100B binds calcium-dependently to GFAP destabilizing GFAP intermediate filament assembly. However, the interaction between S100B and GFAP in a cellular system and the involvement of RAGE in the secretion process of S100B are poorly understood.

Methods and Results: (1) A subset of postnatal cultured primary mouse astrocytes were immunoreactive for S100B whereas microglia cells and hippocampal neurons were S100B-negative. S100B knockout cells served as negative controls. GFAP expression was reduced in S100B positive astrocytes. Activation of astrocytes with IL-1beta caused an increased GFAP expression and a time and concentration dependent enhanced S100B secretion measured by ELISA.

(2) RAGE immunoreactivity was present in all astrocytes and neurons. In unstimulated astrocytes S100B and RAGE were uniformly distributed in the cytoplasm. Stimulation with IL-1beta and IFN-gamma caused a redistribution of RAGE at several cell surface membrane spots and S100B was co-localized around RAGE. Due to subsequent secretion of S100B the number of S100B positive cells were reduced after 48 h of stimulation.

Conclusions: Low intracellular S100B levels are necessary for IL-1beta induced glial activation and GFAP incorporation in intermediate filaments. Co-localization of RAGE and S100B during S100B secretion indicates an involvement of RAGE in the secretion process of S100B. S100B secretion facilitates glial activation representing a potentially crucial factor for impaired synaptic plasticity.

L-Tryptophan and inhibitors of L-Tryptophan metabolism influence developing antigen-specific immunity

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Extracellular L-tryptophan and its cellular uptake and intracellular degradation by cells like monocytes and macrophages are crucial regulatory elements in controlling activation and proliferation of immune cell populations, since L-tryptophan is difficult and energy-intensive to synthesize. As L-tryptophan is also the precursor of the neurotransmitter serotonin, its metabolism represents an important crosslink between the immune and the neuroendocrine system. Various functional studies have already investigated the role of L-tryptophan metabolism in different animal models of clinical disease: inhibition of L-tryptophan degradation has been shown to provoke detrimental effects like fetal rejection, allograft rejection or increased severity of autoimmune disease. No study, however, has so far investigated the direct effects of the presence of L-tryptophan and specific metabolic inhibitors on developing adaptive immunity. Here, we show that after injection of ovalbumin (OVA) in incomplete Freund's adjuvant (IFA) in C57.BL/6 mice, the presence of L-tryptophan, the antimetabolite 1-methyl-tryptophan (1-MT) and the L-tryptophan transporter blocker BCH in the adjuvant can influence strength and character of the antigen-specific immune response as measured by ELISPOT. L-Tryptophan alone increased the average frequencies of interferon-gamma-producing cells. Average frequencies of interleukin-2 producing cells were elevated by the presence of all three reagents. In addition, both 1-MT and BCH led to increased average frequencies of interleukin-4 and interleukin-5 producing cells. These results indicate that L-tryptophan metabolism might be a crucial element in the interplay between adaptive and innate immunity in guiding the adaptive immune reaction.

Neuroimmune-Endocrine Network in Psychiatric Disorders

The role of viral infections during neurodevelopment in schizophrenia pathogenesis

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Objective: To provide an overview of the evidence that viral infections during neurodevelopment play a role in schizophrenia.

Methods: The viral hypothesis of schizophrenia is reviewed based on recent findings in virology, immunology, epidemiology, and developmental neurobiology.

Results: There are converging lines of evidence to support both viral and neurodevelopmental origins for schizophrenia. Nevertheless, specific causal links between viral pathogens and schizophrenia have been difficult to discern because of the long latency between the developmental insult and the manifestation of the psychiatric symptoms. The importance of animal models in elucidating latent effects of viral infections on developing neurons is illustrated by recent studies in our laboratory suggesting that virus-induced interleukin-1 causes a delayed loss of neurons in the hippocampus.

Conclusions: Schizophrenia is probably the result of multiple interacting risk factors. Viruses may be acting through pathophysiological pathways that are shared by other environmental triggers. One intriguing hypothesis is that viruses could disrupt the development of inhibitory neurons in the immature brain. This could occur directly, or via the induction of proinflammatory cytokines. We suggest that the loss of inhibition could then set the stage for a slowly evolving excitotoxicity that could then entrain other neurons or circuits into a pathological cascade.

HLA-J Polymorphism in schizophrenia patients

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Objective: There are reciprocal regulations between the central nervous system (CNS) and immune system. Human Leucocyte Antigens (HLA) are important for a proper response against infections and therefore for the interaction between CNS and immune system. Linkage studies and Single-nucleotide polymorphism studies have identified several genes in the HLA region as candidates for schizophrenia associated genes. Our earlier study found that HLA-A10 allele is positively associated with susceptibility to schizophrenia. In order to identify if such schizophrenia-associated genes exist also in the HLA-CLASS Ib region, we have studied HLA-J polymorphism in schizophrenic patients.

Methods: HLA-J was typed by sequencing based typing in 81 schizophrenic patients and 126 healthy controls. HLA-A was typed serologically in the same panel. Statistics were performed using the SPSS program.

Results: We found a decreased frequency of HLA-J M80468/80469 in schizophrenic patients as compared to normal control individuals (9/81 vs. 38/126, Pc = 0.0045 RR = 0.315). An increased frequency of HLA-J M80469/M80469 was also observed in schizophrenia (66/81 vs. 77/126, Pc = 0.01 RR = 2.8). Because HLA-A10 was positively associated with susceptibility to schizophrenia in our previous study, we analyzed HLA-J polymorphisms in carriers of HLA-A10. We noticed an significant increased frequency in carriers of HLA-A10, who were homozygous for HLA-J M80469/M80469 (8/52 vs. 1/68 OD = 12.18, 95% CI = 1.472–100.817, Pearson Chi-square = 8.223, P = 0.0052, Pc = 0.021).

Conclusions: HLA-J polymorphisms contribute significantly to the susceptibility to schizophrenia. The possible explanation of the strong association between the pseudogene HLA-J in the HLA-CLASS Ib region and schizophrenia will be discussed.

TAP1 but not TAP2 genotypes are associated with high risk of schizophrenia

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Objective: Several studies documented an influence of genetic and environmental factors in the pathogenesis of schizophrenia. Linkage analysis points to several chromosomal regions including the HLA cluster on 6p21. The aim of this study was to investigate whether the cross between microbial environmental and immune genetic factors, especially genetic factors responsible for processing (LMP2 and LMP7) and transportation (TAP1 and TAP2) of microbial proteins are associated with schizophrenia.

Methods: 77 schizophrenic patients and 162 healthy control individuals were investigated by ARMS-PCR (LMP2, LMP7), sequence analysis (TAP1, TAP2) and serological HLA class I typing.

Results: No linkage disequilibrium was found between LMP, TAP and HLA alleles. Neither LMP nor TAP2 but TAP1 allele and genotype frequencies were significantly altered: a high significant increase of the TAP1*A1/TAP1*B1 (3.3×10^{-5}, fisher's exact test, two-tailed) genotype frequency was found in the patient panel as well as a reduction of the TAP1*A1/TAP1*B4 genotype. This culminated in a 14.7 fold relative risk for carriers of the TAP1*A1/TAP1*B1 genotype to develop schizophrenia.

Conclusion: Carriers of the TAP1*A1/TAP1*B4 genotype have a 5.5 fold reduced relative risk to develop schizophrenia, carriers of the TAP1*A1/TAP1*B1 genotype have a 14.7 fold increased relative risk. Because TAP*B1 and TAP1*B4 differ in the predicted peptide binding region of the TAP1 molecule, selection of transported and finally presented peptides could contribute to the schizophrenic syndrome by influencing immune response and type of produced cytokines. It has been demonstrated that distortion of cytokine patterns can induce symptoms of schizophrenia.

Immuno-genetic and immuno-phenotypic gender differences in schizophrenic patients

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Objective. Schizophrenia is a heterogenous disorder with a polygenic mode of transmission and additionally contributing environmental factors. An immune-related pathoetiologic mechanism has been proposed. To the extent that there are reliable gender differences in schizophrenia, gender may be a potential factor contributing to both phenomenological and etiological heterogeneity. We therefore investigated single nucleotide polymorphisms (SNPs) in immunologically relevant genes, and antibody titers against potentially neurotropic viruses in a case-control study with respect to gender differences.

Methods: SNPs: The -C589T SNP in the IL-4 gene promotor; the -G308A SNP of the TNF-α gene promotor; the G241A SNP of the ICAM-1 gene. We recruited 262 (113 female, 149 male) schizophrenic patients (33.7 ± 12 years) and 279 (138 female, 141 male) control persons (40.5 ± 15 years). Antibody titers against cytomegalovirus (CMV) and Epstein-Barr virus (EBV) were determined in a subgroup of 87 schizophrenic patients (36 female, 51 male; 30.2 ± 9 years) and 87 healthy controls (39 female, 48 male; 27.8 ± 7.4 years).

Results: In comparison to healthy male control persons, male schizophrenic patients showed a significantly lower frequency of the IL-4–589T allele (p = 0.033) and of the TNF-α –381A allele (p = 0.028), and a higher frequency of the polymorphic ICAM-1 241A allele (p = 0.016), while female patients did not differ from the female control group (IL-4: p = 0.181; TNF: p = 0.526; ICAM-1: p = 0.976).

There was a higher occurrence of positive anti-CMV titers (p = 0.001), but a lower rate of positive anti-EBV titers (p < 0.0001) in male schizophrenic patients. In contrast, female patients did not differ from female controls regarding anti-CMV titers (p = 0.081) and the lower rate of anti-EBV positive titers was less pronounced (p = 0.018) than in male patients.

Conclusions: In sum, male schizophrenic patients differed from the male control group in the immuno-genotypic markers of ICAM-1, IL-4, and TNF-α and in the antibody titers against CMV and EBV, while female schizophrenic patients only showed a reduced occurrence of anti-EBV titers. Thus, our results are in accordance with the hypothesis that gender differences in schizophrenia reflect differential risks for different subtypes of the disorder. More specifically, our results suggest a higher proneness for an immune-related pathoetiology in male schizophrenic patients.

Cyclooxigenase-2 inhibition in schizophrenia: Advances for therapy

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Signs of an imbalance of the type-1 and type-2 immune response have been repeatedly described in schizophrenia. Aside from other findings, a decreased cutaneous skin reaction, e. g. measured by the Multitest Merieux, and decreased levels of soluble ICAM-1, a marker of the type-1 immune response, point to a decrease of the type-1 response. The increased levels of IL-6 especially in treatment resistant and chronic patients are discussed to be associated with an increased type-2 immune response. Prostaglandin E2 stimulates the IL-6 production. Since it is known that cyclooxigenase-2 (COX-2) inhibits the prostaglandin E2 secretion, COX-2 inhibition might also be associated with a decrease of IL-6 and in particular with a re-balancing of

the type-1/type-2 immune response. Therefore we analysed the clinical effects of COX-2 to schizophrenia. A prospective, randomised, double-blind add-on study of Celecoxib in schizophrenic patients showed significant favourable effects of add-on treatment of COX-2 inhibitors to risperidone. However, this effect was most pronounced during the weeks 2, 3 and 4, while during the 5th week this effect was attenuated. Therefore we replicated the study over an 8 week period in 40 schizophrenic patients. First results of this double-blind, randomised, add-on replication study will be discussed.

Interaction between the HPA-axis and the innate immune response system in depression

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Objective: The innate immune system and the HPA system are closely related and both have been reported to be altered in major depression. It still remains unclear, whether the changes in immune parameters are a secondary consequence of HPA-dysfunction and whether these changes are of any clinical relevance for the patients.

Methods: To gather more detailed information about underlying regulatory mechanisms we measured the immunomodulatory properties of glucocorticoids in a series of experiments in depressive patients and healthy controls. Plasma cytokine levels were determined following oral dexamethasone intake and during experimental changes in plasma cortisol levels. Additionally, in depressive patients we examined cytokine levels and HPA function measured by combined DEX/CRH test in parallel.

Results: Small doses of dexamethasone reduced the plasma levels of TNF-a and IL-6 both in healthy subjects and in patients with major depression, and also an experimental increase in plasma cortisol levels had a comparable effect. Moreover, in depressive patients the circulating cytokine levels were influenced by the amount of HPA system activation: TNF-a levels were significantly lower in patients with a more robust HPA-activation.

Conclusions: To conclude, the important interaction between glucocorticoids and the innate immune system seems to be intact in depressive patients, which fits very well to the fact, that these patients usually do not suffer from a clinically significant impairment in immune function. On the other hand some small immunological abnormalities as they have been reported in depressed patients might in part be explained by HPA-dysfunction.

The Mineralocorticoid receptor system and Tumor-necrosis factor-alpha secretion in Major Depression

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Objective: Since corticosteroid receptor and immune alterations have been shown in major depressive disorder (MDD), we here studied the mineralocorticoid receptor (MR)-modulated production of the cytokine tumor-necrosis factor-alpha (TNF-alpha) in major depressive disorder.

Methods: The production of TNF-alpha in human peripheral blood cells of patients with MDD and healthy controls was examined after in vitro incubation with the MR-antagonist spironolactone (SPIRO), the MR/GR agonist cortisol and the GR-agonist dexamethasone.

Results: In samples of controls, blockade of the MR by SPIRO caused an increase in the secretion of TNF-alpha, whereas a decrease was evoked by mixed MR and GR activation. In the samples of the patients, no alterations of TNF-alpha production were detectable after MR blockade.

Conclusions: Our data suggest that the MR system affects the secretion of TNF-alpha under normal conditions, but not in MDD. Thus, these data further support the assumption of a corticosteroid receptor dysfunction in MDD, leading to an increase in TNF-alpha levels under certain conditions with consecutive alterations in the psychopathology and the endocrine system.

CD26-like enzymatic activity provides a novel molecular basis for anxiety regulation via differential degradation of NPY

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Objective: The enzyme and binding protein dipeptidyl-peptidase IV (DPPIV; CD26) is involved in immune cell activation and in cell adhesion processes. Furthermore, CD26 exerts a unique enzymatic specificity in cleaving dipeptides from chemokines and neuropeptides such as neuropeptide Y (NPY). This results in NPY3–36, which lacks affinity for the NPY Y1 receptor subtype. The NPY Y1 receptor mediates anxiolytic-like effects of NPY. Thus, differences in CD26-like activity may result in a differential degradation of NPY with consecutive differences in the anxiety-like phenotype. Interestingly, CD26 is known to be upregulated during inflammation. This may contribute to behavioural changes associated with sickness behavior and depression.

Methods: The wildtype-like F344 substrain [F344/Crl(Por)] was compared with the CD26-deficient F344 rat substrains [F344/DuCrj(DPPIV-) and F344/Crl(Ger/DPPIV-)]. NPY was administered intracerebroventricularly at different doses (0.0, 0.2, 1.0 nmol) and behavioral responses were screened in tests for anxiety-related behaviors (elevated plus maze and social interaction).

Results: NPY administration was found to be dose-dependently more potent in CD26-negative substrains in exerting anxiolytic-like effects (increased social interaction time in the social interaction test) and sedative-like effects (decreased motor activity in the elevated plus maze).

Conclusions: These data demonstrate for the first time a more potent Y1 receptor-like behavioral response profile of centrally applied NPY in a rat model of CD26-deficiency. Since these results provide direct evidence that NPY-mediated effects in the CNS are modulated by CD26-like enzymatic activity, a novel molecular basis for the regulation of anxiety is provided.

Decreased expression of proinflammatory cytokines in whole blood cell cultures of Alzheimer patients

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Objective: There is growing evidence for a role of a locally induced chronic inflammatory response in Alzheimer's disease (AD). Recent data on systemic immunological changes could up to now not confirm the hypothesis of a general inflammatory process in AD. We investigated the expression of proinflammatory cytokines in blood of AD-patients in order to examine possible systemic alterations of immunological activity in AD.

Methods: 22 patients and 26 healthy aged volunteers were included in the study. Whole blood samples were cultured and stimulated with LPS and PHA for 48 h respectively 96 h. Cytokine concentrations in the supernatants were measured using commercially available high sensitive ELISA kits. Statistical evaluation was done using the non-parametric Wilcoxon test.

Results: Levels of TNFα which is secreted both by T helper cells and macrophages on an 48 h activation by LPS were significantly reduced in AD patients with $p < 0.0147$. Also, the concentration of IFNγ on stimulation with PHA, secreted by T helper cells, and IL-6 were decreased, though without statistical significance.

No correlation was found between the decrease and the degree of dementia.

Conclusions: A reduced secretion of IFNγ and TNFα reflects an attenuated activity of T helper (TH) 1 cells and, subsequently, of the monocyte/macrophage system in Alzheimer's disease. This could be the result of the stimulation of the hypothalamic-pituitary-axis

(HPA) which may lead via negative feedback to a peripheral immunosuppression. On the other hand, an underlying deficiency of the innate immune response – as it is observed in the aged immune system – may result in a deficient removal of debris and subsequently in an increase of Aβ depositions leading to the local inflammatory reactions. We postulate a premature immunosenescence as one pathogenetic factor in AD leading to an attenuated removal of pathological Aβ protein in the AD brain.

Peripheral Neuroimmune Interactions

Th1 and Th2 immune responses in the periphery activate distinct neuronal pathways in the CNS

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The incidences of immunoregulatory disorders (allergies, autoimmunity and inflammatory bowel disease) have been increasing rapidly in rich developed countries. Diminishing exposure to harmless environmental microorganisms, that were ubiquitous throughout mammalian evolution (The "Old Friends"), is leading to deficient priming of regulatory T cells (Treg). The "Old Friends" prime Treg via components of the innate immune system, and polymorphisms of several of these (NOD2, TLR2) influence the likelihood that an individual living in the modern clean environment will develop an immunoregulatory disorder. This concept has led to the identification of microbial materials that drive Treg maturation *in vivo* or *in vitro*, and to successful clinical trials in allergy. However the use of Th1 and Treg-inducing microbial material in man has revealed unexpected changes in mood, coping and pain perception, and in animals the same Th1 + Treg-inducing material causes changes in cfos expression in serotonergic neurones in parts of the brain involved in mood and in coping behaviour, and alters the pattern of cfos expression seen following a minor surgical stress. A quite different pattern was seen in animals preimmunised with a Th2 bias. The signals from the peripheral immune system pass via vagal sensory afferents. We postulate that the "Hygiene Hypothesis" should be expanded into the area of psychiatric disease, because changes in cytokine balance, secondary to changes in Treg activity (they secrete IL-10) can alter the pattern of background signalling from the periphery to the brain. Correlations between allergies and depression in twin studies, and the observation that vagal stimulation can alleviate depression, add support to this view.

Astrocytes protect the CNS by downregulating T cell responses via CTLA-4 upregulation

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Objective: Astrocytes are the first cells that T cells invading the CNS encounter while crossing the blood-brain barrier. In neuroinflammatory diseases, astrocytes can express major histocompatibility complex class II (MHC-II) and B7 molecules and, thus, function as antigen-presenting cells (APCs) in the CNS, possibly contributing to inflammation. Here we investigate their influence on T helper cell activation, proliferation and effector function.

Methods: Primary murine astrocyte cultures were prepared from the cortex of neonatal B10.PL mice and cultured for 10–12 days. Th1 and Th2 cell lines were established from MBP-T cell receptor-(TCR-) transgenic mice. Flow cytometry was used for the analysis of T cell activation and proliferation. Cytokine expression in T cell-astrocyte cocultures was determined by ELISA.

Results: Astrocytes suppressed Th1 and Th2 cell activation (exemplified by CD25 upregulation), proliferation and effector function, including production of interleukin (IL)–2, IL-10, and indirectly interferon (IFN)-gamma. In contrast, activation-induced T cell receptor (TCR) downregulation remained intact. The inhibition of T cell proliferation induced by astrocytes was mediated by upregulation of CTLA-4. This upregulation was induced independently of cell-cell contact between astrocytes and T cells but not of prior T cell activation.

Conclusion: CTLA-4 has been shown to play a role in multiple sclerosis and other autoimmune diseases. Here we show its upregulation induced by astrocytes as a novel mechanism of securing the immune privilege of the CNS with implications for treatment of T cell-mediated inflammation.

Expression profiles of dopamine receptors on immune cells

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Objective: Dopamine is one of the principal neurotransmitters in the central nervous system and acts via five G-protein coupled receptor subtypes, classified into D1-like (the D1 and D5) and D2-like (the D2, D3, and D4) receptor subtypes. Radio-ligand binding studies had shown that dopamine receptors are also expressed on human leukocytes. The aim of this study was to elucidate which receptor subtypes are expressed on the different leukocyte populations.

Methods: Leukocytes of healthy donors were isolated and subdivided into neutrophils, monocytes, B cells, natural killer cells and CD4 + and CD 8 + T lymphocytes by cell sorting. The expression of all five dopamine receptor subtypes on these highly purified leukocyte populations was studied by Real Time RT-PCR.

Results: RT-PCR analysis showed that D1 and D2 receptors are not expressed in leukocytes. The D3 receptors are expressed in T cells and natural killer cells, the D4 receptors are expressed only on CD4 + T cells. The D5 receptor subtype is expressed in all lymphocytes subtypes as well as neutrophils and monocytes.

Conclusion: These results show that each leukocyte population has its distinct expression profile of dopamine receptors indicating specialized functions of individual receptors. Preliminary experiments involving gene array and gene expression studies suggest functions of these receptors on immune cells.

Catecholamines increase Interferon-gamma production of CD4 and CD8 positive T cells following preincubation with Interleukin-2

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Objective: The density and regulation of beta2-adrenergic receptors (beta2-R) differs between CD4 and CD8 positive T cells. To evaluate the impact of IL-2 on beta2-R function, CD4 and CD8 positive T cells were preincubated with IL-2 before activation and stimulation of beta2-R.

Methods: CD4 and CD8 positive T cells from healthy subjects were isolated by negative selection using the MACS technology. T cells were stimulated by anti-CD3- and anti-CD28-antibodies together with Interleukin-2. Aliquots of CD4 and CD8 positive T cells were incubated with high (5 ng/ml) or low dose (0.5 ng/ml) IL-2 for 16 or 72 h before stimulation. Co-incubation was carried out with epinephrine (10 microM). IFN-gamma and IL-10 were determined in the culture supernatant of stimulated cells after 48 h with a sandwich ELISA. Intracellular cAMP of CD4 and CD8 positive T cells was measured after incubation with 10 µM isoproterenol using an ELISA.

Results: Co-incubation with epinephrine decreased IFN-gamma

and IL-10 production of activated CD4 and CD8 T cells. In contrast, epinephrine increased IFN-gamma synthesis in CD4 positive T cells preincubated with high dose, but not low dose IL-2. The synthesis of IL-10 was not altered, however. The isoprotereol induced increase of cAMP was abrogated following pre-incubation with IL-2.

Conclusions: Epinephrine differentially mediates the synthesis of IFN-gamma and IL-10 in human T cells pre-treated with IL-2. Possible mechanisms include the alteration of signal transduction via cAMP.

Transcription pattern analysis of adrenergic immunoregulation in vivo

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Neuroimmune interactions are highly complex, which may contribute to the low reproducibility of in vivo results. A way to tackle this problem may be molecular pattern analysis instead of the collection of single parameter data, as has been largely done so far. Here we investigated the effects of a 12 hour norepinephrine (NE) treatment on the transcription of 200 immunologically relevant genes in the mouse. We used a cDNA chip (Memorec, Germany) containing the sequences of the major cytokines, their receptors and all CD-antigens of the mouse. Three independent experiments were performed, in each of which two Balb/c mice received subcutaneously implanted retard tablets containing 1.7 mg NE, while control animals were treated with placebo tablets. In each experiment splenic RNA was prepared after 12 hours, transcribed into fluorescently labeled cDNA, and hybridized on two cDNA arrays. Consistent results were obtained with a group of five genes involved in the activation of the monocyte/macrophage compartment and in leukocyte migration. In the NE-treated animals four genes (CXCR4, VCAM1, IL-1R2, CD 14) were upregulated (2–8 fold) as compared to the placebo group, whereas CCR3 was found downregulated (< 0.5 fold). These alterations in gene expression profiles were confirmed using reverse transcriptase Real Time PCR performed with individual splenic RNA preparations from seven animals per experimental group. These first in vivo results of transcription pattern analysis point to the relevance of catecholamines in the regulation of inflammatory reactions. Experiments are under way to determine kinetics, receptor specificity and functional relevance of this effect.

beta2-Adrenoceptor-Mediated Inhibition of Growth and Activation in Cultured Human Intestinal Mast Cell

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Objective: To characterize beta2-adrenoceptor (b2AR)-mediated modulation of growth and activation of human intestinal mast cells (MC).

Methods: MC were isolated from intestinal surgery specimens, purified up to 95 % using the MACSä system, and subsequently cultured for 14–21 days in the presence of stem cell factor (50ng/ml) with or without addition of adrenaline, noradrenaline, or salbutamol. Mediator release was induced by IgE receptor crosslinking following incubation with mAb22E7 in the presence or absence of b2AR agonists. Histamine in the supernatant was measured by RIA. MC adhesion assays were performed using fibronectin coated plates and human umbilical vein endothelial cells (HUVEC). Actin polymerization in the activated MCs was assessed by flow cytometry following labeling with fluorescent phallotoxin.

Results: b2AR agonists dose-dependently reduced MC growth to 23.4 % (salbutamol), 25.3 % (adrenaline), and 40.5 % (noradrenaline) of control conditions (all at 10^{-6}M, n = 5). Activation-induced histamine release was inhibited to 4.1 % (salbutamol), 21.6 % (adrenaline), and 35.8 % (noradrenaline) of control conditions in a dose-dependent fashion (all at 10^{-6}M, n = 5). Moreover, presence of b2AR agonists strongly inhibited MC adhesion to HUVECs as well as activation-induced adhesion to fibronectin (n = 3). To address possible mechanisms of b2AR agonist effects in MCs we also investigated mAb22E7-induced actin polymerization in MCs that was abolished by salbutamol and significantly reduced by adrenaline (n = 2).

Conclusion: Our data show that b2AR agonists profoundly regulate human intestinal MC survival and function. Such findings may be of importance for our understanding of intestinal neuroimmune functions and of therapeutic strategies used in allergic disorders.

The sympathetic nervous system stimulates collagen-induced arthritis (CIA) in the induction phase and inhibits CIA in the late effector phase in DBA-1 mice

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Objective: The sympathetic nervous system plays an immune modulatory role during immune reactions. The data on systemic sympathectomy and arthritis are contradictory concerning immune stimulating and immune suppressing effects. We show in the collagen induced arthritis model in DBA-1 mice that systemic sympathectomy mediates a bimodal effect depending on the time point of sympathectomy.

Methods: Arthritis was induced in female DBA-1 mice by intradermal injection of 100 µg of bovine type II collagen (CII). In one group sympathectomy was done 7 days prior to arthritis induction by intraperitoneal injection of 6-hydroxydopamin (6-OHDA, 80 mg/kg KG) on three following days (early sympathectomy = eSx). These animals received further injections of 6-OHDA i. p. every 10 days to sustain sympathectomy. Another group of animals received 6-OHDA at day 59 post induction of arthritis as mentioned above (late sympathectomy = lSx). To confirm the effect of late systemic sympathectomy with another method, we used anti-dopamin-beta-hydroxylase (DBH) antibodies conjugated with saporin (5µg/animal) i. p. at day 40. The antibody is taken up by the neuron and transported retrogradely, followed by the liberation of saporin in proximity of the sER, where it irreversibly inactivates rRNA leading to neuronal death.

Results: The cumulative arthritis probability according to the log-rank test in the Kaplan-Meier statistics was higher in lSx (= 100 %) compared to eSx (= 46.9 %) (p = 0.032).

The arthritis score of both experimental groups was higher in lSx compared to eSx (general linear model: $p < 0.001$). In a further analysis we investigated different phases of the arthritis course: From day 28 (first symptoms appeared) to 59 (before late sympathectomy), arthritis scores were higher in lSx (acts as a control group until 59 days) compared to eSx ($p \leq 0.001$). Arthritis scores in lSx rose markedly after sympathectomy between day 60 and 85 (R-rank = + 0.450, $p < 0.001$) whereas arthritis scores in the eSx-group did not show any significant changes over time. The anti-DBH-antibody treated animals showed a significant increase in arthritis score after sympathectomy ($p < 0.01$).

Conclusions: The sympathetic nervous system has immune stimulating properties at the very beginning and immune suppressive properties at more advanced stages in CIA. In this chronic inflammatory disease, it appears that at early and late time points different immune mechanisms play a dominant role which are either supported or inhibited by the sympathetic nervous system.

Tumor Necrosis Factor Upregulates μ Opioid Receptor Transcription via Nuclear Factor kappa B

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Endogenous opioids and cytokines act within a network connecting the nervous and the immune system. Here, we report that in primary T lymphocytes, Raji B cells, U937 monocytes, primary polymorphonuclear leukocytes and mature dendritic cells the proinflammatory cytokine tumor necrosis factor (TNF) induces transcription of the μ opioid receptor gene, which is silent in unstimulated immune cells. In primary neuronal, μ opioid receptor expressing cells, TNF upregulates μ opioid receptor mRNA levels. Using selective in vivo disruption of possibly involved transcription factors with decoy oligonucleotides, nuclear factor kappa B was identified to be responsible for TNF-induction of the gene in immune cells. Among six putative nuclear factor kappa B elements on the gene's promoter, three cis-active elements were identified at nt –2174, –557 and ?207, using transfection experiments of reporter genes, electrophoretic mobility shift assays and in vivo binding studies with decoy oligonucleotides. An allelic variation within the ?557 element significantly reduced its trans-activating potency, which may affect regulation of the gene in individuals carrying this mutation. It is known that the opioid system is activated during inflammation as a physiological feedback mechanism to attenuate inflammatory pain. Via TNF-induced upregulation of μ opioid receptors the effects of opioids in neurones innervating inflamed tissue could be maximized, which may contribute to inflammation-induced analgesia. In the immune cells, opioid receptors are the structural basis for the diverse immunoregulatory effects of opioids. This study contributes to the understanding of the multiple immuno-neuronal interactions and describes an additional function of TNF within this network.

Primary sensory neurons in dorsal root ganglion function as endotoxin sensors via the toll-like receptor 4

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Objective: Bacterial lipopolysaccharide (LPS) besides evoking a profound immune response is also known to induce hyperalgesia. To determine whether LPS can affect sensory neurons directly we examined the expression of the Toll-like receptor genes 4 (TLR4), and 2 (TLR2) and CD14 in rat dorsal root ganglion (DRG).

Methods: RT-PCR analysis was performed on laser-capture microdissected neuronal and non-neuronal cells from frozen DRG sections of normal and LPS-treated animals. Dual *in situ* hybridization (ISH) with cRNA probes for substance P (SP) and the vanilloid receptor (VR1) was employed to identify the neuronal phenotypes.

Results: Constitutive expression of TLR4 could be demonstrated in microdissected DRG neurons by RT-PCR. TLR4 transcripts were detected in small to medium-sized primary afferent neurons. Dual ISH revealed the peptidergic SP- and VR1-positive cells as the major neuronal phenotypes expressing TLR4. After LPS treatment, TLR4 mRNA levels were not significantly changed, but CD14 mRNA and TLR2 mRNA levels increased dramatically. Strong ISH signals for CD14 and for TLR2 were seen in non-neuronal cells, but not in neurons.

Conclusions: Our results strongly suggest that pain transmitting neurons may function as innate immune sensors by direct activation via TLR4 and, thus, point to the pivotal role of the peripheral sensory nervous system as part of the first line defense coacting with the immune system in the detection and control of microbial infections.

The effects of Vagus Nerve Stimulation on peripheral pro- and anti-inflammatory cytokines in humans

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Objective: Vagus Nerve Stimulation (VNS) is a novel therapy in resistant epilepsy and depression. The mechanism of action of VNS is assumed to be due to modulation of deep brain structures via its afferent connections. We hypothesise that an additional mechanism may occur via efferent vagal action on (particularly hepatic) cytokine synthesis and secretion as well as altered cytokine activation of peripheral vagal afferents. This novel study in humans explores this possibility by examining cytokine levels in patients pre- and post-VNS

Methods: Patients (n = 11) with resistant depression were sampled in the weeks prior to and following insertion of a vagus nerve stimulator. Sufficient time (three months) elapsed for recovery from surgery. No medication changes were made during the course of the study. Plasma was frozen. High sensitivity ELISA kits to measure basal IL-1B, IL-6, TNFa, TGF-b, and IL-10 were performed.

Results: There were highly significant increases in the levels of IL-6, TNF-A and TGF-B. Data on IL-10 and IL-1B are awaited, as is analysis of simultaneously obtained cerebrospinal fluid.

Conclusions: VNS appears to be associated with marked changes in pro- and anti-inflammatory circulating cytokines. This finding was contrary to our expectations and may relate to a complex interplay of opposing anti-inflammatory efferent and pro-inflammatory afferent effects, as will be discussed.

Neuroendocrinology and Immune Function

Inflammatory control of the hypothalamo-pituitary-adrenal axis

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In recent years there has been increasing interest in the possibility that the gases nitric oxide (NO) and, even more recently, carbon monoxide (CO), can function as intercellular messengers in a completely novel manner, providing a medium of cellular communication quite distinct from the classical neuroregulators. These agents will diffuse according to their physicochemical characteristics, in evanescent 'puffs' with spherical limits, unconstrained by formal cell boundaries. Most work has concentrated on their roles in inflammation and control of the vascular tree, but there are now extensive data attesting to a complex involvement in the neuroendocrine axis, particularly the hypothalamo-pituitary-adrenal (HPA) axis. We have established that both NO and CO are powerful and specific inhibitors to the release of corticotrophin releasing hormone (CRH) and vasopressin from the rat hypothalamus in vitro, and in particular in attenuating stimulation of these peptides by cytokines. The enzymes for both gases are also present in the nuclei of origin of these releasing hormones. Further studies demonstrated that exposure of hypothalami to lipopolysaccharide in vitro led to an unexpected fall in both CRH and vasopressin. However, while blockade of NO synthase merely attenuated the fall in CRH, while CO antagonism led to a highly significant stimulation of vasopressin release. Blockade of NO synthase in vivo leads to the enhancement of ACTH and corticosterone release in response to inflammatory stressors, while antagonism of CO synthesis leads to an exaggerated rise in endotoxin-stimulated vasopressin, but not of corticosterone. These results suggest that both NO and CO are important components of the HPA axis, and may act to counter-regulate stimulation of this axis in response to inflammatory stimuli. CO in particular is a major regulator of vasopressin release. In the human, it seems likely that a failure of formation of CO in acute porphyria may be responsible for some of its clinical manifestation, such as paroxysmal hypertension and the syndrome of inappropriate antidiuretic hormone.

I am most grateful to all my collaborators in these studies particularly Mary Forsling (Dept. of Physiology, UMDS, London, UK), Pierluigi Navarra (Dept. of Pharmacology, Catholic University, Rome, Italy), and Alfredo Costa (Institute of Neurology, IRCCS C. Mondino, Pavia, Italy).

Prolactin, growth hormone and corticosteroid receptor antagonists modulate T helper 1/T helper 2 in-vitro cytokine production in human T cells

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Objective: The release of pituitary hormones and corticosteroids is a subject to profound regulation by sleep. In addition these hormones are known to be involved in the regulation of the immune responses. Here, we examined their role for in-vitro production of T helper 1 (Th1) and Th2 cytokines. We hypothesize that increase in prolactin and growth hormone together with decrease in cortisol as seen during early nocturnal sleep could be responsible for a shift towards Th1 cytokines during this time.

Methods: Whole blood was sampled in the morning after regular sleep from 15 healthy humans and was activated in-vitro with ionomycin and two concentrations of phorbol myrestate acetate (PMA, 8 (low) and 25 (normal) ng/ml) in the absence or presence of prolactin, prolactin antibody, GH, glucocorticoid receptor antagonist RU-486, or mineralcorticoid receptor antagonist spironolactone. Production of T cell derived cytokines – interferon-gamma (IFN-gamma), interleukin-2 (IL-2), interleukin-4 (IL-4), and tumour necrosis factor-alpha (TNF-alpha) – was measured at the single cell level using multiparametric flow cytometry.

Results: Generally effects were more pronounced after stimulation with 8 than 25 ng/ml PMA. The following reached significance ($p < 0.05$). Prolactin (vs. prolactin antibody) increased TNF-alpha+ and IFN-gamma+ producing CD4+ and CD8+ cells and IL-2+ CD8+ cells. Compared with control prolactin increased IFN-gamma+ CD4+ cells. GH remained ineffective. Ru-486 (vs. control) increased TNF-alpha+ and IFN-gamma+ producing CD4+ and CD8+ cells and IL-2+ CD8 cells. Spironolactone (vs. control) strongly increased IFN-gamma+ and IL-2+ producing CD4+ and CD8+ cells as well as TNF-alpha+ CD8+ cells. No effects on IL-4+ CD4+ cells were observed, while the IFN-gamma/IL-4 ratio shifted towards Th1 after Ru-486 and spironolactone.

Conclusions: The results indicate possible role of prolactin and corticosteroid receptor inactivation for enhancing Th1 cytokines after activation in-vitro with low concentrations of PMA.

Effect of hyperprolactinemia on glucocorticoid-induced changes in immune parameters in vivo.

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Objective: Recent in vitro experiments suggest that immunomodulating hormone prolactin (PRL) may interfere with glucocorticoids action on immune cells. Controversy remains whether interaction between PRL and glucocorticoids occurs also in vivo. In the present investigation we studied short-term effect of pharmacologic hyperprolactinemia on hydrocortisone-induced redistribution of immune cells and production of Th1/Th2 cytokines.

Methods: Single dose of hydrocortisone (40 mg per os) was administered to eleven healthy female volunteers one hour after domperidone (10 mg per os) or placebo administration. Immune cells subsets and expression of adhesion molecules was assessed by flow cytometry at baseline, 4 and 6 hours after hydrocortisone administration. Intracellular staining of interleukin 4 (IL-4) and interferon-gamma (IFN-gamma) production in CD4 + lymphocytes after phorbol myristate acetate and ionomycin stimulation was performed at the same time points.

Results: Hydrocortisone administration was followed by significant increase of cortisol levels, numbers of leukocytes, granulocytes and percentage of CD16+, CD19+, CD11a+, CD11a+ CD8+, CD11b+, CD11b+ CD8+ cells. The number of lymphocytes, monocytes, percentage of CD3+, CD4+, CD4+/CD8+ ratio, CD62L+, CD54+ and CD54+ CD16+ cells decreased, while the percentage of CD8+ was unaffected. Domperidone administration resulted in a significant increase in PRL concentrations. During hyperprolactinemia hydrocortisone-induced increase of CD11b+ CD8+ cells was significantly ($p < 0.05$) attenuated, at 4 hours. Hydrocortisone-induced changes in other immune parameters remained unaffected. No significant changes in intracellular production of IL-4 and IFN-gamma in CD4+ lymphocytes were observed after single dose of hydrocortisone alone or during hyperprolactinemia.

Conclusions: The study shows attenuated hydrocortisone-induced increase of CD11b+ CD8+ cells in peripheral blood of healthy females during hyperprolactinemia. Our in vivo observations may suggest that proposed short-term interactions occur between PRL and glucocorticoids on selected immune functions. Further studies are needed for confirmation of these results.

Functional role of bacterial endotoxin receptor (Tlr4) in pituitary pathogenesis

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Members of the Toll receptor (Tlr) family are critically involved in the innate immune response after bacterial infections. Effects of bacterial endotoxins (LPS) of gram-negative bacteria are mediated predominantly by Tlr 4. We have shown that LPS stimulated IL-6 secretion in pituitary folliculostellate (FS) cells via Tlr4 receptors and the p38-alfa- MAP kinase/nuclear factor-kB pathway. However, we detected Tlr4 expression also in corticotrophic (AtT20) and gonadotrophic (alfaT3–1) cell lines suggesting that Tlr4 is also expressed in endocrine epithelial pituitary tumour cells. Therefore we looked for Tlr4 expression in 3 normal human pituitaries and in 67 pituitary adenomas. In pituitary adenomas, Tlr4 mRNA expression was observed by ISH in 24 adenomas. mRNA expression of Tlr4 was not found in 43 pituitary tumors by ISH. In the latter, no Tlr4 protein expression could be detected by ICH in 39 adenomas: only 30 % of hormone-inactive adenomas were positive, 47 % somatotothrophic, 33 % lactotrophic and 50 % corticotrophic and thyreotrophic. Immunohistochemical investigations and in situ hybridisation confirmed abundant Tlr4 expression in normal and tumoral pituitary epithelial cells. Since most pituitary adenomas produce IL-6, the effect of LPS on intratumoral IL-6 secretion was investigated in primary cell cultures of pituitary adenomas. The IL-6 release was enhanced dramatically by LPS in a dose- and -time-dependent manner in 9 IL-6 producing adenomas. The specific p38-alfa-MAP kinase inhibitor SB203580 dose-dependently suppressed the LPS-induced IL-6 secretion indicating that the signal transduction pathway might be similar to FS cells. Moreover, dexamethasone dose-dependently inhibited LPS-induced IL-6 production in all pituitary adenoma cell cultures. Since IL-6 is supposed to represent a growth factor of pituitary adenoma cells, we speculate that bacterial endotoxins could support pituitary tumour progression during infectious or inflammatory processes. However, elevated cortisol levels may counteract LPS-induced adenoma progression by suppressing intratumoral IL-6 production.

Stress and Addison's Disease evidence of the biological function of acute cortisol increases in response to stress?

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Objective: Despite intensive research endeavors, the biological function of acute cortisol increases in response to stress is a matter of controversy. One possibility to answer this question is to examine adrenalectomized animals. Looking for a human model we investigated patients suffering from Addison's Disease (AD).

Methods: Addison patients (AD-NaCl) and healthy control subjects (CG-NaCl) were exposed to the 'Trier Social Stress Test' (TSST). Salivary free cortisol, plasma catecholamine levels, NF-kappaB binding activity, as well as glucocorticoid (GC) sensitivity of IL-6 production were measured before and after stress. A second group of AD patients was treated with hydrocortisone intravenously (AD-HC) immediately after TSST.

Results: Untreated AD patients had no cortisol response to TSST. Norepinephrine levels increased in all groups, while epinephrine levels were only detectable in the healthy controls. GC sensitivity decreased significantly immediately after TSST and returned to baseline 1 hour later only in the CG-NaCl and the AD-HC group; placebo treated patients showed a prolonged decrease. NF-kappaB binding activity increased in healthy controls one hour after stress and returned to baseline 1 hour later. The AD-NaCl group showed increased NF-kappaB activity 2h after TSST. Interestingly, the NF-kappaB response pattern was restored by HC injection.

Conclusions: These preliminary results show the relevance of the cortisol response to acute stress as one important contributor to homeostasis. These first data revealed also that additional HC medication after stress could help to improve the medicinal therapy of patients suffering from Addison's disease and could thereby reduce their risk of additional inflammatory diseases.

Central Catecholamines Effect on Leukocyte's Proliferation is Completely Abolished by Prior Peripheral Sympathectomy

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Objectives: To analyze the role of peripheral catecholamines in the immunosuppressive effect induced by central catecholamine depletion.

Methods: The proliferative capacity of splenocytes from rats with central catecholamines depletion by 6-hydroxidopamine (6-OHDA) i. c. v. treatment with prior peripheral sympathectomy (6-OHDA i. p.) were analyzed together with appropriate vehicle controls.

Results: Central catecholamines depletion by 6-OHDA i. c. v. treatment in rats induced an inhibition of splenic and blood lymphocyte proliferation and splenic cytokine production and expression (interleukin-2 and interferon-gamma) seven days post-injection. In addition, central treatment with 6-OHDA reduced the percentage of splenic and peripheral blood NK (CD161+) cells, and T-cytotoxic (CD8+) cells in peripheral blood.

Furthermore, the reduction in splenocyte proliferation is not associated with alterations in glucocorticoid plasma levels but is completely abolished by prior peripheral sympathectomy.

Conclusions: These data demonstrate a crucial role of central and peripheral catecholamines modulating peripheral immune functions.

The prevalence of Semaphorine 3C, a repellent of the sympathetic nervous system, is increased in the synovial tissue of patients with rheumatoid arthritis

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Objectives: We have recently shown a distinct reduction of the sympathetic nervous system in the synovial tissue of RA patients (Miller et al., FASEB J 2000;14:2097–2107.). The presence of selective nerve repellents i. e. semaphorine, could be responsible for the observed reduction of the sympathetic innervation in the synovial tissue of patients with RA.

Methods: In situ hybridizations with Dig-labeled RNA probes directed against different semaphorines showed that only semaphorine 3C mRNA was present in the synovial tissue of all investigated patients (RA, N = 8; Osteoarthritis (OA), N = 3). In order to investigate the abundance of semaphorine 3C protein (S3C) in 10 RA, 10 OA and 5 control synovial tissue, a polyclonal antiserum directed against S3C was produced by immunizing a rabbit with two peptides specific for S3C.

Results: In RA and OA, all in-situ hybridizations showed the presence of S3C mRNA but no other investigated semaphorine (e. g. against sensory nerve fibers). A quantitative analysis of the S3C protein staining showed an increased density of S3C positive cells in the synovial tissue of RA patients (339 ± 65 cells/mm^2) in comparison to OA patients (168 ± 27 cells/mm^2; $p = 0.029$ vs RA) and controls (126 ± 26 cells/mm^2; $p = 0.027$ vs RA). Interestingly, a negative correlation existed between density of S3C-positive cells and CD3-positive T cells in RA (R-Rank = –0.648, $p = 0.043$) and OA (R-Rank = –0.802, $p = 0.005$).

Conclusions: These findings suggest that semaphorine 3C, which is selectively directed against sympathetic nerve fibers, could be responsible for the reduced sympathetic innervation observed in RA patients. The inability of the sympathetic nervous system to re-innervate the synovial tissue of RA patients could be contributing to the chronic nature of RA.

Immune-endocrine interactions during the development of pulmonary tuberculosis

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Objective: To explore the role of immune-endocrine interactions for the development of Tuberculosis (TB) in patients with different degrees of lung involvement.

Methods: 37 HIV-negative TB patients were enrolled. Severity was determined according to the degree of pulmonary involvement: Mild (Mi), Moderate (Mo) and Severe (Se). None of the patients were under treatment. Age- and sex-matched healthy volunteers without contact with TB patients were used as Controls (Co).

Results: The plasma levels of IFN-gamma, IL-10 and IL-6 showed a positive correlation with the severity of TB and significant differences were found between TB patients and Co, and also among the TB subgroups. No differences were found in TNF-alfa plasma levels. Cortisol plasma levels were higher in TB patients compared to Co and differed significantly from this group. In contrast, DHEA plasma levels were remarkably low in TB patients and diminished proportionally to the severity of the disease. The Cortisol/DHEA ratio was significantly higher in TB patients and increased significantly according to the severity of the TB. Blood levels of Growth Hormone (GH) were remarkably increased in TB patients; group in which Prolactin levels were also significantly elevated. Both hormonal increases were proportional to the severity of the disease.

Conclusions: In patients with progressive disease, endocrine-immune interactions result in an altered adrenal function as reflected by an increased Cortisol/DHEA ratio, that might serve to exacerbate infection and hence perpetuate disease. The elevation of PRL and GH levels could represent an unsuccessful attempt to counteract the anti-inflammatory effects and the Th2-shift that is favoured by the increased Cortisol/DHEA ratio.

Stress, Behaviour, and Immune Function

Stress, behaviour and Immune function – a review on exercise and immunity

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Cytokines are considered as part of the immune system. Recent studies, however, demonstrate that cytokines also play multiple roles in regulating substrate metabolism and muscle adaptation. Recently, researchers have shown that production of interleukin-6 (IL-6) (independently of muscle damage) is produced by contracting skeletal muscles and induces signal transduction in skeletal muscle. The biological roles of IL-6 are many: 1) activation/inhibition of the transcript of metabolic genes; 2) Induction of lipolysis (independent of other known lipolytic mediating hormones); 3) Inhibition of TNF-induced insulin resistance and 4) Induced cortisol production, thereby influencing leucocyte trafficking during prolonged exercise. Production of IL-6 and hence exercise-induced leucocyte changes are highly influenced by substrate availability. The transcription rate for IL-6 in muscle nuclei isolated from muscle biopsies during exercise is rapid and this is further enhanced when muscle glycogen content is low. In addition, carbohydrate supplementation during exercise has been shown to inhibit the release of IL-6 from contracting muscle, but not hepatic clearance in humans. Carbohydrate loading has also been found to inhibit exercise-induced leucocyte changes, suggesting that this effect is at least partly mediated by an inhibition of muscle-derived IL-6 and cortisol production. The interactions between IL-6 and anti-oxidants are complex. Supplementation with vitamin C and the vitamin E isoform, gamma-tocopherol, inhibits exercise-induced increases in muscle-HSP72, but not muscle-IL-6mRNA. This indicates that HSP does not induce the production of IL-6 in muscle. Supplementation with vitamin C and the vitamin E isoform, alpha-tocopherol, has been shown not to influence transcription of IL-6, but rather inhibit the release of muscle-IL-6 and consequently increase in cortisol levels during exercise. The clinical consequences of dietary modification of the cytokine and leukocyte responses to exercise may include both risk of acquiring infectious diseases, training adaptation and insulin resistance.

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Immune conditioning by psychological stressors in mice

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Objective: Insufficient inflammatory reactivity causes deficient antibacterial defences whereas an exessive hyperinflammatory response can be lethal. Both situations are the result of different regulatory circuits. Psychological stress has been implicated in conditioning immune responses. To investigate the neural-immune interactions we examined the immune alterations after combined acoustic and restraint stress in mice.

Methods: Female BALB/c mice were exposed to the stressors for two hours twice daily for five successive days. After the last session immune phenotyping and functional assays were performed in different compartments of the body. In parallel, the survival rate in a LPS shock model and an experimental polymicrobial sepsis (Colon Ascendens Stent Peritonits, CASP) was monitored.

Results: Stressed mice responded with increased serum cortisol levels ($P < 0.005$), lymphocytopenia ($P < 0.01$), signs of cellular activation (CD69-expression; $P < 0.01$), and reduced ex vivo T cell proliferation ($P < 0.01$). In lymphoid tissue a huge number of apoptotic cells were detectable ($P < 0.01$). This was accompanied by a massive granulocyte invasion ($P < 0.01$). Peritoneal macrophages, but not spleen cells, failed to respond to endotoxin ex vivo with regular TNF synthesis ($P < 0.005$). Moreover, the ex vivo IL10 inducibility of splenocytes of stressed animals was increased ($P < 0.01$). Stress-exposed animals were less sensitive to high doses of endotoxin and showed a reduced mortality rate after CASP in comparison to unstressed mice ($P < 0.01$).

Conclusions: Chronic psychological stress exposure induces a hypoinflammatory bias and therefore results in an immune conditioning which alters immunocompetence.

Changes in the modulation of whole blood natural killer cell activity as a consequence of long-term colony housing in F344 rats

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Social confrontation modulates natural killer cell numbers and in vitro cytotoxicity and substantially increases NK-sensitive tumor metastasis. However, acute stress may pose only a minor threat to the individual's health, whereas the risk of contracting a disease can increase dramatically with stressor duration.

Therefore we investigated two newly formed colonies of F344 rats, each containing 5 male-female pairs. Blood samples were taken one week before, as well as one, three and six weeks after colony formation for flow cytometric analyses and a 4 hour whole-blood chromium-release assay.

Daily behavioral observations revealed the formation of a stable despotic dominance hierarchy within the first week. At the end of the experiment, elevated adrenal masses and doubled adrenal tyrosine hydroxylase activities indicated severe social stress in all subdominant males. Throughout the experiment, colony males were characterized by a stagnation in body mass gain, pronounced granulocytosis and lymphopenia in comparison to undisturbed home cage controls. In contrast, NK cell numbers were only elevated after weeks one and three. In addition, whole blood NK activity was compromised only after week one, but elevated – mainly due to the increased NK cell numbers – after week three. After six weeks in the colony, both NK cell numbers as well as activities no longer differed from controls.

These findings demonstrate that colony housing has profound and long lasting effects on the immune system. However, the temporal dynamics of the effects on NK cells differ from those of other immune cell subtypes and seem to be more transient.

Endocrine and cytokine responses to standardized physical stress in multiple sclerosis

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Background: Since the earliest descriptions psychological and physical stress has been considered a controversial but potentially important factor in the onset and course of multiple sclerosis (MS). During recent years it has become clear that MS patients benefit from physical exercise as performed in aerobic training. As acute exercise has profound effects on immune and endocrine parameters we studied endocrine and immune response to standardized physical stress in MS within a study of aerobic training.

Methods: Fifteen MS patients completed an eight-week aerobic training program, 13 patients were part of a wait-control group. 20 healthy controls were recruited as well. A step-by-step bicycle ergometry was performed to determine individual exertion levels. For the endurance test patients exercised at 60 % VO2max for 30 minutes.

Blood samples were drawn before, directly after and 30 minutes after completion of the exercise.

Results: Heart rate and lactate increased in all groups ($p < 0.0001$). We furthermore saw significant increases in endocrine parameters (epinephrine, norepinephrine, ACTH and β-endorphin; all $p < 0.0001$) in healthy individuals and in MS patients but without a differential effect. Whole-blood stimulated production of IFN-gamma (IFNγ) was induced similarly in all groups ($p < 0.01$). TNF-alpha (TNFα) and IL-10 were less inducible in MS patients (trend).

Conclusion: From these data we could not demonstrate a proinflammatory immune deviation in response to physical stress in MS. The observed trend of hyporesponsive TNFα and IL-10 responses in MS warrants further investigation.

Effect of low grade endotoxemia on cognitive functions in young and elderly people

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Objective: Chronic low-grade inflammation is found even in healthy elderly subjects. A link between chronic inflammation and cognitive impairment in the elderly has been suggested. Short-term immune activation transiently impairs memory and learning in young individuals. On the other hand, IL-1receptor knock-out mice have impaired memory consolidation, indicating that pro-inflammatory cytokines are involved in optimal cognitive function. We investigated the effect of acute very low-grade endotoxemia on cognitive function in young and elderly healthy subjects.

Methods: A bolus of endotoxin (0.2 ng/kg), calculated to induce 4 and 10 fold increases in plasma-TNF and -IL-6, respectively, was administered to 12 young (20–30 yrs) and 12 elderly (65–80 yrs) male subjects.

The study was carried out as a double-blind, fully balanced, randomised, cross-over investigation. Neuropsychological assessment and blood sampling were performed at baseline, and at t = 1.5; 5.5 and 24 hours. Neuropsychological assessment included testing of declarative memory and learning, working memory, attention and executive functions. Mood was assessed using a Visual Analogue Mood Scale.

Results: The project is ongoing with the last experiment scheduled for September 3rd. Subjective symptoms and body temperature do not differ between placebo and endotoxin infusion. During endotoxemia all subjects show a transient increase on leucocyte count. Preliminary analyses reveal a tendency to verbal and non-verbal memory functions being improved 5.5 hours following endotoxin administration, whereas attention and executive functions were not affected. Data analysis on age-effect and relation to degree of inflammation will be finalised and presented at the GEBIN symposium.

Sleep modulates T helper 1/T helper 2 cytokine balance in humans

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Objective: Recent human studies suggested a supportive influence of regular nocturnal sleep on immune responses (e. g. after vaccination). We hypothesize here that sleep could ease such responses by shifting the balance between T helper 1 (Th1) and T helper 2 (Th2) cytokine activity towards Th1 dominance thereby favouring cellular over humoral responses to infection.

Methods: We compared the Th1/Th2 cytokine balance in 14 healthy men during nocturnal sleep from 2300 to 0700 h and while remaining awake during the same interval, in a within-subject crossover design. Production of T cell derived cytokines – interferon-gamma (IFN-gamma), interleukin-2 (IL-2), interleukin-4 (IL-4), and tumour necrosis factor-alpha (TNF-alpha) – was measured after stimulation with ionomycin and phorbol myrestate at the single cell level using multiparametric flow cytometry. Also, several immunoactive hormones (e. g. prolactin, growth hormone (GH), cortisol) were measured, the release of which is regulated by sleep.

Results: Compared with wakefulness, early nocturnal sleep induced a shift in the Th1/Th2 cytokine balance towards increased Th1 activity, as indicated by an increased ($p < 0.05$) ratio of IFN-gamma/IL-4 producing T helper cells. However, the Th1 shift was only of moderate size and replaced by Th2 dominance during late sleep ($p < 0.05$). Though unexpected, the most pronounced effect of sleep was a robust decrease in TNF-alpha producing CD8 + cells. Both prolactin and GH were distinctly increased during sleep ($p < 0.001$) while cortisol concentrations tended to be reduced.

Conclusions: Our data suggest a modulating effect of sleep on Th1/Th2 cytokine balance which can have an effect on the course of infection or vaccination. Sleep presumably acts via specifically regulating hormone release.

Prenatal immune activation alters offspring's endocrine and immune responses in adulthood

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Maternal stress disrupts the development of the fetal hypothalamic-pituitary-adrenal (HPA) axis resulting in a hyper-responsive axis in adulthood. A common stressor during pregnancy is maternal infection. This study investigated whether prenatal exposure to bacterial endotoxin would result in adult offspring that were hyper-responsive to stress. Given the links between the HPA axis and the immune system it was hypothesised that should such alterations occur, they should also be associated with changes in the immune systems of adult offspring. Pregnant dams were injected with bacterial endotoxin (salmonella enteriditis (SE), 200 µg/kg, i.p) or the vehicle on gestational days 16, 18 and 20. Serum endocrine (corticosterone) and immunological (interleukin-1(IL-1)) responses to a physiological stressor (20 min restraint stress) and an immunological stressor (SE, 200 µg kg, ip), were assessed in adult male offspring. Offspring of endotoxin-treated (ET) dams exhibited significantly increased corticosterone levels at baseline and immediately following restraint stress ($p < 0.05$) when compared to offspring of saline-treated (ST) dams. Offspring of ET dams also exhibited significant increases in corticosterone at 90 and 360 min. post-endotoxin treatment in adulthood ($p < 0.05$). These same offspring exhibited significantly higher plasma IL-1 responses to endotoxin administration ($p < 0.05$), but not to restraint stress. These findings add to our understanding of the long-term consequences of immune activation during pregnancy and may add to our understanding of the genesis of stress-related illness in later life.

Neuro-Endocrino-Immune Interactions in Neurology and Psychiatry

In Vitro Adrenergic Modulation of Cellular Immune Functions in Experimental Autoimmune Encephalomyelitis (EAE)

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Objectives: To analyze the effects in vitro of alpha- and beta-adrenergic agonists on splenocyte proliferation and proinflammatory cytokine production in splenocytes and peritoneal macrophages in different stages of EAE.

Methods: Splenocytes and peritoneal macrophages (MF) were harvested from rats in the acute phase of EAE and in remission, and from controls. The beta-agonist terbutaline, the alpha$_1$-adrenergic agonist methoxamine, and the alpha$_2$-agonist UK-14304 were added in concentrations of 10^{-6} to 10^{-9}M together with ConA (in splenocytes cultures) or lipopolysaccharide (LPS) (in MF cultures). TNF-alpha and IFN-gamma contents in supernatant were determined together with splenocytes proliferation.

Results: In response to terbutaline, a significant and concentration-dependent suppressive effect on TNF-alpha production by LPS-stimulated peritoneal MF was observed in all groups, with a maximal suppression at the highest terbutaline concentration to an average of 26.9 ± 1.9 % of original production. UK-14304 also significantly diminished MF TNF-alpha production in a concentration-dependent manner. However, compared to EAE remission animals and controls, EAE acute phase rats were resistant to the suppressive effect of UK-14304 on MF TNF-alpha production. In contrast, methoxamine did not affect TNF-alpha production by MF in either group. Both IFN-gamma and TNF-alpha production by ConA-stimulated splenocytes were significantly suppressed by terbutaline. While no group differences were found in the TNF-alpha response, we observed significantly reduced terbutaline-induced inhibition in EAE acute phase and remission animals. No agonist effects on ConA-stimulated splenocyte proliferation were found in either group, and no effects of alpha-adrenergic agonists were found on ConA-stimulated IFN-gamma or TNF-alpha production by splenocytes.

Conclusions: Alpha$_2$-adrenergic sensitivity of macrophages is decreased in the acute phase of EAE, while beta$_2$-adrenergic modulation appears to remain unaffected. Regulation of splenic IFN-gamma by beta-adrenergic pathways during EAE may be less efficient than in healthy animals. Resistance to inhibition of IFN-gamma cannot simply be explained by reduced adrenergic receptor signaling, and might therefore be located at another level, e. g. transcription factors.

Substance P induced gene expression pattern in astrocytes

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Substance P (SP) has been shown to be involved in the etiopathology of different clinical conditions such as neurogenic inflammation, pain, asthma and emesis. More recently, SP has been postulated to be involved in the etiopathology of affective disorders.

Objectives: We were interested in the potential effects of substance P on gene expression patterns in cells of the CNS.

Methods: Human U373 MG cells, a cell line known to express substance P receptors (NK-1), were treated with substance P and gene expression patterns investigated by macro-array technique.

Results: We found various genes to be up- or down-regulated by substance P in U373 MG cells. We found the corticotropin releasing factor receptor (CRFR) to be strongly up-regulated by substance P which was confirmed by using RT-PCR and Western-blots.

Conclusion: Our data suggest a link between substance P and the HPA axis including the CRF system.

Experimental infection of TNFα-Transgenic mice with the neurotropic Borna disease virus

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Objective: TNFα-transgenic mice were infected experimentally with the neurotropic Borna disease virus in order to analyse the effect of neuronal overexpression of TNFα on an immunopathological virus infection of the CNS.

Material: TNFα-transgenic CBA7J x C57/Bl6 mice were infected neonatally with a mouse-adapted strain of Borna diesease virus (BDV). Nontransgenic mice were treated similarly. BDV-infected mice died or were euthanized between 42 and 62 days p. i. The brains were stained with H&E or used for immunohistology for the detection of the viral nucleoprotein, microglial activation and reactive astrogliosis.

Results: Neither TNFα-transgenic mice nor nontransgenic mice showed obvious clinical signs. The TNFα transgenic mice inoculated with BDV developed a nonpurulent meningoencephalitis with perivascular and parenchymal mononuclear immune cell infiltrates, strong microglial activation and reactive astrogliosis. Virus antigen was detected throughout the brain. In brains of nontransgenic BDV-infected mice, a similar viral distribution was detected, but strong inflammatory lesions and microglial activation were absent. They may develop a mild reactive astrogliosis.

Conclusions: Neuronal overexpression of TNFα does not affect viral spread in brains of BDV-infected mice, however is necessary for a strong activation of microglial and astroglial cells and invasion of immune cells into the CNS. This might indicate that TNFα-expression in the CNS makes mice more susceptible for virus induced immunopathological reactions in the CNS.

Cognition in the early stage of Multiple Sclerosis – a neuropsychological test battery

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Objective/Background: Cognitive function is often impaired in patients with MS. 45–50 % of all MS patients suffer from cognitive dysfunctions. Frequently quality of life is affected. 22–31 % of the patients present memory and learning deficits, 22–25 % show deficits in attention and 12–19 % have executive dysfunctions. There is only little knowledge about cognitive impairment especially in the early stage of MS and its treatment.

The aim of the study was to find out specific patterns of cognitive dysfunctions in the early stage of MS.

Methods: We assessed 21 MS-patients (7 male, 14 female, RRMS, PPMS), with an onset of neurological symptoms within two years (mean 15 months) preceding the study and 22 healthy controls.

The neuropsychological test battery consisted of tests for assessing memory and learning deficits (TAP, WMS-R, VLMT, RVDLT, ROC-Figure), attention deficits (TAP), executive functions (SLP, WCST-Nelson) and spatial functions (ROC-Figure), completed with a depression scale (ADS) and intellectuell capabilities (WST). The examination of each patient and healthy control was randomised.

Results: Our findings show few cognitive dysfunctions in the MS-patients. As a result we can clearly demonstrate a decreased reaction time, nonverbal memory deficit and planning deficit in most of the patients compared with healthy controls.

Conclusions: We found that even in the early stage of MS cognitive dysfunctions are evident. In the next step we do a study to compare cognitive functions at the point of diagnosis of MS with the extent of cerebral lesions in MRI and MR-Spectroscopy as well.

Severe affective psychosis in a Borna virus seropositive patient improved by CSF filtration

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Objective: Borna disease virus (BDV) infection may induce mild autoimmune encephalitis underlying affective and schizophrenic type psychoses (Bechter 2001). CSF filtration (CSFF) was effective in Guillain Barré syndrome, a neurological autoimmune disorder (Wollinsky et al. 2002). Experimental CSFF inquired therapy resistant psychoses (Bechter et al. 2000), however question of placebo was raised, this a recent single case appears elusive.

Methods: CSFF was performed as in neurological patients. 300 ml CSF filtrated daily over 5 days (lumbar catheter, automatic pump, PALL-CSF1-E-Filter). Psychopathology measured by HAMD, MADRS, BPRS, PD-S; test performance by ZVT, d2-test, reaction times and finger tapping, repeatedly over 10 months before and after CSFF.

Results: A 65 year-old-patient with affective psychosis progressively deteriorated over one year despite (inpatient) treatments, including antidepressants, mood stabilizers and augmentation therapies. Patient fulfilled (November 2001) criteria of an ongoing CSFF study in therapy resistant affective psychoses (approved by ethical committee, University of Ulm), but preferred, before CSFF, another lithium and amantadine augmentation trial, however worsened further. When CSFF was performed in March 2002, patient status improved rapidly, patient remaining oriented since, gradually improving further (psychopathology, test performance). Medication was unchanged 4 weeks before filtration, during filtration and nearly 8 weeks after filtration. Catamnesis of more than one year shows further recovery.

Conclusion: In a rare case of rapidly progressive affective psychosis in a BDV seropositive patient, eventually presenting with demented status over months rapid improvement under CSFF was observed. Placebo effect is excluded because of severe psychopathology before CSFF.

A Th2-shift in a subgroup of schizophrenia: primary results of an immunological exploration

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Goal: Various immune alterations in schizophrenia have been described for decades. Among the immunological findings in schizophrenia research, a hyper- or hypo-production of different cytokines belonged to the most frequently reported indications. Despite of a few controversies, the majority showed that an imbalance between these two T-helper systems with a shift towards the Th2-system might occur in a subgroup of schizophrenia. This study attempts to discover subgroups of schizophrenia with a Th2-shift.

Subjects: 67 schizophrenic patients (37 men and 30 women) and 77 healthy controls (42 men and 35 women) were included in the study. The diagnostic subgroups for 54 of the 67 schizophrenic patients were: 3 disorganized, 6 catatonic, 41 paranoid, 2 schizophreniform, 1 residual, and 1 brief psychotic disorder. All patients and controls were free of acute or chronic infections or any severe medical diseases. None of them were under any allergic medications in the past month. All patients met DSM-IV criteria for schizophrenia.

Methods: Whole blood from all subjects was diluted with medium [1, 16], then stimulated with PHA (end concentration = 5 µg/ml), and incubated at 37°C/5 % CO_2 for 48 h. The supernatants were kept frozen at –80°C until assessment. The Th1/Th2 parameters measured consist of IFN-γ, IL-4, and IL-10. Cytokine concentrations were determined by Cytometric Bead Assay (CBA) kits. A Th2 shift is defined as a decreased IFN-γ/IL-4 as well as a reduced IFN-γ/IL-10 ratio. Statistics applied were χ^2, t-test, cluster centre analysis, Mann-Whitney-test, and one-way ANOVA.

Results: (1) Like most previous studies, the schizophrenia patients as a whole group, compared to the healthy controls, revealed a reduced IFN-γ in vitro production. No significant differences were found between both groups concerning their IL-4 and IL-10 in vitro production. Both IFN/IL4 and IFN/IL10 ratios merely showed a trend to significance. (2) The schizophrenic patients were first divided into 3 subgroups according to their IFN-γ/IL-4 values: a high IFN/IL4 (M = 3674; SD = 1857; N = 6), a middle high IFN/IL4 (M = 1284; SD = 303; N = 14), and a low IFN/IL4 (M = 418; SD = 186; N = 47). Both the high and the low IFN/IL4 groups were very significantly different from that of healthy controls (M = 1283; SD = 1418; N = 77). (3) Then the patients were again classified into three IFN/IL10 subgroups: a high IFN/IL10 (M = 82; SD = 12; N = 7), a middle one (M = 42; SD = 9; N = 19), and a low group (M = 17; SD = 6; N = 41). Similar to the results from the IFN/IL4 clusters: the high and the low IFN/IL10 groups also significantly differed from their healthy counterparts (M = 39; SD = 26; N = 77). (4) Altogether, there were a total number of 51 schizophrenic patients, who had low IFN-γ/IL-4 or IFN-γ/–10 ratios. Thirty-seven of those 51 schizophrenia had a low IFN-γ/IL-4 as well as a low IFN-γ/IL-10 ratio. (5) 19 of the 41 paranoid, 1 of the 3 disorganized, and all the patients of the other diagnostic subgroups exhibited a low IFN/IL4 as well as a reduced IFN/IL10 ratio, i. e. a shift to Th2.

MIF, a proinflammatory cytokine and anti-glucocorticoid, is elevated in schizophrenia

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Objective: Schizophrenia has been associated with infections during neurodevelopment as well as immune/endocrine alterations in adults. Macrophage migration inhibitory factor (MIF) is a unique cytokine and anti-glucocorticoid molecule that is induced by infections and psychological stress. Moreover, MIF undergoes marked regulatory changes at the maternal-fetal interface, and it has been implicated in dopamine metabolism. The objective of this study was to determine if plasma MIF is abnormal in schizophrenia, and to ascertain whether MIF is changed as patients switch from conventional to atypical antipsychotic medications.

Methods: MIF levels in plasma samples obtained from patients with schizophrenia (n = 9) or schizoaffective disorder (n = 4) were assessed longitudinally as the patients switched from conventional antipsychotics to atypical (risperidone or olanzapine) medications. MIF levels were compared with values from mentally healthy controls (n = 21) matched for age and sex with the patient (SCH) group.

Results: The mean MIF level in the control group was 10.4 ng/ml (SEM = 2.4), compared to 51.4 ng/ml (SEM = 9.6) in SCH patients taking conventional antipsychotic medications ($p < 0.005$ by t-test). Moreover, after patients switched to atypical medications, MIF remained elevated, 55.4 ng/ml (SEM = 9.3) ($p < 0.0004$ by t-test).

Conclusions: MIF is markedly elevated in SCH patents regardless of whether they are taking conventional or atypical antipsychotics. This could indicate that diverse antipsychotic agents are capable of increasing MIF, and suggests that MIF could constitute a therapeutic target for schizophrenia. Another possibility is that immunogenetic abnormalities predispose some individuals to heightened MIF responses. Higher MIF levels could precede schizophrenia, possibly playing a role in neurodevelopmental abnormalities and/or maternal-fetal responses to infections.

Interference of psychopharmacological agents with substance P-induced gene expression and expression of the receptor for substance P

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The neuropeptide substance P (SP) has been hypothesized to be involved in the etiopathology of affective disorders. This hypothesis is based on the findings that SP-receptor antagonists have antidepressant effects in depressed patients and that SP may worsen mood.

Objectives: We investigated the effect of different psychopharmacological agents including antidepressants, neuroleptics, and mood stabilizing agents on SP-induced expression of interleukin-6 and expression of the SP-receptor.

Methods: The human cell line U373 MG, western blots, ELISA, and Taqman-PCR were used.

Results: We found that the mood stabilizer valproic acid (VPA) dose-dependently inhibited SP-induced IL-6 synthesis via inhibition of protein kinase C epsilon activation. Furthermore, VPA as well as imipramine and haloperidol downregulated the expression of the

substance P receptor (neurokinin(NK)–1-receptor) as assessed by real time PCR and Western blotting.

Conclusion: Whether both mechanisms contribute to the clinical effectiveness of these substances has to be evaluated in further studies.

Body weight and the plasma levels of leptin and endogenous immune modulators during treatment with carbamazepine or lithium

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Several psychopharmacological agents induce weight gain. Recent studies suggest that the fat-cell-derived hormone leptin and the tumor necrosis factor-α (TNF-α) cytokine system are pathophysiologically involved.

To assess whether carbamazepine and lithium have effects on weight and circulating levels of cytokines we weekly measured plasma levels of TNF-α its soluble receptors sTNF-R p55 and p75, interleukin 6 (IL-6), leptin and weight in 25 inpatients receiving lithium (N = 10) or carbamazepine (N = 15) during the first four weeks of treatment.

Carbamazepine treatment led to a significant increase in weight ($p = 0.002$) across four weeks, preceded by a significant increase of TNF-α ($p = 0.021$) plasma levels in the first week of treatment. Patients treated with carbamazepine gained a mean weight of 1.3 kg within four weeks of treatment. Plasma levels of sTNF-R p55 and p75, leptin and IL-6 levels were not affected significantly during the first week of treatment.

Lithium treatment led to a mean increase of body weight of 1.5 kg within 4 weeks, but this increase did not reach statistical significance, the increase of body weight was preceded by a significant increase of TNF-α ($p = 0.005$), sTNF-R p55 ($p = 0.046$) and leptin ($p = 0.018$) plasma levels in the first week of treatment. Plasma levels of sTNF-R p75 and IL-6 levels were not affected significantly during the first week of treatment.

The present results support the notion that the activation of the TNF-α cytokine system is an early marker of weight gain induced by psychotropic agents. The dosage during the first week of carbamazepine treatment seems to play a role regarding the amount of further weight gain.

Effect of clozapine and haloperidol on microglia in vitro

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It was shown in peripheral paradigms that clozapine and haloperidol differentially modulate secretion of cytokines and soluble cytokine receptors (e. g. TNF-alpha, soluble TNF receptors (sTNFR's), IL-6), and it was suggested that this immunomodulation is involved in the mechanism of action [1, 2]. Microglia, originating from the same source as peripheral blood monocytes [3], might be the major target in the CNS. Thus, we investigated the effect of haloperidol and clozapine on the secretion of TNF-alpha, sTNFR's and IL-6 as well as on cell death in vitro in microglial cell lines.

According with the data on peripheral cells published to date, we hypothesize that in microglial cells clozapine modulates secretion of the above mentioned cytokines while not promoting cellular death whereas haloperidol induces cellular death while not modulating cytokine secretion.

A human and a murine microglial cell line were set up with and without lipopolysaccharide (LPS) and combined with dilution series of clozapine or haloperidol. Cell viability was detected by MTT assay and cytokine secretion was measured by commercial ELISA in supernatants.

The investigation is ongoing. Preliminary results support that clozapine significantly modulates secretion of sTNFR-p75 and TNF-alpha in our in vitro paradigm whereas haloperidol does not. If CNS born cells respond differentially to antipsychotic substances by modulating cytokine secretion this would further support the hypothesis that immunomodulation is a mechanism of action of these drugs.

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Effects and side effects of adoptive immunotherapy in patients with schizophrenia and depression

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Objective: Many scientists assume that unrecognized or underestimated chronic infections play a major role in the disease development of mental diseases. Chronic infections can disarrange the balance of cytokines used for communication between immune cells. Gradual elimination of chronic infections by adoptive immunotherapy should therefore restore distorted cytokine balances and improve the status of mental illnesses.

Methods: Patients were treated with in vitro CD3-activated immune cells and with immune cells primed with activated immune cells (cascade primed "CAPRI" cells).

Results: Positive results will be reported on patients with schizophrenia (N = 4) and depressive disorders (N = 3) and compared with previous results (Wank, Medical Hypotheses 2002; 59:154–8). Three of four schizophrenic patients (two males, age 28 and 30y, the female 28y) resumed occupational activities within short time of treatment after several years of occupational interruption. The fourth patient (46y) lost aggressive behavior against his mother and acknowledged parental efforts. All three patients with depressive syndromes (one male, 68y, two females, 50y and 63y) received professional psychiatric treatment for several years. In the two older patients a complete remission occurred months after adoptive treatment was finished. Relatives of both patients confirmed that the patients have not required drugs for four and six years respectively. The third patient as well substantially improved ("I regained creativity and body strength") although treated now only the fourth week. It is, however, too early for final judgement.

Conclusions: Adoptive immunotherapy can be recommended as adjuvant therapy in patients with schizophrenia and depressive syndromes.

T- and B-lymphocytes in subjects with paranoid schizophrenia in acute psychotic episode and the course of the treatment

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Introduction: Schizophrenia has been observed to be associated with alterations of the immune system. In this study we monitored B- und T-lymphocytes in the course of antipsychotic treatment.

Methods: 23 patients diagnosed with an acute exacerbation of paranoid schizophrenia were tested before and during treatment with antipsychotics. Percentages of B- und T-lymphocytes were measured by FACS analysis (anti-CD3 Ab for T-cells, anti-CD19 Ab for B-cells). 20 healthy volunteers served as controls.

Results: In the acute state of psychosis a significant elevation of the B-lymphocyte fraction was observed, while the percentage of T-lymphocytes was decreased. These values levelled to those of the control group in the course of treatment.

Conclusion: The findings in the acute state of schizophrenia – which consist in a suppression of the cellular immunity and a coexisting dominance of the humoral immunity – are described as typical for the effect of catecholamines on the immune system and could be seen as a non-specific stress reaction or an effect of the altered metabolism of neurotransmitters. On the other hand the observed changes in the immune system could be due to infectious diseases or autoimmune processes provoking the symptoms of schizophrenia.

The astroglial protein S100B is increased in chronic schizophrenic patients with predominant negative symptoms

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Objective: S100B, a calcium binding cytokine produced by astroglial cells, evolves paracrine and autocrine effects on neurons and glial cells. It regulates the balance between proliferation and differentiation in neurons and glial cells affecting protective and apoptotic mechanisms. Post mortem studies demonstrated a deficit in synapses and dendrites in brains of schizophrenics. Recent studies showed increased S100B levels in medicated acutely psychotic patients with schizophrenia as well as unmedicated or drug naive schizophrenics. One study reported a positive correlation between negative symptoms (cognitive impairment, social and emotional withdrawal, affective flattening) and S100B.

Methods: S100B serum levels (quantitative immunoassay) and psychopathology (PANSS) were examined at intake, after 12 and 24 weeks of standardized treatment in 98 chronic schizophrenic patients with predominant negative symptoms.

Results: Compared to age- and sex-matched healthy controls the schizophrenic patients showed significantly increased S100B concentrations on admission, after 12 and after 24 weeks of treatment. High PANSS negative scores were correlated with high S100B levels. Regression analysis including psychopathology subscales and S100B identified negative symptomatology as the predicting factor for S100B.

Conclusions: S100B is not only elevated in an acute stage of disease but remains increased for at least half a year after acute exacerbation. Considering psychopathology negative symptomatology appears to be the predicting factor for the S100B concentration. This might indicate that S100B in schizophrenic patients either promotes apoptotic mechanisms itself or is released from astrocytes attempting to repair a degenerative or destructive process.

Autoantibodies to Serotonin (5-HT) in Serum of Patients with Psychiatric disorders

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Objective: Autoantibodies to serotonin were first detected in fibromyalgia (FMS) and appear to be characteristic for this disease. FMS is often associated with psychiatric disorder. Preliminary results then proved occurrence of these antibodies in psychiatric disorder without FMS also. The question is as to whether these antibodies could indicate an autoimmune etiology of psychiatric disorders or are an epiphenomenon maybe useful as a biochemical marker of these disorders.

Methods: Antibodies to serotonin in serum were investigated by ELISA in patients with paranoid schizophrenia (N = 27), schizoaffective psychosis (N = 38), depression (N = 67), Alzheimer's disease (N = 21), chronic alcoholism (N = 43), rheumatoid arthritis (N = 25), multiple sclerosis (N = 16), and in healthy volunteers (N = 60). Diagnosis was done according to ICD 10.

Results: Increased antibody reactivity to serotonin was found in schizoaffective psychosis, chronic alcoholism, and rheumatoid arthritis. Decreased antibody reactivity to serotonin was found in multiple sclerosis and depression.

Conclusions: These anti-serotonin antibodies belong to the class of so-called natural antibodies. Alterations of these antibodies could indicate a disturbance of the immune system. Natural autoantibodies could act as an autoimmune defence against intruding antigens with mimicry of important epitopes. It is possible that these antibodies could also influence receptor function. We could demonstrate anti-serotonin receptor antibodies also.

Perhaps these antibodies could be a biochemical marker for schizoaffective psychosis or alcoholism.

Multitest Merieux in schizophrenic patients and controls

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Introduction: Immunological abnormalities have long been implicated in the pathogenesis of schizophrenia. There is evidence that Type-1 mediated immune reactions may be deficient in schizophrenia patients. We therefore hypothesized that the type-IV delayed skin hypersensitivity reaction, which is mediated by the Type-1 immune reaction as well, is attenuated in schizophrenia patients.

Methods: A prospective case-control study was performed to assess skin reactivity of patients versus healthy controls after the application of defined antigens. A standardized device (Multitest Merieux) with seven different antigens (Tetanus, Diphtherie, Streptokokkus Candida, Tuberkulin, Proteus u. Trichophyton) was applied intracutaneously; after 48 hours, skin reactions were quantified as the average of the transversal and longitudinal diameters.

Results: Schizophrenia patients showed attenuated positive skin reactions (diameter ≥ 2 mm) to antigen stimulus when compared to healthy controls.

Conclusions: Type-1 mediated cellular immunity was significantly attenuated in schizophrenia patients. This is consistent with other results observed in schizophrenia patients. Possible therapeutical implications are being discussed.

Peripheral Neuro-Immune Interaction, Neuro-Endocrinology, and Behaviour

Neuroendocrine and Cellular Immune Responses to Nutrient Challenge In Women with Irritable Bowel Syndrome (IBS) Compared to Ulcerative Colitis (UC) and Healthy Controls

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Objective: To investigate the neuro-immune axis in irritable bowel syndrome (IBS) by analyzing the neuroendocrine and cellular immune responses to nutrient load compared to UC in remission and healthy controls (HC).

Methods: In the fasting state and 20, 40, 70 and 100 minutes following nutrient load, blood samples were collected and cardiovascular recordings were accomplished in 15 female IBS patients, 12 women with UC in remission and 15 healthy women. Plasma norepinephrine, cortisol, and blood pressure and heart rate responses were analyzed. The distribution of peripheral leukocytes and lymphocyte subpopulations and the in vitro production of tumor necrosis factor alpha (TNF-alpha) and interleukin 6 (IL-6) after whole blood stimulation with lipopolysaccharide (LPS) were analyzed.

Results: IBS patients demonstrated significantly greater postprandial increases in plasma norepinephrine and systolic blood pressure compared to UC and controls. A postprandial redistribution of circulating leukocytes and lymphocyte subpopulations was observed in all

groups, including significant increases in the numbers of leukocytes and granulocytes and significant decreases in the numbers of monocytes, T-cells and natural killer (NK) cells. However, IBS patients demonstrated significantly greater postprandial increases in leukocytes and granulocytes, while changes in the numbers of monocytes and NK cells were significantly diminished compared to controls. IBS and UC pts failed to show the postprandial decrease in the in vitro TNF-alpha production observed in controls. Postprandial norepinephrine concentrations were negatively correlated with NK cell numbers in IBS patients ($r = 0.58$, $p < 0.05$) but not controls or UC patients. In the fasting state, IBS and UC pts demonstrated significantly reduced TNF-alpha concentrations compared to controls, and IBS pts showed reduced numbers of $CD3^-CD16^+CD56^+$ and $CD3^+CD8^+$ lymphocytes compared to controls and UC pts.

Conclusions: Autonomic responses to nutrients are markedly enhanced in IBS. The absence of similar findings in UC suggests that this phenomenon is unique to the pathophysiology of IBS. Decreased fasting numbers of peripheral natural killer and T-helper cells, diminished postprandial redistribution of these cells, along with markedly decreased basal production of the proinflammatory cytokine TNF-alpha, provides first evidence that cellular immune functions are altered in IBS and UC in remission. Thus, disturbances of the brain-gut axis may also involve the neuro-immune axis in patients with functional and inflammatory bowel disorders.

The role of leptin in hypoandrogenicity in patients with systemic lupus erythematosus and rheumatoid arthritis

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Objective: Hypoandrogenicity is a common phenomenon in obesity and in chronic inflammatory diseases such as systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA). Adrenal androgens such as androstenedione (ASD) and dehydroepiandrosterone (DHEA) sulphate (DHEAS) are low which partly depends on the influence of TNF in chronic inflammatory diseases. Since leptin is stimulated by TNF and leptin is associated with hypoandrogenicity in non-inflammatory conditions, we aimed to study the interrelation between serum levels of leptin and adrenal steroids in SLE and RA.

Methods: In a retrospective study with 30 patients with RA, 32 with SLE, and 54 healthy control subjects (HS), we measured serum levels of leptin, ASD, DHEA, and 17-hydroxyprogesterone (17OHP) by ELISA and serum levels of cortisol by radioimmunoassay.

Results: In SLE and RA but not in HS, serum levels of ASD correlated negatively with serum levels of leptin ($p < 0.01$). This was independent of prior prednisolone treatment in patients with SLE ($p = 0.013$) and tended to be independent of prednisolone in patients with RA ($p = 0.067$). In a partial correlation analysis, this interrelation remained significant after controlling for daily prednisolone dose in both patient groups. Furthermore, in both patient groups, serum leptin levels were negatively correlated with the molar ratio of serum ASD/serum cortisol and serum ASD/serum 17OHP, and leptin levels were positively correlated with the molar ratio of serum DHEA/serum ASD.

Conclusions: The negative correlation of serum leptin and ASD or, particularly, ASD/17OHP together with its known anti-androgenic effects indicate that this obesity hormone is also involved in hypoandrogenicity in patients with SLE and RA. This pilot study indicates that leptin may be an important link between chronic inflammation and the hypoandrogenic state.

Regulation of granulocyte function by neuropeptide Y (NPY)

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Objective: The neurotransmitter neuropeptide Y (NPY) exerts several immunological and physiological functions in the periphery. Among these, NPY improves survival of rats in lipopolysaccharide-induced septic shock. The mechanisms of this protective-like effect are still unknown. The activation of neutrophil granulocytes is a hallmark in the inflammatory reaction during sepsis. Here, we investigated the effect of NPY on the superoxide anione production during the respiratory burst of neutrophil granulocytes in in vivo/ex vivo/in vitro models.

Methods: LEW/Ztm rats were equipped with a chronic intravenous (i. v.) catheter. Rats received different doses (0, 0.01, 0.1, 1, 10, 50, 100 µg/kg) of NPY i. v. and blood samples were taken 10min thereafter. Granulocytes were incubated with E. coli ex vivo. The respiratory burst was measured by flow cytometry. In a parallel approach, human granulocytes were co-incubated in vitro with selective agonists and antagonists for NPY receptor subtypes and the respiratory burst was measured accordingly.

Results: In vivo application of NPY modulated dose-dependently (bell-shape-like) the respiratory burst of rat granulocytes after in vitro challenge with E. coli. Low (0.01 and 0.1µg/kg) and very high (100 µg/kg) doses exerted an inhibitory effect while medium doses significantly stimulated granulocyte function. In vitro stimulation of human blood granulocyte NPY revealed a receptor profile characteristic for the proposed Y3 receptor, which is not yet cloned.

Conclusions: The respiratory burst of human and rat neutrophil granulocytes is dose- and receptor-specifically modulated by NPY. This effect may contribute to the protective-like effects of NPY found in septic shock.

Dopamine modulates survival and cellular immune functions during systemic inflammation independent of prolactin release

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Objective: The immunomodulatory effects of prolactin have been demonstrated. The release of prolactin is modulated via pituitary dopamine receptors. We therefore investigated the effect of dopamine and metoclopramide, two clinically used drugs that modulate pituitary prolactin release on the survival and cellular immune functions during sepsis.

Methods: A continuous infusion of dopamine (0.06 mg/kg/h ip) with or without the concomitant administration of the central dopamine antagonist metoclopramide (0.5 mg/kg ip) was administered in male NMRI mice after induction of a polymicrobial sepsis by cecal ligation and puncture (CLP). The survival, cellular immune functions (immune cell distribution, splenocyte proliferation, apoptosis and cytokine release) and prolactin serum concentrations were determined 48 hrs after CLP.

Results: Infusion of dopamine significantly increased sepsis-induced lethality, augmented the sepsis-induced depression of splenocyte proliferation and IL-2 release, increased splenocyte apoptosis but did not affect circulating prolactin levels. Administration of metoclopramide did not affect lethality, improved cellular cytokine release and increased prolactin serum concentrations. The effect of a combination of dopamine and metoclopramide on the survival and on splenocyte proliferation, apoptosis, and cytokine release were comparable to those observed in the dopamine-treated group. However, co-administration of dopamine and metoclopramide was paralleled by a significant increase of serum prolactin levels.

Conclusions: Administration of dopamine during systemic inflammation is associated with a decreased cellular immune function and an increased lethality in a murine model of systemic inflammation. This effect is independent of circulating prolactin concentrations and cannot be antagonized by concomitant administration of metoclopramide.

Patients with RA and SLE demonstrate increased renal excretion of mitogenic estrogens in relation to endogenous anti-estrogens

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Objective: In patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), 17beta-estradiol was thought to play a dual pro- and antiinflammatory role depending on its concentration or probably conversion to downstream mitogenic 16alpha-hydroxyestrone or naturally occurring anti-estrogens such as 2-hydroxyestrone. This study aimed to compare renal excretion of these two types of estrogens of healthy subjects (HS) with patients with RA and SLE.

Methods: In a prospective study with 30 patients with RA, 32 with SLE, and 54 HS, we measured urinary levels of 16alpha-hydroxyestrone and 2-hydroxyestrogens by enzyme immunoassay. We studied renal excretion in order to estimate the time-integral of hormone production.

Results: Urinary concentration and total urinary loss of 2-hydroxyestrogens was 10 times higher in HS as compared to either patients with SLE or RA irrespective of prior prednisolone treatment or gender. The urinary concentration and loss of 16alpha-hydroxyestrone did not differ between HS and RA/SLE. Furthermore, the ratio of urinary 16alpha-hydroxyestrone/2-hydroxyestrogens was more than 20 times higher in RA and SLE as compared to HS irrespective of prior glucocorticoid treatment or gender.

Conclusions: This study in RA and SLE patients clearly demonstrates a huge shift to mitogenic estrogens in relation to endogenous anti-estrogens. Both steroids are converted from the precursor 17beta-estradiol and estrone. In patients with RA and SLE, the magnitude of conversion to the mitogenic 16alpha-hydroxyestrone is extremely upregulated which most likely contributes to the maintenance of the proliferative state in these diseases.

Prior incubation of PBMCs with CRH abrogates the suppressive effects of cortisol on NKA

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Objective: Activation of the hypothalamic-pituitary-adrenal (HPA) axis is associated with generalised immunosuppression. There is both in vitro and in vivo evidence that corticotrophin-releasing hormone (CRH) is immune stimulating. This experiment undertook to examine the comparative effects of CRH and cortisol in vitro, on natural killer activity (NKA) both alone and in combination.

Methods: Peripheral blood mononuclear cells (PBMCs) from six healthy subjects were prepared from fresh samples of whole blood. Triplicate samples, at three effector:target ratios were prepared for a standard 4hr. chromium-51 release assay, to estimate NKA. Physiologically relevant concentrations of cortisol and CRH were incubated alone or together with PBMCs for 2hrs. prior to the assay.

Results: As expected, cortisol significantly diminished NKA. Incubation with CRH alone led to significant stimulation of NKA. Pre-incubation with CRH prior to the addition of cortisol, significantly attenuated the suppressive effects of cortisol on NKA. However addition of CRH to the cortisol-effector mixture had minimal such effects.

Conclusions: The HPA axis derived predecessors of cortisol, CRH in particular, appear to have an effect on immunity that opposes that of cortisol itself. Here we show for the first time that, for CRH, this effect is only significant in cells that have not been previously exposed to high levels of cortisol. Implications for the in vivo milieu are discussed.

An analysis of salivary cortisol in patients with early breast cancer

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Objective: An earlier study* examined the relationship between salivary cortisol, innate immunity and psychological morbidity in patients with early breast cancer. This paper explores the relationship between magnitude of cortisol response (as a measure of HPA activation) and variability of cortisol response (as a measure of HPA dysregulation.

Methods: Patients (n = 70) were sampled just prior to primary surgery. Fasting saliva was collected at midnight and at three points in the early morning. Cortisol was measured by radio-immunoassay, modified for saliva. Magnitude and variability of cortisol response were calculated by area under the curve (AUC) and standard deviation from the mean (SD), respectively and correlations were performed.

Results: There was a satisfactory progressive rise in cortisol levels between midnight and the three morning samples. Values for all time points were several times higher than normative data. There was a strong correlation (r = 0.78) between AUC and SD in the sample. However, when morning only samples were considered, this relationship disappeared (r = 0.07).

Conclusions: This was clearly an acutely distressed population, but interpretation of cortisol responses was hugely dependant on considering the midnight measure. On the one hand it appears that a robust HPA response (high AUC) is associated with HPA axis integrity/plasticity (high SD). If one considers the morning values only, it appears one can mount a robust cortisol response regardless of the state of tone of the HPA axis. The importance of obtaining a true diurnal sample in psycho-biological research is emphasised.

* Submitted for publication

In vivo release and gene upregulation of hypothalamic prolactin during stress in rats

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Objective: Brain prolactin (PRL) is involved in the regulation of neuroendocrine and behavioral stress responses [1]. PRL is expressed in the brain, however evidence for its intracerebral release is lacking. Here, PRL release within the paraventricular nucleus (PVN) and the medial preoptic area (MPOA) of the hypothalamus, as well as hypothalamic PRL gene expression during stress was monitored.

Methods: Virgin female and male rats were fitted with a guide cannula (21 ga) above the MPOA or the PVN for subsequent push-pull perfusion (PPP; 30-min periods, Ringer solution, 20µl/min), and a jugular vein catheter for blood sampling. The hypothalamus of diestrous female rats was isolated before and 0, 30 or 90 min after immobilization, and total RNA was extracted, retrotranscribed and Real-Time PCR performed.

Results: PPP with depolarizing fluid (56mM K^+) triggered PRL release within the PVN ($p < 0.05$) and MPOA ($p < 0.05$) indicating release from intact neuronal structures. Administration of hypertonic saline (3M, ip) triggered peripheral, but not intracerebral PRL release, demonstrating independent release patterns. A 30-min immobilization period triggered PRL release in both PVN ($p < 0.05$) and MPOA ($p < 0.05$) of virgin female and male rats. The same stressor resulted in a significant increase in hypothalamic PRL mRNA after 30 and 90 min.

Conclusions: This study demonstrates the stimulus-dependent, site-specific release of PRL from intact neuronal structures within selected brain regions. Further, a stress-induced activation of PRL release, and gene upregulation within hypothalamic neurons is observed supporting the physiological relevance of this neuropeptide in context of stress coping.

Reference
1. Torner et al. (2001) J Neuroscience 21:3207–3214

Repeated exposure to an emotional stressor during pregnancy: Differential effects in high vs. low anxiety rats

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Objective: In pregnancy and lactation, the responsiveness of the HPA axis to various stressors is severely attenuated. While repeated exposure to stressors during pregnancy has frequently been used as a model for prenatal stress, consequences of pregnancy-stress on the maternal organism in lactation have never been monitored.

Methods: Female rats bred for high (HAB) or low (LAB) anxiety-related behaviour were daily exposed to psychosocial (maternal defeat) and emotional (restraint) stressors between days 5 and 20 of pregnancy and characterized with respect to behavioural and neuroendocrine parameters between days 3–6 of lactation.

Results: Behavioural tests on the elevated plusmaze and the hole-board revealed a significantly higher anxiety in lactating HAB compared to LAB rats independent of pregnancy stress indicating that the innate level of anxiety persists in lactation. However, pregnancy stress further increased anxiety-related behaviour in HAB, but not LAB rats. Maternal care including maternal aggression was not affected by pregnancy-stress in lactating HAB rats, while LAB rats spent less time with their pups but showed more offensive behaviour towards an intruder rat during maternal defence test. Despite the severely attenuated ACTH responses in both lactating HAB and LAB rats to a similar degree, repeated stress in pregnancy significantly elevated the ACTH secretory response to novel environment in lactating HAB, but not LAB, rats indicating a differential vulnerability of the HPA axis to repeated pregnancy-stress exposure.

Conclusions: The data show that rats bred for different levels of anxiety in combination with relevant stress procedures during pregnancy may provide a useful model for future studies on stress-coping mechanisms during the female reproductive cycle.

Acute stress effects on local inflammatory responses to bacterial pathogens in vivo

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Objective: One aim of PNI research is to elucidate psychological influences on local inflammatory responses. Human models, however, allowing for analyses of *local in vivo* responses to pathogens are rare. In previous presentations at GEBIN we proposed a minimal invasive human model to assess such effects. This model, which we originally tested in the context of prolonged academic stress, has now been transferred to an experimental acute laboratory stress condition.

Methods: Twelve students (6 male, 6 female) refrained from oral hygiene in two antagonistic quadrants for 28 days and thereby allowed for increasing microbial stimulation of the respective sites. In remaining quadrants students maintained good oral hygiene. At day 27 and 28 students were subjected to either a 30 minute laboratory psychological stress or a control condition (cross-over). Gingival crevicular fluid (GCF; a transudate of healthy or exudate of inflamed gingival tissue, respectively) was taken immediately after stress and 60 minutes later for later analysis of Il-1β and PGE2.

Results: Repeated measures (stress × time × hygiene) ANOVA revealed significant stress ($p = 0.014$) and hygiene ($p = 0.038$) effects on GCF-Il-1β. Stress induced an increase of Il-1β as did plaque accumulation. No effects on PGE2 were found.

Conclusions: The advantages and disadvantages of the proposed model as compared to other strategies to assess stress effects on local inflammatory responses are discussed. It is concluded that the proposed model is well suited for the assessment of the net effects of stress on inflammatory responses *in vivo* in humans under experimentally controlled conditions.

Tolerance to bacterial endotoxin does not prevent conditioned-taste aversion

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Objectives: To determine the effects of LPS tolerance on changes in water consumption and taste preference. To determine possible relation with TNF response to LPS.

Methods: Tolerance was induced in DA rats with injections of increasing doses of LPS (0.1, 0.2, 0.5, 0.5, and 0.5 each day). Saccharin-flavored water was given in 15 minute sessions preceding each injection. Water consumption was measured eight hrs. later. Control animals received saccharin and LPS in a non-contingent way. Another group started receiving the saccharin-flavored in the second day. All animals were re-exposed to saccharine taste in three sessions. TNF response in plasma after each LPS injection was determined (ELISA) in an independent group of animals.

Results: TNF response to LPS drops to undetectable levels after the first LPS injection. Water consumption decreases after the first LPS injection, but increases as LPS tolerance is established. Tolerant animals show a conditioned aversion to saccharin taste not shown by controls. Animals that start receiving saccharin when tolerance is already established (day two) also acquire a strong CTA.

Conclusions: TNF is not essential for the immune-to-brain communication required for CTA acquisition, and behavioral consequences of LPS challenge may occur in the absence of detectable TNF response. However, it remains to be analyzed whether other cytokines like IL-1 may be involved.

HIV-positive men differ in immunologic but not catecholamine response to an acute psychological stressor

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Objective: Acute psychological stress in humans induces sudden alterations in catecholamine plasma levels and in the distribution of peripheral blood lymphocytes. The purpose of this study was to investigate whether infection with the human immunodeficiency virus (HIV) had an impact on the psychoneuroimmunologic axis.

Methods: Twelve asymptomatic HIV-positive homo- or bisexual men (CD4 cell counts $> 400/mm^3$) and 13 healthy HIV-negative control subjects were exposed to an acute psychological stressor consisting of a 30 min semi-structured stress interview using emotion- and client-centered conversation techniques surrounded by two relaxation periods. Changes in neuroendocrine and immunological, as well as cardiovascular parameters, were intermittently monitored.

Results: Under the influence of the stressor plasma norepinephrine (NE) levels increased significantly in HIV-positive patients (30.6%; $p < 0.05$) and in HIV-negative individuals (17.5%; n. s.). These changes were paralleled by significant increases in blood pressure and heart rate. Plasma cortisol decreased continuously from initially high levels during the entire experimental setting in both groups, compatible with an adaption reaction. Concomitantly, WBC and neutrophilic granulocytes increased significantly in HIV-negative subjects, while they were blunted in HIV-positive patients. Interestingly, NK cell increases were significantly higher during the stress experiment in HIV-positive patients than in HIV-negative controls. CD4(+) and B cell counts remained unaffected by the stressor.

Conclusion: This study suggests that catecholamine secretion induced by an acute psychological stressor is preserved in HIV-infected patients with the responsiveness of WBC and neutrophilic granulocytes being diminished, while NK cells showed an increased response.

Changes in Immune Parameters in the Blood and Lymph Nodes of Socially Stressed Laboratory Rats

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Over the last decade studies have shown that both acute and chronic social stress can affect immune parameters in the peripheral blood of male laboratory rats. However, the majority of immuno-competent cells are not found in the blood but in the secondary lymphoid organs, such as the lymph nodes and spleen. Therefore, we looked at whether the changes seen in the peripheral blood are reflected in the lymph nodes, the proliferative centres of the immune system.

We employed the resident-intruder confrontation model to induce social stress. Hereby an adult male (intruder) was placed into the home enclosure of an established male-female pair (residents) for a particular length of time. The frequency of several aggressive behavioural elements (e. g. full defensive posture, flight) clearly indicated social defeat of the intruder. Directly after the confrontation peripheral blood samples were taken and the lymph nodes were processed to yield cell suspensions. These were used to analyse lymphocyte proliferation and lymphocyte subpopulations.

Lymphocyte function and cell numbers were clearly affected following social stress. Depending on the original derivation of the lymphocytes, the effects seen differed. For example, whilst an increase in the proliferation of lymphocytes derived from the cervical lymph nodes was seen in the intruders in comparison to home cage controls, a decrease in the proliferation of blood lymphocytes was observed.

These and ongoing investigations should serve to offer an insight into the immune changes which occur during social stress, not just in the blood, but also in the lymphoid organs of an individual.

Lesioning the Ventromedial Nucleus of the Hypothalamus Impaires the Recall, but not the Acquisition of Conditioned Immunosuppression in the Cellular Immune Response

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Objective: To investigate the role of the Ventromedial Nucleus of the Hypothalamus (VMH) in the acquisition and recall of conditioned immunosuppression.

Methods: DA rats received bilaterally excitotoxic (kainic acid) lesions by stereotaxic surgery. The effect was evaluated in the acquisition (lesioned before conditioning) and in the recall (lesioned between the conditioning and the reexposure) of conditioned immunosuppression with cyclosporin A (UCS) and a novel tasting saccharin solution (CS). Fluid consumption was monitored to look for the conditioned taste aversion. Splenocytes' proliferative capacity after mitogen stimulation (Con A) and interleukin-2 and interferon-gamma contents in supernatant were measured. Lesions were confirmed by histological analysis.

Results: Both VMH sham-lesioned groups (lesioned before conditioning and lesioned between conditioning and reexposure) showed a regular conditioned immunosuppressive response. This was indicated by lower proliferative capacity and reduced interleukin-2 and interferon-gamma production of splenic lymphocytes, as well as a conditioned taste aversion to the saccharin. When conditioned animals were lesioned between the acquisition and recall phase, the immunosuppressive response was abrogated. However, conditioned animals lesioned before the acquisition phase demonstrated no difference to sham-lesioned animals, they also displayed a pronounced immunosuppression.

Conclusions: These results demonstrate the VMH being essential for evoking the conditioned cellular immune response (recall), but not for the association of the conditioned and unconditioned stimuli (acquisition).

Anxiety-like behaviour in rats and cytokine mRNA in the brain

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Objective: There is evidence that interleukin (IL)–2 in the brain may be related to anxiety-like behaviour in the elevated plus-maze (EPM), and that IL-2 interacts with the striatal serotonergic system. Our previous experiments have shown that Wistar rats can differ systematically in EPM behaviour, which was related to the neurotransmitter serotonin in the ventral striatum. Moreover, we showed that rats with high compared to low anxiety-like behaviour in the EPM had higher striatal levels of IL-2 mRNA, but did not differ significantly in expression of other striatal cytokine mRNA. Here, we investigated whether these expression effects are anatomically specific to the striatum. We asked whether EPM behaviour may also be related to endogenous levels of cytokine mRNA in the hippocampus.

Methods: Based on open arm time in the EPM, male Wistar rats were divided into subgroups with either low or high anxiety-like behaviour. Then, IL-1beta, IL-2, IL-6, and tumor necrosis factor (TNF)-alpha cDNA levels were measured post mortem in hippocampal tissues using semi-quantitative, competitive, reverse transcription polymerase chain reaction (RT-PCR).

Results: Rats with high and low anxiety-like behaviour in the EPM did not show significant differences in hippocampal IL-1beta, IL-2, IL-6, and TNF-alpha mRNA expression. Hippocampal cytokine mRNA showed no relationships with its corresponding striatal cytokine mRNA. Hippocampal IL-1beta, IL-6, and TNF-alpha mRNA were significantly higher expressed than in the striatum, whereas IL-2 showed an opposite trend.

Conclusions: These results provide new evidence indicating that specific cytokine mRNA patterns in the striatum but not in the hippocampus may be associated with EPM behaviour in Wistar rats. *Support by: DFG Schw 559/5–1*

Modulation of anxiety and airway hyper-responsiveness in F344 rats by postnatal stimulation or deprivation

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Objective: Specific experiences in the early postnatal period as well as the genetic background modulate the adult behavioral phenotype along with the disease susceptibility for inflammatory and autoimmune disease models in rats. In humans, the postnatal social and microbiological environment has been demonstrated to influence the incidence of asthma in children. Here we investigated whether the behavioral phenotype and the susceptibility for an ovalbumin (OVA)-induced model of airway-hyper-responsiveness (asthma) is modulated by postnatal handling stimulation or repeated maternal deprivation in adult stress-high-responding F344 rats.

Methods: Male F344/Ztm rats were daily handled (maternal separation + exposure to novelty, 5 min/day), maternally deprived (maternal separation for 2 hours/day), or left undisturbed from postnatal day 1–28. At the age of 3–5 months animals were subjected to a test battery for activity/anxiety/exploratory-like difference. At the age of 5 months, the animals were immunized with OVA and consecutively challenged by intratracheal instillation of the same antigen. Numbers of eosinophils in the bronchoalveolar lavage were used to quantify allergen-induced inflammation.

Results: Measuring home-cage activity, open-field, elevated plus maze, social interaction, and hole board exploration revealed a markedly reduced behavioral stress-responsiveness to novelty, anxiety and increased exploratory behavior in handled F344 rats while maternally deprived animals exhibit increased locomotor activity and reduced exploratory behavior.

Preliminary analysis of the number of eosinophils revealed pro-inflammatory effects by postnatal handling stimulation while maternal deprivation reduced this parameter of allergen-induced inflammation within the airways.

Conclusions: Postnatal handling and maternal deprivation exert long-lasting and specific influence on activity, anxiety and exploration in F344 rats. These differences are associated with a differential allergic responsiveness.

Stress-induced increases of intracellular cytokine profiles of CD4+ and CD8+ T-lymphocytes in atopic dermatitis: A reanalysis with attention to IGE levels

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Objective: Multiple exogenous and endogenous provocation factors such as psychological stress can induce the exacerbation of atopic dermatitis (AD). In addition to CD4+ T-helper cells and CD8+ T-lymphocytes IgE is suggested to be important for the pathophysiology of AD. Thus, the focus of this study is to compare the effects of acute psychological stress on illness-specific immunological parameters in AD patients with high IgE levels and healthy controls (HC).

Methods: Patients with AD (n = 15) and HC (n = 15) were exposed to a brief laboratory stressor (public speaking and mental arithmetic). In vitro analyses were done 1 hour before, immediately after and 1 hour after stress exposure. Lymphocytes and intracellular cytokines in blood-derived lymphocytes were analysed by flow cytometry. Previously published Data comparing the whole groups of AD patients and HC (n = 15 each) were reanalysed by dividing the AD sample into two subgroups, discriminating patients with a high level (n = 6) versus a low level (n = 9) of IgE (cutoff = 1000 kU/l).

Results: A significant interaction effect for stimulated IL-5 positive CD4+ and CD8+ T cells could be shown ($pI < 0.05$), indicating a significant elevated number and a pronounced stress-induced increase of these cell types in AD patients with high IgE levels compared to HC. In addition, a trend ($pI < 0.1$) was observed for IL-4 in the supernatant with more marked stress-induced changes in AD patients.

Conclusions: This reanalysis shows that there are different immunological influences of psychological stress in patients with high and low IgE levels.

Tumor necrosis factor, motion-sickness, and classical conditioning interventions

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Objective: The proinflammatory cytokines IL-1β and TNF-alpha are regarded as immunological correlates of sickness behavior (Dantzer, 2001, BBI, 15, 7–24). TNF-alpha is also involved in cachexia, reduction of food intake (Plata-Salamàn, 2000, Nutrition, 16, 1009–1012) and conditioned taste aversion, thus related to symptoms occurring during cancer and chemotherapy. In our group, we examine mechanisms of conditioned nausea and immunomodulation in cancer chemotherapy (Stockhorst et al., BBI, 2000, 14, 198–218) and derive prevention techniques from the conditioning etiology model. In order to test these interventions prior to clinical use, we established a motion-sickness model in healthy subjects with rotation as the nausea-inducing stimulus.

Method: Subjects are exposed to 1-axial, vertical rotation (120 degrees/sec) plus head movement (maximum: five 1-min rotations per session). Repeatedly per session, they have to rate nausea-related sickness symptoms (e. g., nausea, urge to vomit, dizziness, general discomfort, tiredness), and to collect saliva for the measurement of cortisol and TNF-alpha. In a first screening study, we found an increase of TNF-alpha after rotation, and a positive correlation between TNF-alpha and nausea (respectively urge to vomit) in 50 healthy subjects. We now conduct experimental studies and use latent inhibition (context preexposure) and overshadowing (application of a salient scapegoat CS) as the intervention techniques that should prevent conditioned symptoms to the context (CS) associated with rotation (US). Data analyses addressing (a) the correlation between TNF-alpha and symptoms, and (b) the question whether the behavioral interventions also affect TNF-alpha and cortisol are in progress.

Results and Conclusions: Results will be presented and discussed at the meeting. (Supported by the Wellcome Trust).

Gene expression in peripheral blood monocytic cells of schizophrenic patients

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Objective: Schizophrenia is a multifactorial neuropsychiatric disorder with complex gene-environment interactions. Thus new profiling-techniques such as cDNA microarrays, which screen for disease-associated gene expression patterns at the mRNA level, represent a new and powerful tool in the research of schizophrenia as their approach is independent of whether pathology is primarily genetic or/and environmental determinated. With regard to several studies that document a potential involvement of the immune system in the etiopathogenesis of schizophrenia, we performed cDNA microarray analysis on peripheral blood monocytic cells of schizophrenic patients compared to a pool of matched healthy controls.

Methods: Five schizophrenic patients and 12 healthy control persons were included. For every microarray, mRNA of one schizophrenic patient and pooled mRNA of 4 matched controls has been extracted from peripheral monocytic cells, reverse transcribed into cDNA, labeled using two different fluorescent markers and then hybridized simultaneously to the 2400 immobilized DNA spots on the array surface. After the slide had been washed in order to remove non-specific hybridisation it was scanned by means of a laser-detection-system. The measured intensity values of the fluorescent dyes represented the gene expression levels and a comparison between the two samples has been derived from the resulting intensity ratios.

Results: 4 of the 5 schizophrenic patients showed an explicit down-regulation of the HLA-B39 gene compared to the different control groups.

Conclusion: HLA-B39 has not yet been investigated in the context of schizophrenia. HLA-B39 is reported to be associated with several autoimmune disorders like juvenile rheumatoid arthritis. Thus, our finding could contribute to the understanding of the well-known negative association between rheumatoid arthritis and schizophrenia. Interestingly, the HLA cluster resides on chromosome 6p. Several studies have suggested linkage of this region to schizophrenia, underlining the relevance of our herein described finding of a marked down-regulation of HLA-B39 in schizophrenia.

Decrease of amyloid β1–42 Antibodies in Serum of Patients with Alzheimer's disease

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Amyloid β (Aβ) is the histopathological hallmark of Alzheimer's disease (AD). Antibodies against Aβ occur naturally and are used in promising therapeutical approaches. To assess the diagnostic value of serum Aβ-antibodies for AD, we compared serum Aβ-antibody levels of 96 patients with AD, 14 with Frontotemporal Dementia, 8 with Lewy-Body-Dementia, 18 with Vascular Dementia (VD), 31 with Major Depression (MD) and of 30 healthy controls (HC) using a newly developed immunoassay by Brahms. We found a highly significant decrease of Aβ-antibody levels in AD compared to HC ($p = 0.001$) independent of age, cognitive status and ApoEε4-carrier-status. Aβ-an-

tibody levels correlated to gender in AD with higher levels in women. Applying ROC-Analysis, sensitivity/specificity was 81.3%/46.7% comparing patients with AD to HC. There was no significant difference of Aβ-antibody levels comparing AD to Non-AD-Dementia or MD. Serum Aβ-antibodies may be related to AD, but appear not to be a useful diagnostic marker for AD.

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CSF-levels of amyloid β-peptide antibody in differential diagnosis of Alzheimer's disease

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The accumulation of β-amyloid (Aβ) in neuritic plaques is thought to be the causative agent for the progression of Alzheimer's disease (AD) [1]. Recently, both active immunization and passive administration of Aβ antibodies dramatically attenuated amyloid plaque deposition, neuritic dystrophy, astrogliosis and behavioral deficits in transgenic animals [2]. In addition, it was found that titers of naturally occurring Aβ antibodies in the CSF of AD patients are significantly lower than those in age-matched controls [3].

To assess the use of Aβ-antibodies as a marker of differential diagnosis for AD we compared levels of Aβ-antibodies in CSF of 84 AD patients with 32 major depression (MD) patients and 19 healthy control subjects. The results show significant lower Aβ-antibody levels in MD patients compared to age-matched AD patients.

Despite the significant group separation by CSF-Aβ-antibodies sensitivity and specificity are not sufficient for the use as a marker of differential diagnosis between AD and MD. The interpretation of the results leads to the assumption that levels of Aβ-antibodies in CSF are influenced by multiple factors additional to amyloid plaque deposition.

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