STRESS, BEHAVIOUR AND IMMUNE FUNCTION

Psychological, psychopathological characteristics and cytokine pattern in melanoma patients

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It has been demonstrated that Interferon alpha and IL-2 treatment could worsen depressive symptoms in melanoma patients and that the initial mood/affective state of patients before cytokine therapy is a critical condition for the development of cytokine induced clinical depressive symptoms. It is important to assess psychosocial characteristics of patients at the time of the diagnosis in order to select subgroups of patients that could more easily develop psychological disturbances during the course of cytokine therapy for melanoma or whose psychological distress could deteriorate the compliance to treatment and follow up schedules. Anxiety, alexythymia, depression and other psychological characteristics have been variably correlated with several cytokines such as IL-1 beta and alpha-MSH (anxiety), IL-4 and TNF-alpha (alexithymia), IL-1beta, IL-18 and IL-6 (depression). In order to investigate a relationship between particular cytokine pattern and psychological characteristics in melanoma patients we submitted a battery of self-administered questionnaires to patients receiving the diagnosis of melanoma and correlated it with serum concentration for IL-1beta, IL-4, TNF-alpha, IL-18, alpha-MSH and IL-6. The questionnaires were dealing with Quality of life (SF36, Skindex 29), Psychological variables such as depression (BDI), alexithymia (TAS-20), general mental health (SCL-90, GHQ-12), Anxiety and rage (STAI, STAX-I), and some psychosocial variables such as Self-esteem (RSES), social support (MSPSS), hypochondriasis (WIH) and coping strategies (Brief Cope). We confirmed the relationship between some cytokines pattern and psychological conditions or preconditions that could affect patient's response to treatment.

Body mass index affects cardiovascular and immune cell responses to psychosocial stress in women with polycystic ovary syndrome

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Obesity in combination with insulin resistance and hyperandrogenism are amongst the characteristic features of the polycystic ovary syndrome (PCOS), affecting 6% women of reproductive age. A markedly increased proportion of PCOS patients meet the diagnostic criteria for the metabolic syndrome at a relatively young age, accordingly, the long-term cardiovascular risks are substantially increased. Although there is evidence to support a role of stress and chronic low-grade inflammation in cardiovascular risk, no data exist thus far regarding stress responsiveness in PCOS. Therefore, we analysed the neuroendocrine and immune responses to acute psychosocial stress in women with PCOS. To specifically address the role of obesity, we compared overweight PCOS patients with lean PCOS and with lean healthy controls. Responses to public speaking stress were analysed in 17 PCOS patients with a BMI ≥ 25 (overweight PCOS), 12 PCOS patients with a BMI < 25 (lean PCOS), and 16 lean healthy females (controls), all without psychiatric comorbidity. At baseline, during, and 10- and 45-min after stress, state anxiety, cardiovascular responses, cortisol, ACTH, catecholamines, as well as circulating leukocyte subpopulations were measured, together with hsCRP and cytokines levels at baseline (IL-6, TNF-α, IL-10, IFN-γ), and at 45 min poststress (IL-6). Whereas lean PCOS did not differ in any immune measure from controls, overweight PCOS demonstrated increased basal hsCRP, increased white blood cell count, elevated IL-6, and lower IL-4, IL-10, and IFN-γ concentrations. In response to stress, all groups showed a significant increase in IL-6 and a redistribution of leukocytes and lymphocyte subpopulations, along with significant neuroendocrine and cardiovascular activation. However, the increase in lymphocyte numbers and particularly natural killer cell ($CD56^+$) cells was significantly enhanced in overweight PCOS, who also demonstrated significantly more pronounced cardiovascular responses. In conclusion, enhanced cardiovascular responses to psychosocial stress may play a role in the long-term cardiovascular risks associated with the diagnosis

of PCOS, particularly in obese patients. Chronic low-grade inflammation and effects of stress on immune functions may constitute additional mechanisms by which psychological risk factors, including depressive symptomatology, may play a role in cardiovascular risks in PCOS.

Alterations in amounts and function of white blood cells measured by FACS-analysis in healthy volunteers after dexamethasone administration is subject to circadian time of administration

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The non-specific immune-system and the hypothalamo-pituitary-adrenal (HPA)-axis are closely related. Treatment with glucocorticoids is known to be associated with alterations in differential leukocyte counts, mainly due to robust increases in granulocyte counts. Moreover, glucocorticoids are important modulators of cytokine-release during infection and inflammation. Although glucocorticoids are used as immunosuppressants for years it is not definitely known, how oral glucocorticoids alter immune function in immunocompetent cells and whether the alteration depends on circadian rhythms. We measured the influence of the oral intake of 1.5 or 3.0 mg dexamethasone at 0900 or 2100 h on the basal and ex vivo endotoxin-stimulated exprimation of CD 16b and CD18 in 40 healthy male volunteers. Dexamethasone had potent immunomodulatory properties, resulting in an increased amount of CD16b-positive cells (granulocytes), and moreover in the activation of the granulocytes as it is represented by increasing CD18 density (MnI X) on the cell surface. Independent of dose DEX decreased expression of CD18 in subjects 12 h after application only when administered in the morning suggesting immunological suppression. Administration at night resulted in increased CD18 expression 24 h thereafter suggesting a rebound effect. The significant immunomodulatory effect in the morning compared to evening administration could be explained by overcoming a glucocorticoid-threshold in summation of the defined amount of DEX and the circadian originated higher amount of physiological cortisol in the morning.

Central nervous and systemic reactive oxygen species in endotoxemic shock in mice

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Severe septic shock is often associated with impairment of brain function. Little is known about the effects of endotoxemic shock on central nervous damage caused by the local generation of reactive oxygen species (ROS) due to the lack of a suitable method to differentiate between local central nervous and systemic release. Therefore the aim of our investigation was to differentiate *in vivo* between the formation of reactive oxygen species (ROS) in central nervous system and systemic release by combination of a microdialysis probe and a venous catheter. ROS were detected by electron spin resonance spectroscopy (ESR) infusing 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH 5 mM) as spin label. Stereotactic implantation of the microdialysis probe (CMA® 12) was performed according to coordinates of Paxinos and Watson (Lewis rat 250–300 g; hippocampus, striatum or amygdala) or Paxinos and Franklin (balb/C mouse 22–25 g; striatum). Primarily CMH was infused via the microdialysis probe (Sampling time 15 min, infusion rate 5 μl/min). After 2 h the rats were killed and blood samples were taken immediately. In brain areas high radical concentrations and in peripheral blood no paramagnetic signal could be detected. Reverse results with systemic ROS and no brain radical formations were detected when CMH was applied via a venous catheter and the microdialysis probe flashed with ringer. The different brain areas in rat; shown significant differences in formation of reactive oxygen species: striatum 181 ± 11, hippocampus 103 ± 15 or amygdala 82 ± 16 AU. Mouse experiments were 3 h with a 90 min control period in rats and 1.3 μl/min in mice, followed of a LPS (100 μg/kg) period of 210 min. Sampling time was every 30 min. LPS enhances radical formation up to $35 \pm 7\%$ ($n = 4$) and was not detectable in blood when CMH was infused via the probe. LPS increases peripheral ROS up to $26 \pm 4\%$ ($n = 5$) after venous CMH application. The method used is suitable to differentiate between central nervous and systemic released ROS. Although the blood brain barrier seems to be intact there is a local increase of ROS in central nervous system after LPS infusion, which might be the mechanism of brain damage in septic shock.

Beta$_1$ adrenergic receptors on immune cells impair innate defenses against listeria

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Cold-restraint (CR) for 1 h elicits a psychological and physiological stress that inhibits host defenses against *Listeria monocytogenes* (LM). Previous analyses indicated that this inhibition is not due to depletion of NK or T cells, but is instead dependent on signaling through beta-adrenoceptors (βARs). We now show that impaired host-resistance by CR cannot be accounted for by a decrease

in LM-specific (LLO91-99 tetramer$^+$) effector CD8$^+$ T cells; this result is consistent with previous observations that CR-induced effects are mainly limited to early anti-LM responses. β2AR$^{-/-}$ FVB/NJ and wild-type (WT) FVB/NJ mice had equivalent anti-LM defenses, whereas β1AR$^{-/-}$ FVB/NJ mice had lower levels of LM even when subjected to CR-stress. Additionally, host-resistance competency of β1AR$^{-/-}$ mice could be transferred to irradiated WT mice reconstituted with β1AR$^{-/-}$ bone marrow progenitors and spleen cells, indicating that β1AR signaling on immune cells reduces anti-LM responses. β1AR$^{-/-}$ mice had improved cellular (DTH) responses while β2AR$^{-/-}$ mice had improved humoral responses (IgG1, IgG2, IgM); a result that further explains the strain differences in LM defenses. CR-induced expression of β1AR and β2AR mRNA was assessed by real time PCR. CR treatment significantly increased βAR mRNAs in Ficoll-purified and F4/80$^+$-enhanced liver but not splenic homogenates, demonstrating an organ-specific effect of stress that alters host defenses. Finally, CR treatment induced early increases in perforin expression that may enhance immune cell apoptosis and interfere with LM clearance. In conclusion, β1AR-signaling has immunomodulatory effects on early cell-mediated immune responses; a lack of β1AR-signaling improves anti-listerial defenses and cell-mediated immunity, in general.

IL-1 plays a key role in the development of stress-associated glucocorticoid resistance in mice

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The stress-associated secretion of glucocorticoids (GC) evokes metabolic, cardiovascular and immune changes that are considered to adapt the organism to new environmental demands. However, frequent or chronic stress can result in an altered GC responsiveness of target cells. We previously reported that repeated social defeat in mice, experimentally induced by daily confrontations with an aggressive opponent, was associated with adrenal hypertrophy, increased plasma GC levels, and reduced GC sensitivity of immune cells in the spleen. Specifically, lipopolysaccharide-stimulated splenocytes of mice subjected to six cycles of social stress were less sensitive to the anti-inflammatory actions of GC as evident from an increased production of proinflammatory cytokines and enhanced cell survival. The development of this functional GC resistance was accompanied by the accumulation of CD11b$^+$ cells in the spleen. Molecular studies showed that the CD11b$^+$ splenocytes of stressed mice exhibit impaired nuclear translocation of the GC receptor and show a lack in the transcriptional suppression of NF-κB. Similar impairments in GC receptor function have been observed after *in vitro* treatment of different cell lines with the proinflammatory cytokine interleukin (IL)-1. Thus, the aim of this study was to elucidate whether IL-1 might be involved in the development of the stress-associated GC resistance in the murine spleen. In the first experiment, we investigated if social stress alters the plasma level and the tissue gene expression of IL-1α and IL-1β. It revealed that recurrent exposure to the stressor significantly increased splenic mRNA and plasma protein levels of IL-1β but not IL-1α. In the next step, IL-1 receptor I (IL1R1)-deficient mice were subjected to the stressor and both the tissue distribution of CD11b$^+$ cells and the GC sensitivity of the splenocytes were compared to wildtype mice. Mice lacking the IL1R1 exhibited adrenal hypertrophy and thymic involution in response to stress but did not show an accumulation of CD11b$^+$ cells in the spleen and failed to develop GC resistance. These findings demonstrate for the first time that IL-1 plays a key role in the development of the social stress-associated GC resistance in the murine spleen.

Pre-treatment levels of sTNF-R1 and sIL-6R are associated with a higher vulnerability for IFN-alpha induced depressive symptoms in patients with malignant melanoma

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Background: Immunomodulatory therapy with IFN-α (often leads to neuropsychiatric side effects, especially depression. An activation of the immune system is discussed to trigger neurotransmitter changes and depressive illness. So far few data is available about biological markers, who may predict the individual risk for developing depressive symptoms during IFN-α therapy. The aim of the present study was to investigate the predictive role of certain immunological markers for the development of IFN-α induced depression. We hypothesized that patients characterized by a pro-inflammatory and Th1 accentuated immune response before treatment might have an increased risk for developing depressive mood changes.

Methods: Thirty-three melanoma patients were prospectively investigated during adjuvant treatment with

IFN-α(-2a/2b (3x3 Mio units/week). Depressive mood changes were assessed with the self-rating-depression scale (SDS, Zung-scale) before and during IFN-α treatment. Serum concentrations of sTNF-R1, sIL-6R, sIL-4R and neopterin were measured before and after 3 months of treatment.
Results: sIL-6R, which was negatively associated with SDS scores, significantly predicted higher depression scores in the first three months of IFN-α treatment. sTNF-R1, which was positively associated with SDS scores, significantly predicted the development of late depressive symptoms after six months of therapy. Conclusions: In contrast to the initial hypothesis, patients characterized by high sTNF-R1 and low sIL-6R baseline levels, indicating an anti-inflammatory condition prior to therapy, had a higher vulnerability for depression during IFN-α therapy.

Alterations of the host defense system after sleep deprivation are followed by impaired mood and psychosocial functioning in healthy humans

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In healthy humans, sleep deprivation (SD) has consistently been demonstrated to impair different parameters of the host defense system and of psychosocial functioning. However, the individual timing of these alterations and their possible association have remained unknown so far. We therefore investigated functional measures of the individual host defense system as well as of subjective well-being and psychosocial performance in 10 healthy male adults before and after SD as well as after recovery sleep. In detail, we examined the number of leukocytes, granulocytes, monocytes, lymphocytes, B cells, T cells, T helper and cytotoxic T cells, natural killer (NK) cells as well as the interleukin-1 beta (IL-1 beta) release from platelets after serotonin (5-HT) stimulation. Subjective well-being was determined by the Adjective Mood Scale and psychosocial performance (excitement, energy, ability to work and timidity) was measured by visual analogue scales. Taken together, SD induced a deterioration of both mood and ability to work, which was most prominent in the evening after SD, while the maximal alterations of the host defense system could be found twelve hours earlier, i.e. already in the morning following SD. Our findings therefore suggest an SD-induced alteration of these psycho-immune response patterns in healthy humans preceding deterioration of mood and psychosocial functioning.

Alterations of the serotoninergic system after sleep deprivation are followed by impaired mood and psychosocial functioning in healthy humans

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Sleep deprivation (SD) in healthy humans is known to worsen individual mood and psychosocial performance. However, the timing of these impairments with respect to their physiological – in particular serotoninergic – underpinnings has remained unclear so far. We therefore investigated measures of subjective well-being and psychosocial functioning in 10 healthy male adults before and after SD as well as recovery sleep. The serotoninergic response pattern to SD was characterized by assessing platelet 5-HT_{2A} receptor functioning and MAO-B activity. Subjective well-being was determined by the Adjective Mood Scale and psychosocial performance (excitement, energy, ability to work and timidity) was measured by visual analogue scales. Taken together, SD induced a deterioration of both mood and ability to work, which was most prominent in the evening after SD, while the maximum increase of serotoninergic activity was observed twelve hours earlier, i.e. already in the morning following SD. Our findings support the hypothesis that the deterioration of mood and psychosocial functioning after SD in healthy humans can be related to a preceding upregulation of the serotoninergic system as measured by the platelet 5-HT_{2A} receptor function.

Seasonal differences in the murine neuroendocrine regulation of inflammatory responsiveness

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All individuals are continuously confronted with environmental changes like seasonality. Seasonal variations of behaviour and physiology are well documented, though seasonal differences in the susceptibility to inflammatory stimuli and to psychological stress are less well studied. Using the colon ascendens stent peritonitis (CASP) as a model of hyperinflammatory shock and chronic psychological stress as a model of immunosuppression we found that seasonality is accountable for the amplitude of inflammatory responsiveness. Mice held with constant photoperiodicity all over the year had a higher risk to suffer from lethal septic shock due to polymicrobial peritonitis during

summer or autumn. This was associated with an exaggerated TNF response when compared with experiments performed in spring or winter time. Consistently, the severity of stress-induced immunosuppression was less pronounced in mice that were exposed to chronic psychological stress in the summer compared with experiments carried out in winter. During cold seasons, however, mice responded with enhanced stress-induced elevation of plasma corticosterone levels, lymphocytopenia, splenocyte apoptosis and antiinflammatory cytokine bias compared with summer time. The HPA axis response was hightly correlated with depression-like behaviour ($r_p = 0.7696$; $P = 0.0430$). And, the behavioural changes that were pronounced in winter time showed correlation with the ex vivo inducibility of IL10 of splenocytes ($r_p = 0.699$; $P = 0.05$). These results imply that seasonal differences of the hypothalamus-pituitary-adrenal axis response and of immune reactivity constitute the nature of the inflammatory response, and therefore, influence the susceptibility to infections and to stress. Thus, seasonality could play an important role in the pathogenesis of infectious complications.

Mineralocorticoid receptor modulation by spironolacton and fludrocortisone under a stressful learning task: Relationship between cortisol secretion and learning performance

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The hypothalamus-hypophysis-adrenal (HPA-axis) axis is the major stress responsive system of the organism, which is under the control of hippocampal mineralo- and glucocorticoid receptor function. Furthermore, animal studies suggest that glucocorticoid- and the mineralocorticoid- receptor systems are involved in learning and memory processes. Therefore, in this study we examined the effects of a modulation of the mineralocorticoid receptor system on basal and stress induced cortisol secretion and, in addition, on learning and memory function. In an intraindividual placebo controlled completely balanced repeated measurement design 24 healthy male subjects (age 20–30 years) received a single oral dosage of either 200 mg spironolacton, 0.1 mg fludrocortisone or placebo 3 h before a stressful learning task. In this task subjects had to learn 10 associations between letters and numbers (e.g. A-7) based on a trial and error task with immediate feed back (wrong/correct). This task had to be performed with (stress condition under placebo, fludrocortisone and spironolacton) and without (baseline) a time limitation. For the augmentation of stress subjects could achieve an additional 30,- € reward for an excellent and quick performance. The number of trials, correct and false answers during the learning task and the number of correctly recalled associations after the end of the experiment were assessed. Cortisol samples were obtained from saliva before (twice) and until 120 min after the beginning of the stress task. The application of 200 mg spironolacton significantly increased baseline cortisol secretion compared to placebo and fludrocortisone. Under fludrocortisone baseline cortisol secretion was descriptively reduced compared to placebo. In addition, under fludrocortisone cortisol response to stress terminated earlier and there was a trend for a faster learning performance than under spironolacton. No differences for memory retrieval could be observed between conditions. Thus, spironolacton is able to increase the basal tone of the HPA-axis reflected by an increased basal cortisol secretion and stress induced cortisol appeared to be prolonged by spironolacton compared to fludrocortisone. These observations and the faster learning performance under fludrocortison compared to spironolacton suggest that the MR-function is related to learning performance probably mediated by stress induced cortisol.

Allergic disease reduces stress-coping skills: indications from a mouse model

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Stress is reported to be an important factor that contributes aggravation of allergic diseases such as atopic dermatitis. Atopic sufferers, chronically exposed to distressing symptoms, show specific psychoneuroimmunologic changes and behavioural traits that are characterized by an altered HPA-axis reactivity, depression, tension, and anxiety. To examine whether affected individuals react to stress differently from healthy ones, we used a combined mouse model of experimental allergic dermatitis (AD) and stress. AD was induced in C57BL/6 mice by double sensitization (i.p.) and an intradermal challenge using chicken egg ovalbumin. Animals were additionally exposed to sonic stress for 24 h prior to challenge. Alterations in anxiety- and depression-like behaviour, locomotor activity, exploration, and 'approach/avoid conflict' behaviour were assessed using elevated plus maze and tail suspension test. We observed increased avoid conflict behaviour in AD-induced mice compared with non-treated mice. Interestingly, stress itself had an anxiolitic effect. It promoted locomotion and exploration, and at the same time suppressed an 'avoid conflict' behaviour. This effect of stress was reduced in stressed AD mice. Taken together, it we conclude that AD impairs coping with a new stressful situation as represented by the plus-maze. Enhancement of stress-coping skills therefore appears a useful measure to balance AD-induced behavioural changes.

Stress modifies specific immunity in allergic disease via substance P dependent mechanisms

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Atopic dermatitis is a chronically relapsing, neuroinflammatory skin disorder, characterised by an imbalance in T-cell immune responses. Stress plays an important role in the pathogenesis and aggravation of the disease. It modulates peptidergic cutaneous innervation thereby having an impact on skin immune cells' activation. To examine how stress exposure influences antigen presentation and the function of skin dendritic cells in atopic dermatitis, we used a mouse model of experimental allergic dermatitis (AD) and stress. In epidermal sheets from biopsies of treated skin cultivated over three days *in vitro*, we found significantly more dendritic cells, mainly Langerhans cells, that emigrated from the epidermal sheets of AD animals exposed to stress. Interestingly, the effect of stress was abrogated when animals were treated with NK1 antagonist prior and after stress application. Using flow cytometry we found that these cells migrate to the draining lymph nodes, where dendritic cells (CD11c+) from stressed AD animals show significant up-regulation of co-stimulatory molecules CD80 (B7-1) and CD86 (B7-2). Cells expressing CD86 also expressed enhanced VLA-4, an adhesion molecule involved in eosinophilia and TH2-differentiation, while CD80+ cells expressed enhanced LFA-1, an adhesion molecule involved in AD worsening and TH1-differentiation/chronification. Taken together, our data show that stress has an activating effect on dendritic cells in allergic dermatitis. We further suggest that altered release of Substance P, among other stress-mediators, may account for the observed changes being an important enhancer of dendritic cell migration in allergic disease.

Neuro-immune idiosyncrasy: individual's immune history modifies peripheral immune stimuli perception and its association

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Single administration of bacterial lipopolysaccharide (LPS) induces a rapid peripheral increase in pro-inflammatory cytokines (e.g. tumor necrosis factor alpha: TNF-α, and interleukin-1β). Repeated LPS administration induces a state of immune-tolerance characterized by a drastic reduction in cytokine response. Importantly, it has been demonstrated that a single pairing of a taste stimulus (conditioned stimulus: CS) with the first encounter to LPS (unconditioned stimulus: US) induces a strong and long lasting associative learning, evidenced by a reduction of the ingestive behaviour (conditioned response) after subsequent CS re-exposures. Within the present study we investigated if saccharin (0.2%)-LPS (0.5 mg/kg i.p.) associative learning is inducible in LPS-immune-tolerant animals. Our behavioural data clearly indicate that the immune history indeed affects associative learning when the US employed is transduced by the immune system. Saccharin-LPS associative learning in LPS-immune-tolerant animal was less pronounced (i.e. low avoidance behaviour and fast extinction) than in LPS-naïve animals, indicating that in LPS-immune-tolerant animals the 0.5 mg/kg LPS i.p. injection was differently perceived by the CNS (i.e. different cytokines and/or lower concentration), and thus associated in a different way. These data indicates, that similar immune challenges might result in different neuro (behavioural)-endocrine consequences depending on the immune status of each individual.

Effect of chronic interferon alpha application in mice on behaviour and immune response

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Interferon (IFN)-α is a cytokine generally used for the treatment of chronic viral infections and malignancies in humans. Here, the onset of depression-like symptoms has been described as the most frequent side effect, which occasionally even necessitates discontinuation of IFN-α therapy. Markedly, the pathways by which IFN-α induces depression-like symptoms still remain elusive and a skew of the immune hemostasis towards immunity – subsequently perpetuating cell migration – may play a role in the pathogenesis of such depression-like symptoms. To address this hypothesis, we injected murine IFN-α (60 000 U/kg) i.p. daily over a period of 7 days into male Balb/c mice, followed by forced swim test to identify depressive behaviour. Furthermore, flow cytometry was used to identify an IFN-α induced immune bias of blood and brain cells. We observed that this chronic IFN-α application resulted in an increased percentage of the adhesion molecule leukocyte function associated antigen (LFA)-1 and the activation marker CD25 on blood lymphocytes. Also, an increased percentage of CD11c+ microglia cells and CD4+ lymphocytes could be detected upon chronic IFN-α administration. Our data

confirm that chronic IFN-α application also induces depression-like behaviour in mice. We propose that, besides neural stimulation, an IFN- α advanced immunity may render the blood brain barrier more permeable for an enhanced migration of inflammatory cells, subsequently resulting in depression-like behaviour. We further advocate that mice treated with IFN-α may serve as a model which will not only allow to develop therapeutic approaches for IFN-α induced depression in patients with malignancies or chronic viral infections, but also permits a glimpse on pathways of how peripheral immune response pathways talk to the brain.

Stress and allergic disease: re-evaluating psychoneuroimmunologic findings in atopy

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Since the early days of psychosomatic thinking, atopic disease was considered exemplary. In the 70s and 80s clinical observations were supported by numerous reports on increased anxiety, depression, ill stress-coping and other characteristic psychopathological findings in atopic patients. However, patient groups were small and diverse and controls rare. Therefore, the question remained whether these psychopathological findings were a pathogenetic prerequisite or if any chronic disabling disease could cause such symptoms. Recently, the discussion has been revived and refocused by psychoneuroimmunological findings. We now know, that atopic disease is characterized by an imbalance in the response of the classical stress-axis, the hypothalamus-hypophysary-pituitary axis and that this can be induced e.g. by dermatitis. This imbalance can be found shoulder by shoulder with enhanced expression of newly emerging neuro-endocrine stress mediators such as substance P (SP) and nerve growth factor. Together they can alter the inflammatory as well as the stress- response on several levels. In skin, the immediate inflammatory response involving neuropeptide release and mast cell degranulation, in short neurogenic inflammation, is enhanced by stress. Systemically, antigen-presentation and cytokine balance are altered and centrally, the stress axis responsiveness is reduced. Imbalanced stress-responsiveness through overload may therefore be at the core of stress-exacerbated allergic disease and deserves re-evaluation of therapeutic options such as neutralisation of SP-signalling by antagonists against its receptor NK1, cortisol treatment as supplementation and relaxation techniques to counteract unbalanced stress-responses.

Stress exposure during pregnancy may program allergic asthma and behaviour of the progeny

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Environmental factors encountered during pregnancy may act as fetal programming agents thus modulating the genetic predisposed susceptibility towards allergic diseases. Among many other factors, maternal stress perception during pregnancy has been postulated as one of these non-genetic programming agents for allergic diseases. Here, we aimed to investigate if and how the environmental factor 'stress perception' during pregnancy perpetuates allergic diseases and behaviour as non-genetic programming agent. Syngenic pregnant BALB/c mice were exposed to sound-induced stress on gestation days 12 and 14. Six weeks after birth, offspring were sensitized and airway challenged with a model allergen, Ovalbumin (OVA). Sensitized and challenged offspring from non-stressed dam served as control. Stress exposure during pregnancy increased allergen-induced airway hyperresponsiveness as well as airway inflammation. This aggravation of asthma symptoms was accompanied by enhanced Th2-immune responses, which correlated with an up-regulation of the chemokine receptor CCR3 on lymphocytes in the lung. Stress exposure during pregnancy let also to a state of hyperanxiety and depression in the progeny, which was accompanied by a down-regulation of CRH in the paraventricular nucleus of the brain. The data show that the susceptibility for allergen-induced immune reactions and certain behaviour may already be set in *utero* by stress exposure during pregnancy. This offers new insights into the mechanisms involved in the development of allergic diseases and points towards possible new strategies for allergy prevention.

The effect of chronic subordinate colony housing on depression, anxiety and colonic inflammation: is there a causal link

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Chronic subordinate colony housing (CSC; 19 days) has recently been shown to be an appropriate model for assessing the effect of chronic psycho-social stress in male mice. This is underlined by the finding that CSC effects on various behavioural, neuroendocrine, and immunological parameters are robust and reproducible. Additionally, there

is a growing numerous of animal studies linking chronic stress to the development of depression like symptoms. Therefore, the aim of the present study is to investigate whether exposure to CSC results in depression-like behaviour. Following the CSC mice will be subjected to a number of tests examining different facets of depression: namely saccharine preference test, tail suspension test and the forced swim test. Additionally, mice will be assessed in the elevated plus maze and social avoidance test to determine whether CSC also increases anxiety. Further, we will examine the effect of chronic antidepressant (imipramine) or anxiolytic (diazepam) treatment throughout the CSC on behaviour and the previously reported CSC-induced increase in colonic inflammation. In conclusion, the present study will determine whether exposure to CSC induces depression and/or anxiety and if this depressive state is involved in the onset of spontaneous colonic inflammation after prolonged CSC exposure.

Activation of pro- and anti-inflammatory signaling pathways in caregivers of cancer patients

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Chronic stress is has been shown to be associated with adverse health outcomes across a wide range of medical conditions. Disinhibition of inflammatory pathways, possibly mediated by alterations of HPA axis activity, may be an important mechanism through which chronic stress exerts its effects. However, little is known about the molecular signaling pathways through which stressors bring about excessive inflammation. Therefore we investigated 18 familial caregivers of brain cancer patients and 17 healthy adults without caregiving responsibilities or major stressors (mean age = 50 years, 66% female). Caregivers participated in the study an average of 9.5 weeks after the patients' first surgery. To assess activation of the inflammatory response, production of IL-6 was measured in endotoxin-stimulated whole blood cultures. Furthermore, mRNA for NF-kappaB, I-kappaB, and the glucocorticoid receptors (GR) alpha and beta were measured to investigate signaling pathways using real-time PCR. Basal HPA axis activity was assessed by collecting six daily cortisol samples over three consecutive days. Caregivers showed more psychological distress compared to controls, as indicated by higher scores on perceived stress and symptoms of depression ($P < 0.05$). Caregivers showed signs of pro-inflammatory activation, with a trend toward higher LPS-stimulated IL-6 production ($P = 0.08$) and significantly higher quantities of NF-kappaB mRNA ($P < 0.05$). There was also evidence of activation of anti-inflammatory processes: caregivers showed higher GR alpha/beta ratios and increased quantities of I-kappaB ($P < 0.05$). There was no significant group difference in diurnal cortisol secretion ($P > 0.05$). We conclude that the chronic stress of caregiving not only activates pro-inflammatory signaling cascades, as hypothesized, but also activates counterregulatory anti-inflammatory responses. Diurnal cortisol secretion itself seems not to be the mediator of increased inflammatory activity, and it does also not seem to be the main compensatory mechanism. Our findings highlight the molecular signaling pathways through which stressors act.

The influence of social stress on collagen-induced arthritis in laboratory rats: study outline and first results

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Rheumatoid arthritis is a multi-factorial autoimmune disease whose aetiology and progression is influenced by a wide range of immunological, neuroendocrine, and psychosocial factors. A growing number of epidemiological studies suggest that psychological stressors can affect autoimmune diseases. Thus, the aim of the present study is to systematically investigate the effects of social stress on the clinical, histological, and immunological manifestation of this collagen-induced arthritis (CIA) in Wistar rats. CIA is an experimental model of autoimmune disease that is induced by immunization with type II collagen. In a series of experiments, we exposed male Wistar rats to psychosocial stressors during their pre-, peri-, or postnatal life. In a first experiment, we focused on the effects of postnatal stress experienced by adult rats (120 days) immediately before the induction of CIA. Social stress was induced in male Wistar intruders by repeated social confrontation with resident male opponents. The behaviour of the animals during confrontations was recorded by infrared cameras. After induction of CIA, a regular assessment of the clinical symptoms (fore- and hind paws) was conducted. At various time points after induction of CIA (day 0, day 7, day 14, day 28, day 42) blood samples for immunological (such as lymphocyte subsets, IFN-γ and TNF-α production profiles) and endocrine measurements (corticosterone and adrenal catecholamines) were collected. The present study also includes histological analysis of the tarsal joints, spleen and adrenal glands. The results will provide new insights into the impact of psychological and neuroendocrine factors on the susceptibility and progression of collagen-induced arthritis in rats which might also help to develop new therapies for rheumatoid arthritis in humans.

Central nervous formation of reactive oxygen species in endotoxemic shock in NO-synthase treated mice

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Systemic application of LPS results in enhanced inflammatory processes, characterized by a stimulation of neutrophils and macrophages, this in turn leads to organ diseases or even organ damage. Septic shock is often associated with impairment of brain function. Little is known about the effects of endotoxemic shock on central nervous damage caused by the local generation of interleukin 6 (IL-6) and reactive oxygen species (ROS) in NO-synthase (NOS) treated animals. Therefore the aim of our investigation was to analyse *in vivo* the formation of reactive oxygen species (ROS) in central nervous system and systemic release. ROS were detected by electron spin resonance spectroscopy (ESR) infusing 1-hydroxy-3-methoxycar- bonyl-2,2,5,5-tetramethylpyrrolidine (CMH 5 mM) as spin label. Stereotaxic implantation of the microdialysis probe (CMA® 12) was performed according to coordinates of Paxinos and Franklin (balb/c mouse 18–24 g; striatum). Central nervous generated IL-6 was quantified using a immunoassay kit from R&D Systems®. NOS inhibition was performed by i.p. injection of LNAME (10 mg/kg). A 120 min control period followed by a 3 h LPS (100 μg/kg) period. We started the experiments by infusion of CMH via the microdialysis probe (1.3 μl/min) Sampling time was every 30 min. LPS enhances striatum radical formation up to 35 ± 7% (n = 6) LNAME abolish the LPS induced ROS formation. IL-6 increased initially up to 300 ± 30% (basal: 10 ± 3 pg/ml) and return to a steady state of 180 ± 45%. At the end of each experiment, blood was taken by heart puncture and analysed for blood born ROS by incubation with CMH, ROS increased 21 ± 5% after 3 h LPS. The method used is suitable to differentiate between central nervous and systemic released ROS. As shown in NOS inhibition experiments, enhanced ROS can be blocked in central nervous system in endotoxemic shock which might a protection against brain damage in septic shock.

Anticipatory cognitive stress appraisal is associated with altered proinflammatory cytokine inhibition in response to stress

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Objective: Anticipatory cognitive appraisal can affect the stress-induced release of stress hormones which in turn can modulate monocyte cytokine release. We investigated whether anticipatory cognitive appraisal processes would predict changes in monocyte cytokine release following psychosocial stress in relation to stress hormone release.

Methods: Forty-four men (mean age 43 ± 2 years; mean arterial blood pressure (MAP) 102 ± 2 mmHg; mean body mass index (BMI) 26 ± 4 kg/m^2) completed the Primary Appraisal Secondary Appraisal (PASA) scale before undergoing the Trier Social Stress Test (combination of mock job interview and mental arithmetic task). *In vitro* monocyte tumor necrosis factor (TNF)-α and interleukin (IL)-6 release following lipopolysaccharide (LPS)-stimulation were assessed immediately before and after stress, and during recovery up to 60 min post-stress. Moreover, we repeatedly measured salivary cortisol as well as plasma epinephrine and norepinephrine levels.

Results: Stress hormones increased and cytokines decreased following stress (all $P < 0.05$). Correlation analyses showed that the PASA 'stress index' was negatively associated with total LPS-stimulated TNF-α (r = –0.33, P = 0.03) and IL-6 (r = –0.32, P = 0.33) area of the inhibition curve (AIC) between rest and 60 min post-stress. While controlling for age, BMI, and MAP, TNF-α stress change was significantly predicted by the primary PASA scale 'control expectancy' (β=0.42, P = 0.003). IL-6 stress change was predicted by both, 'control expectancy' (β=.32, P = 0.031) and 'challenge' (β = –0.30, P = 0.046). None of the stress hormones predicted stress change of any cytokine.

Conclusions: The findings suggest that anticipatory cognitive stress appraisal predicts the amount of LPS-stimulated cytokine inhibition following stress independent of stress hormone release.

NEUROIMMUNOLOGY IN THE CNS

TNFα expressed by a recombinant rabies virus (RV) prevents lethality and enhances innate and adaptive immune responses in the brain

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Rabies Virus (RV) infection is a central nervous system (CNS) disease that is almost invariably fatal. Considering the fundamental albeit still controversial role of TNFα in many neurological diseases we tested whether TNFα contributes to protection against lethal RV infection through a mechanism that inhibits virus load or stimulates inflammatory responses in the brain or both. Recombin-

ant RV strains were engineered to express soluble TNFα (SPBN-TNFα(+)), insoluble membrane-bound TNFα (SPBN-TNFα(MEM) or which carries an inactivated TNFα gene (SPBN-TNFα(–)). TNFα knockout mice were infected with RV non-invasively via the intranasal route. Growth curves derived from infection of mouse neuroblastoma NA cells *in vitro* revealed significantly lower virus spread and production of SPBN-TNFα(+) than SPBN-TNFα(MEM) or SPBN-TNFα(–). Expression of soluble or membrane-bound TNFα was independent from NA cell viability indicating a direct antiviral effect of TNFα. Brains of mice infected intranasally with SPBN-TNFα(+) showed significantly lower virus load than did mouse brains after SPBN-TNFα(–) infection. None of the SPBN-TNFα(+)-infected mice succumbed to RV infection, whereas 80% of SPBN-TNFα(–)-infected mice died. Reduced virus load in SPBN-TNFα(+)-infected mouse brain was paralleled by enhanced CNS inflammation including T cell infiltration and microglial activation. The extent of inflammatory reactions, reactive microgliosis, and T cell infiltration induced by TNFα correlates inversely with RV load in the brain and the lethality of the infection. These data suggest that TNFα exerts its protective activity in the brain directly through an as yet unknown antiviral mechanism and/or indirectly through induction of inflammatory processes in the CNS.

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Modulation of cyclooxygenase-2 expression and prostaglandin E_2 production by purinergics in activated microglia

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Purinergic receptors are expressed in both the central and peripheral nervous system, where they mediate various forms of intercellular communication and modulate several biological functions. Large amounts of ATP can be rapidly released from different cell types following hypoxia, stress, and tissue damage. Some reports indicate that ATP is able to modulate pro-inflammatory pathways in immune cells. In this study, initially we investigated the effects of ATP on cyclooxygenase-2 (COX-2) expression and prostaglandin E_2 (PGE_2) production in primary microglial cells prepared from rat and mouse pups. We characterized the expression of different P2 receptors on rat and mouse microglia. We found a strong expression of P2X1, P2X7, P2Y2 and P2Y6, moderately expressed P2X4 and a very weak expression of P2X2, P2Y1 and P2Y7 in rat microglia. Primary mouse microglia strongly expressed P2X7 and P2Y1. In rat microglia, exogenously added ATP induced COX-2 transcription, but PGE_2 release occurred only with co-stimulation with LPS. Similarly, ATP synergistically enhanced LPS-induced COX-2 protein levels. On the contrary of what was found in rat microglia, ATP dose-dependently produced a significant reduction in COX-2 expression and PGE_2 levels in LPS-treated primary mouse microglial cells. Later inspired by these results we also studied effect of purinergics in microglia obtained from human post mortem brains on the expression of above inflammatory markers. These findings indicate that there are species-specific differences in the effects of purinergics on COX-2 induction and PGE_2 formation mediated by LPS. Although at this stage, the exact molecular mechanism(s) explaining these findings are unknown, a different P2 receptor profile might be involved. These results could have important implications for future studies investigating the effects of purinergics on activated microglia, which could be relevant to neuroinflammatory processes occurring in neurodegenerative disorders.

IL-6-induction in the striatum of the mouse by adenosine, evidence for the role of the A_2B-receptor

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IL-6 and adenosine are two neuromodulators, which are known to interact with each other in the CNS. Moreover they both are thought to be important in the neurobiology of several psychiatric neurological diseases, especially in major depression, Alzheimer disease, epilepsy, stroke and traumatic brain injury. However the relationship and function of these two substances in such pathologies is at the present not clear enough. It was demonstrated that adenosine stimulate IL-6 synthesis in astrocytes culture. This induction could be involved in the therapeutic effect of the sleep deprivation and electroconvulsive therapy in MD as soon as in the pathogenic of AD. Nevertheless it has not been proved *in vivo* so far. Therefore, the aim of this study was to quantify the IL-6 synthesis after stimulation with different adenosine agonists and antagonists in freely moving mice. A CMA/12® guide cannula was implanted stereotactically into the left striatum of Balb'C mice (Paxinos and Franklin, 2001). After 7 days the microdialysis probe (CMA/12, 100 000 Da) was inserted and the experiments were performed. Ten perfusates were collected every 30 min for a period of 5 h at a constant flow rate of 2 μl/min. Perfadex®(ringer dextran 40) was perfused during the first hour and the drug/control solution during the next 4 h. NECA (10-4 M), NECA (10-4 M) +DPCPX (10-6 M) and NECA (10-4 M) +MRS17O6 (10-5 M) were diluted in

Perfadex® and used as drug solution. IL-6 concentrations in the perfusates were quantified with the Quantikine®-Kit (IL-6 Mouse Immunoassay). NECA (10-4 M) (potent but non-specific A2b-agonist) enhanced the release of IL-6 significantly ($P < 0.05$). This effect was inhibited with the addition of the A2b-antagonist (MRS1706), but not with the A1-antagonist (DPCPX). In conclusion, our results confirm the IL-6 release after A2b stimulation, a phenomenon that could contribute both to the pathophysiology of AD and to the antidepressant effects of SD and ECT.

A pivotal neuroimmune regulatory role of fractalkine and its receptor during lentivirus Infection in the rhesus monkey brain

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Existing data concerning the role of the δ-chemokine fractalkine (CX3CL1) and its receptor (CX3CR1) in lentivirus-induced encephalitis are limited and controversial. Here we explored by quantitative in situ hybridization and immunohistochemistry the cell-specific expressional changes of CX3CL1 and CX3CR1 in rhesus macaque brain during simian immunodeficiency virus (SIV) infection and antiretroviral treatment. Neuronal expression of CX3CL1 was significantly reduced in cortex and striatum of AIDS-diseased monkeys as compared to uninfected and asymptomatic SIV-infected monkeys. CX3CL1 mRNA was increased in some endothelial cells and newly induced in astrocytes and macrophages focally in areas of SIV burden and inflammatory infiltrates. In most CX3CL1-positive astrocytes and macrophages the transcription factor NF-κB was translocated to the nucleus. CX3CR1 was upregulated in scattered, nodule and giant cell-forming microglia/macrophages and mononuclear infiltrates close to CX3CL1-induced cells in the brain. Treatment of AIDS-monkeys with the CNS-permeant 6-chloro-2',3'-dideoxyguanosine fully reversed SIV burden, productive inflammation, nuclear NF-κB translocation as well as focal induction of CX3CL1 in astrocytes and macrophages, and downregulation in neurons. In contrast, diffuse CX3CR1-positive microgliosis and GFAP-positive astrogliosis were partially reversed by 6-chloro-2',3'-dideoxyguanosine. Thus, focally induced CX3CL1 may be a target for therapeutic intervention to limit ongoing inflammatory infiltration into brain in lentivirus infection.

Fingerprints of neural activity after peripheral immunes challenges

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Peripheral immune stimulation has repeatedly been shown to alter neural activity in the brain. However, the mechanisms by which the CNS detects or 'senses' changes are poorly understood. The aim of this study was to elucidate whether peripheral administration of immunosuppressive or immunostimulating agents differently affects central neural activity in specific brain regions which previously were identified to play an important role in immunoregulation. Adult male Dark Agouti rats were stereotaxically implanted with monopolar electrodes within the central amygdala, the insular cortex and the striatum as well as with a reference electrode above the cerebellum. Three weeks after brain surgery, experimental animals received an intraperitoneal injection of either the immunosuppressive drug, cyclosporine A (CsA, 20 mg/kg) ($n = 6$), the bacterial superantigen *Staphylococcus enterotoxin B* (SEB, 1.0 mg/kg) ($n = 5$), or physiological saline ($n = 5$). The neural activity in the targeted brain regions was analysed by recording field potentials in different frequency bands (alpha1, alpha2, beta1, beta2, delta, and theta) via a wireless system (radiotelemetry). In all targeted brain areas, the most prominent changes in neural activity were observed 140 min after CsA injection and 80–110 min after SEB injection, respectively, compared to saline-injected controls. Importantly, the pattern in the different frequency bands was specific for the type of immunomodulating agent as well as for the brain area targeted. These data confirm that the central nervous system is able to detect immune challenges in the periphery throw an afferent pathway (humoral or neural) and modulate his neural activity by providing 'fingerprints' according to the peripheral challenges.

Role of 5-HT7 receptors in neuroinflammation

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Serotonin (5-hydroxytryptamine, 5-HT) is a widely distributed neurotransmitter which is involved in

neuroimmunomodulatory processes. We found 5-HT to induce IL-6 protein synthesis in the human astrocytoma cell line U373 MG. We demonstrate that the 5-HT-induced IL-6 release is mediated by the 5-HT_7-receptor based on several agonist/antagonists that were used. 5-HT-induced IL-6 synthesis is inhibited by the partially selective 5-HT_7 receptor antagonist, pimozide, and the selective antagonist SB269970. Furthermore, IL-6 synthesis was induced by the 5-HT_7-receptor agonist carboxamidotryptamin (5-CT). In addition, we found p38 mitogen-activated protein kinases (MAPK) and protein kinase C epsilon (PKCϵ) to be involved in 5-HT-induced IL-6 synthesis. 5-HT mediated the phosphorylation of both p38 MAPK as well as the PKC ϵ isoform. Besides a new role of 5-HT7 receptors in mediating inflammatory mediators, we demonstrate for the first time, that the expression of 5-HT7 receptors is induced by LPS in primary microglia. Our data suggest an important role of 5-HT7 receptors in neuroinflammation.

Functional downregulation of the neurokinin 1 receptor by antidepressant, neuroleptics and mood stabilizers

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Neurokinin-1/-2-receptor antagonists have some efficiency in the treatment of affective and anxiety disorders. However, recent data from clinical trials are disappointing since large phase III studies could not confirm previous data. Nevertheless, animal models have shown clear 'antidepressant' and 'anxiolytic' effects of these substances in a wide variety of test systems. In the present study, we investigated acute effects (stimulation for 1 h up to 2 days) of the antidepressants imipramine and fluvoxamine, the antipsychotics clozapine and haloperidol as well as the mood stabilizers valproic acid, lithium and carbamazepine on NK-1-receptor expression and receptor binding in human astrocytoma cells, which highly express the NK-1-receptor. We found that imipramine and fluvoxamine, haloperidol as well as valproic acid strongly downregulated NK-1-receptor mRNA-expression (quantitative RT-PCR) and protein synthesis (Western Blot). Furthermore, these drugs also decreased functional activity of the receptor as they downregulated substance P-induced gene expression and led to a decrease of SP binding sites as shown by binding assay studies using ^{3}H-SP. In summary, we have shown that certain antidepressants, antipsychotics and mood stabilizers are able to downregulate the NK-1-receptor with the consequence of decreased SP binding to the receptor and decreased functional activity. This may indicate a new mechanism by which psychopharmacological agents exert their psychotropic effects.

Immunological characterization of a human fetal microglial cell line

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Microglia plays a role not only in host defense in the brain but also in various other functions. Moreover, the function of microglia also depends on the developmental state of the brain. Thus, microglia has been reported to be involved in brain development ruling cell differentiation and cell death/apoptosis. Therefore, we investigated the human fetal microglial cell line CHME-3 for its microglial host defense characteristics. We analysed inducible NO production, spontaneous and stimulated IL-6 secretion, and cell surface markers. We found that CHME-3 cells were not inducible for NO production by any of the measures that induced significant NO production in native newborn rat microglia, such as lipopolysaccharide (LPS), tumor-necrosis-factor-alpha (TNF) and interferone-gamma (IFN-γ). This was not caused by cell death or general inactivity of the CHME cells because cell viability and cell protein production were not impaired. Moreover, CHME-3 cells produced significant amounts of IL-6 in response to the stimulators mentioned. LPS systematically decreased IL-6 secretion under all conditions except for spontaneous versus LPS. The results for the cell surface markers are pending and will be presented at the meeting. However, even at this point we think that our results provide important data for the discussion of the role of fetal versus adult and rodent versus human microglia.

Experimental infection of TNFα-transgenic mice with the Borna disease virus – characterization of the inflammatory reaction

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The aim of the study was to analyse the influence of the proinflammatory cytokine tumor necrosis factor-α (TNF) on the clinical outcome, the inflammatory reaction and the viral spread of non-transgenic, hetero- and homozygous TNF-transgenic mice experimentally infected with the neurotropic Borna Disease Virus (BDV). Clinical and neurological examinations were carried out weekly. After necropsy, the mice brains were analysed for the presence of pathohistological lesions. The viral nucleoprotein (BDV-N) expression, invading immune cells and glial fibrillary acidic protein (GFAP)-reactive astrocytes were visualized using immunohistochemistry. Notably, significantly

lower weight gain was found in BDV-infected non-transgenic mice compared to mock-infected animals. Furthermore, transgenic mice displayed a lower weight gain compared to their non-transgenic counterparts. Spontaneous epileptic seizures were observed exclusively in BDV-infected transgenic animals. These seizures appeared more frequently in homozygous transgenic mice starting at 21 days post infection (dpi). In contrast, seizures started at 42 dpi in heterozygous transgenic animals. The BDV-N showed a disseminated distribution within the brain in all three BDV-infected mice groups. The inflammatory reaction correlated strongly with the transgene status of the animals. Non-transgenic mice showed a mild immune cell infiltration at all time point investigated. In contrast, the inflammatory reaction in both TNF-overexpressing transgenic mice groups caused a progressive severe non-purulent meningoencephalitis with astrogliosis and activation of microglia. Despite a high TNF-transgene-expression, only a minimal inflammatory reaction was present in the hippocampus. Interestingly, an astrogliosis was observed also in non-transgenic mice despite only mild inflammatory lesions. TNF-overexpression correlated with the degree of the meningoencephalitis, but not with the qualitative composition of the immune cell infiltrates. The invading cells are composed mainly of T-cells, macrophages and B-cells. Therefore the main effects of TNF-overexpression consisted of epileptic seizures and severe non-purulent meningoencephalitis with accompanying microglial activation but no obvious influence on viral distribution.

Endogenous brain derived neurotrophic factor modulates the immune response in autoimmunity of the central nervous system

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Brain derived neurotrophic factor (BDNF) is involved in neuronal, glial and probably also thymocyte development and survival. It is mainly produced by neurons, but also by leukocytes *in vitro* and in inflammatory lesions, e.g. in multiple sclerosis. Yet, the functional relevance of BDNF expression by immune cells is still unknown, since conventional BDNF knockout mice die prematurely. We applied the Cre/loxP system to generate mice with a conditional deletion of BDNF in the T-cell lineage (lckCre $BDNF^{fl/fl}$ mice), in myeloid cells (lysMCre $BDNF^{fl/fl}$ mice) or in both lineages together (lysMCre lckCre $BDNF^{fl/fl}$ mice). In these mice, we investigated myelin oligodendrocyte glycoprotein (MOG) peptide 35–55 induced experimental autoimmune encephalomyelitis (MOG-EAE), a model for a 'Th1'- (auto) immune response. Compared to control mice, lckCre $BDNF^{fl/fl}$ mice and lysMCre $BDNF^{fl/fl}$ mice developed a similar disease course of MOG-EAE. In contrast, the early phase of MOG-EAE in lysMCre lckCre $BDNF^{fl/fl}$ mice was less severe with a reduced T cell and macrophage/microglia infiltration in the histologic analyses of spinal cord tissue. After immunization with MOG35-55, the antigen specific T cell proliferation in lysMCre lckCre $BDNF^{fl/fl}$ mice was significantly impaired. Moreover, the production of 'Th1'-cytokines like interferon-gamma, interleukin (IL)-1beta, IL-6 and IL-23p40 in supernatants of primary lymph node cell cultures from lysMCre lckCre $BDNF^{fl/fl}$ mice was clearly reduced. In contrast, levels of IL-10 were increased in lysMCre lckCre $BDNF^{fl/fl}$ mice while IL-5 remained unchanged. Studies investigating the frequencies of regulatory T cells and 'Th17' cells are presently ongoing. In summary, these data reveal a new and unexpected role for endogenous BDNF as an immunomodulator orchestrating T cell and macrophage responses during autoimmune demyelination of the CNS.

IL-10-mediated anti-inflammatory signalling modulates the psychopathological consequences of prenatal infection

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Maternal infections during pregnancy increase the risk for schizophrenia and related disorders in the offspring. Given that the precise immunological response is critically influenced by the genetic background of the infected host, maternal genetic factors are expected to be crucial determinants of the offspring's susceptibility to postnatal brain and behavioural dysfunctions after prenatal maternal infections. Specifically, the anti-inflammatory cytokine IL-10 may have an important role in this context, because it is a fundamental regulator of inflammation in both acute and chronic conditions. In the present study we combined a recently established animal model of schizophrenia, which is based on prenatal viral-like infection by polyriboinosinic-polyribocytidilic acid (PolyI:C; 2 mg/kg, i.v.) on gestation day 9, with a mouse model of genetically driven overexpression of IL-10 by macrophages. We demonstrate that enhanced levels of the anti-inflammatory cytokine IL-10 at the maternal/fetal interface prevent the emergence of multiple schizophrenia-related behavioural and pharmacological abnormalities in the adult offspring after prenatal immune challenge.

In the absence of a discrete prenatal inflammatory event, however, enhanced levels of IL-10 in prenatal life precipitates spatial exploration deficits and learning disabilities. Hence, by regulating the balance between pro- and anti-inflammatory cytokines during early brain development, prenatal IL-10 acts as a developmental modulator of postnatal brain functions in normal and prenatal inflammatory conditions.

The precise time of prenatal infection predicts symptom subtypes in an animal model of schizophrenia

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Maternal infections during pregnancy increase the incidence of neuropsychiatric disorders with a presumed neurodevelopmental origin in the offspring, including schizophrenia and autism. However, this association appears to be critically dependent on the precise times of the prenatal infectious events. In particular, the long-term functional consequences of prenatal immune activation at different times of gestation may be related to differing symptom clusters of schizophrenia. In order to study this temporal dependency in an animal model of prenatal viral-like infection in mice, we administered pregnant dams on gestation day (GD) 9 or GD17 with the viral mimic polyriboinosinic-polyribocytidilic acid (PolyI:C; 5 mg/kg, i.v.) or vehicle solution. The resulting adult offspring were then tested in a number of behavioural and pharmacological paradigms relevant to the positive–negative dichotomy and cognitive symptoms of schizophrenia. Here, we show that whilst deficits in prepulse inhibition (PPI) exclusively emerge after prenatal immune activation on GD9, prenatal immune challenge on GD17 specifically leads to working memory impairments in the Morris water maze. In addition, a potentiation of the locomotor reaction to the NMDA-receptor antagonist dizocilpine (MK-801; 0.15 mg/kg; i.p.) only appears after prenatal PolyI:C exposure in late but not middle gestation, whereas enhanced locomotor responding to systemic administration with the dopamine-receptor antagonist amphetamine (AMPH; 2.5 mg/kg; i.p.) emerges independently of the precise timing of the prenatal immunological manipulation. Our findings here and in previous reports (*Brain Behav. Immun.* 20:378-388, 2006; *J. Neurosci.* 26:4752–4762, 2006) thus indicate that prenatal immune activation in early/mid pregnancy leads to a variety of abnormalities associated with positive symptoms of schizophrenia, whereas prenatal immune activation in late gestation results in the emergence of behavioural and pharmacological dysfunctions particularly associated with negative and cognitive symptoms of this disorder.

Signalling pathways involved in microsomal prostaglandin E synthase-1 (mPGES-1) expression in activated primary microglia

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Activation of microglia commonly occurs in the early response of the brain to a wide variety of pathological stimuli, including trauma, neurodegeneration, and ischemia. One of the hallmarks of neuroinflammation is the dramatic production of prostaglandin E_2 by activated microglial cells. Recent experimental evidences indicate that microsomal prostaglandin E_2 synthase-1 (mPGES-1) is a key contributor to PGE_2 production by microglia and other immune cells. It has been shown that mPGES-1 is highly inducible and its induction parallels that of cyclooxygenase-2 (COX-2), resulting in a coordinate over-expression of these key enzymes involved in PGE_2 synthesis. However, the signal transduction mechanisms involved in mPGES-1 expression in activated microglia are poorly understood. In the present study, by using specific inhibitors of different signalling pathways, we investigated the molecular mechanisms involved in mPGES-1 expression in lipopolysaccharide (LPS)-activated primary rat microglial cells using PCR and immunoblotting. We found a dramatic increase in mPGES-1 mRNA and protein induced by LPS having a peak at 24–48 h of stimulation. This up-regulation was diminished by PD98059 and SB202190 at the RNA level, suggesting the involvement of p42/44 and p38 mitogen-activated protein kinases (MAPKs). Furthermore, blockade of protein kinase C with GF109203X resulted in significant reduction in LPS-induced mPGES-1 protein levels in rat microglia. We also demonstrated that nuclear factor kappaB (NF-kappaB) may play a minor role in the regulation of mPGES-1 in activated microglia. The study of the intracellular signalling pathways involved in mPGES-1 expression in activated microglia opens a new avenue in the search for novel potential targets to reduce neuroinflammation.

Neuropathic pain after thoracolumbar spinal ischemia in the rat depends on the severity of innate immune responses in the brain stem

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The rat model of spinal ischemia is well suited to study central neuropathic pain behaviour and underlying mechanisms. Microglial activation may be an important factor in

the development and chronicity of neuropathic pain. Here we explore whether rats subjected to spinal ischemia that develop allodynia and rats that do not show signs of allodynic behaviour differ in the degree of presumed microglial activation along the pain neuraxis with particular emphasis on the primary afferent relay center of the brain stem gracile ncl. Three groups of rats ($n = 6$ in each group) were investigated; rats subjected to spinal ischemia with allodynia, without allodynia and sham treated rats. After assessing allodynic behaviour with von Frey hairs 2 weeks after photic ischemic lesion of the lower thoracic spinal cord rats were fixed by perfusion with Bouin-Hollande fixative. Brain and spinal cord were postfixed in the same fixative for 24–48 hours and dehydrated in 2-propanol. Deparaffinized serial sections of the lower brain stem bearing the gracile ncl. or spinal cord were immunostained for the microglial marker IBA-1, the microglial complement activation marker C1q and the microglial response gene cyclooxygenase-1 (COX-1). Both allodynic and non-allodynic rats exhibited microglial activation and increased numbers of microglial cells in the gracile ncl. as compared to sham. The increase in the number of IBA-1, C1q and COX-1 positive microglial cells in the gracile nuclei in allodynic rats was significantly higher than that in non-allodynic rats. Our data indicate that the gracile ncl. in addition to the spinal cord is an important pain relay center in which the degree of microglial activation and production of inflammatory mediators such as complement and prostaglandins but not microglial activation per se determines whether neuropathic pain develops or not. Our model offers the unique opportunity to discern microglial factors causing pain by differential gene expression analysis.

Elevated microglial density in schizophrenia and depression is associated with suicide

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Suicide has a high prevalence in patients with schizophrenia and affective disorder. Our recent postmortem study (Steiner et~al. Acta Neuropathol, 2006) revealed increased microglial densities in two schizophrenic patients who had committed suicide. Therefore, the hypothesis of microglial activation during acute psychosis was proposed. Alternatively, 'suicide' could be a diagnosis-independent factor leading to microgliosis. To clarify this question, microglial HLA-DR expression was analysed by immunohistochemistry in the dorsolateral prefrontal cortex (DLPFC), anterior cingulate cortex (ACC), mediodorsal thalamus (MD) and hippocampus of 16 schizophrenics, 14 depressed patients with affective disorder and 10 matched controls. A subgroup of six schizophrenics and seven patients with affective disorder who committed suicide was included. ANOVA revealed no effect of diagnosis on microglial density (DLPFC: $P = 0.469$; ACC: $P = 0.349$; MD: $P = 0.569$; hippocampus: $P = 0.497$). However, significant microgliosis was observed in the DLPFC ($P = 0.004$), ACC ($P = 0.012$) and MD ($P = 0.004$) of suicide patients. A similar trend was seen in the hippocampus ($P = 0.057$). In conclusion, immunological factors may play a hitherto underestimated role in suicide. First, microglial activation might be interpreted as a consequence of presuicidal stress. Second, one might speculate a causal link between microglial activation and suicidal behaviour, such as neuroendocrine factors, cytokines, and nitric oxide, which are released from microglial cells and are known to modulate noradrenergic or serotonergic neurotransmission and thus may trigger suicidality.

NEUROIMMUNE AND NEUROENDOCRINE NETWORK IN PSYCHIATRIC DISEASE

Psychobiological stress reactivity during healthy human pregnancy and its predictive value for post partum depressive symptoms

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Recent human research on pharmacological or physical stress provocation procedures in pregnant women has resulted in inhomogeneous findings regarding the extent of alterations of the hypothalamus-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS) with respect to the progression of pregnancy and the type of stressor. The purpose of this study was to test the endocrine, autonomic and psychological responses to standardized psychosocial stress at different stages of pregnancy and its predictive value for post partum mood states. We exposed 30 healthy pregnant women at the beginning of the second trimester (group 1), 30 healthy pregnant women at the beginning of the third trimester (group 2) and 30 healthy non-pregnant controls (group 3) to the stressor. Stress response was measured by psychometrics, endocrine parameters from saliva samples (cortisol and alpha-amylase as an indirect indicator of norepinephrine), and heart rate for the calculation of heart rate variability. Additionally, we assessed post partum mood states one week after delivery of a healthy baby. Baseline data show elevations of all biological parameters in group 2 in contrast to the other groups. Stimulated responses from SAM and HPA show significant increases in group 1 and 3 following stress exposure, but nearly no responses in group 2. Those women who experienced negative mood swings post partum showed significantly higher cortisol responses to the stress

test compared to the mothers with stable mood post partum, whereas baseline levels during pregnancy did not differ. No group differences were found for prior episodes of psychiatric disorders, obstetrical complications, birth weight or mode of delivery. Our data provide evidence that (a) healthy pregnant women show characteristic stress responses during pregnancy and (b) healthy pregnant women developing postpartum depressive symptoms might be identified already during pregnancy by means of their higher cortisol reactivity and their higher psychological reactivity in response to psychosocial stress.

Effects of experimentally induced panic attacks on neuroimmunological markers

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Neuroimmunological markers have been found to be altered in major psychiatric disorders such as depression and schizophrenia. So far, only few studies have been conducted concerning the regulation of immunological parameters in rapid changing psychiatric states such as panic attacks. Experimental panic induction using CCK-4 has been shown to be a very useful tool in anxiety research and serves as a reliable paradigm for neurobiological changes during acute panic. In this pilot study we investigated the influence of CCK-4 induced panic on the neuroimmunological system. Therefore MIF, sIL-2R, IL-1β, IL-1Ra, TNFα, sTNFα-R1, sTNFα-R2 and IL-6 were determined in a sample of healthy subjects, which underwent experimental panic induction using the CCK-4 paradigm, including a placebo controlled cross over design. Panic reaction was assessed five minutes after injection using the API score. For measurement of immunological parameters, blood samples were taken 45 and 30 min prior to injection, at the time of injection (min 0) and after 5, 20, 60 and 120 minutes. In addition, changes of cortisol were evaluated during the challenge. We did not find any influence of cortisol, the API scores or the substance CCK on serum levels of measured parameters. We could not detect any relevant changes within the marker-systems focused on, only IL-6 showed an unexpected rising at the end of investigation time even under placebo conditions in individuals, expecting a panic reaction because of having been treated with CCK in the first condition. The latter very carefully hints at IL-6 as possible marker for trait anxiety. This is the first investigation concerning rapid changes of serological immune parameters in correlation to acute panic induction under experimental conditions. From our preliminary data we cannot confirm a rapid change of the immune status of a person during panic attacks. Previous data from studies describing altered serological levels of immune markers in Panic disorder may possibly more reflect the long-term effects of unspecific stress.

Cortisol and prolactin response to dopaminergic and serotonergic stimulation, behaviour in an approach-avoidance paradigm and personality traits in patients with anxiety disorders and borderline personality disorder

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Patients with Borderline Personality disorder (BPD) and with anxiety disorders (AD) clearly contrast in personality traits (predominantly impulsivity and anxiety) and behaviour (avoidance vs. impulsive (approach) behaviour). According to Gray (1973) impulsivity and anxiety are independent personality traits which are characterized by a different susceptibility for stimuli of reward and punishment. Furthermore, these two dimensions of personality differ in serotonergic and dopaminergic reactivity. In addition, dopaminergic and serotonergic neurotransmission have been related also to facets of the impulsivity-aggression spectrum. Therefore, a study in patients with BPD and AD, which includes the assessment of personality, behaviour and neuroendocrinology has been conducted. 15 patients with BPD and 15 patients with AD, who all were free of medication, have been examined. All patients had to complete personality questionnaires, such as I7, BIS 11 (impulsivity), STAI, NEO-FFI, TCI. In addition, all patients underwent an approach-avoidance paradigm in which reward and punishment were systematically varied. Furthermore, placebo controlled double blind randomly assigned serotonergic (citalopram 20 mg p.o.) and dopaminergic (1.25 mg bromocriptin p.o.) stimulation tests with the assessment of cortisol and prolactin response were performed in both groups. The results of this study show that patients with BPD present with higher values in scales related predominantly to the impulsivity-aggression spectrum and novelty seeking, but not in neuroticism related scales. Cortisol stimulation by citalopram differs between groups, showing a more pronounced cortisol and prolactin rise in BPD than in AD after citalopram. No major differences in the dopaminergic stimulation test could be detected between both groups. The behavioural approach-avoidance paradigm revealed a significant difference between BPD and AD only in the condition, in which reward could be easily achieved. This results show that beside clear differences in impulsivity-aggression related personality traits- patients with BPD

and AD differ in certain aspects of behaviour related to reward and punishment and in serotonergic neurotransmission reflected by a different stimulation of cortisol and prolactin after application of citalopram.

Effects on cytokine plasma levels during interpersonal psychotherapy (IPT) in individuals with major depression: association with responder status and known confounders

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Cytokines and their soluble receptors have been implicated in the pathophysiology of psychiatric disorders, including major depression. Although sound findings are subtle it is likely that certain cytokines might be altered during major depressive episodes, and successful therapy might normalize these alterations. If this is true for the disorder and its therapy per se and not a mere medication effect such effects are to be found with psychotherapy only, as well. Therefore, we investigated the plasma levels of tumor-necrosis factor (TNF)-alpha, soluble TNF receptor-p55 (TNFR-p55), sTNFR-p75 and leptin in the beginning and at the end in 24 patients undergoing interpersonal psychotherapy (IPT), a proven effective therapy in major depression. We found that therapy response by Hamilton Depression Rating Scale (HAMD) was significantly associated with the relative change over time compared to baseline in TNF and sTNFR-p55. Whereas smoking habit, age and first versus recurrent episode status did not have any effect gender, weight, and prior antidepressive medication did have additional effects. The original figures at baseline and in the end were not affected by the treatment response status but by the confounders as named above. In conclusion, we found that the response status is associated with differential changes in the plasma levels of TNF and sTNFR-p55 supporting a hypothesis that certain cytokines and soluble cytokine receptors might be involved in the pathophysiology of major depressive episodes. Our results further support that there are differential biological effects independent of substance induced changes.

Effects of adrenergic stimulation on cultured microglial cells – is cerebral innate immunity modulated by norepinephrine?

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In the brain, 10% of all cells are microglia, which belong to the lineage of bone marrow derived resident macrophages. In peripheral organs, norepinephrin (NOR) released by sympathetic nerve endings and epinephrine from the circulation influence macrophage behaviour. In the CNS, NOR is released from widespread projections of the nucleus coeruleus and selected damage to these neurons leads to extensive changes in glial reagibility. Prostaglandines synthesized by cyclooxygenase (COX)-1 or COX-2 regulates the glial-glial and the neuron-glial interaction and represent one intercellular signalling system, which may be modulated by NOR. Here we investigated the effects of NOR alone, or in combination with lipopolysaccharide (LPS) on COX expression in cultured rat microglial cells. LPS binds to CD14 in microglia and is a known stimulator of COX-2 expression. COX-1 was constitutionally expressed in cultured microglia and COX-1expression was neither induced by NOR nor by LPS. NOR alone led to a small increase in COX-2 mRNA without any change in COX-2 protein concentration. Stimulation with LPS led to a massive increase in COX-2 mRNA and COX-2 protein. Combining NOR and LPS stimulation led to a 20-fold increase of COX-2 protein concentration compared to LPS alone. Results from experiments with selective receptor agonists and antagonists suggest that both. β_1 and β_2 adrenergic receptors contribute to the augmentation of LPS effects by NOR while α_1 and α_2 receptors are not involved. These experiments demonstrate, that CD14 and β-receptor activated intracellular signalling cascades may finally augment each other leading to a massive amplification of CD14 triggered COX-2 protein synthesis.

Effects of substance P and serotonin on the cytokine production of peripheral blood lymphocytes

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The interactions and close relationships of the immune system and the central nervous system have been objects of interest over the last recent years. Accordingly, the involvement of an immune process in the pathophysiology of psychiatric disorders has been proposed. Serotonin (5-HT) plays an essential role in the pathophysiology and treatment of depression and the neuropeptide substance P (SP) has been discussed in the etiopathology of affective disorders. Co-localisation studies of 5-HT and SP show their close functional relationship and interdependency in various regions of the CNS. On the other hand, both 5-HT and SP receptors are expressed on lymphocytes. Serotonin increases proliferation of lymphocytes and substance P may function as a modulator of the effects of other neurotransmitters or neuropeptides on lymphocytes. However little is known of the functional effects upon cytokine-production. Thus we were interested in the effects of 5-HT and SP on the production of IFN-γ as indicator for the Th1 subset

and IL-4 and IL-13 as Th2 cytokines to investigate the Th1/Th2-balance. Peripheral blood mononuclear cells were gained from blood of 43 healthy individuals by ficoll dense separation. The cells were incubated under standard conditions together with different concentrations of the mitogen PHA, SP, 5-HT or NaCl for 25h on 96-well-ELISpot plates. IFN-γ, IL-4 and IL-13 were measured as spot-forming units using a Biotin-ALP-Streptavidin complex and quantified using ELISpot-microscopy. Serotonin in concentrations of 10^{-4} mol/l significantly decreased the IFN-γ and IL-4 productions, but not the IL-13 production. Neither 5-HT in concentrations of 10^{-5} mol/l nor SP alone altered IL-4 production, but the combination of both significantly increased IL-4 production. We further conducted genetic investigations and could demonstrate that the 590-CT SNP of the IL-4 gene influences the IL-13 production, as the homocygotic CC-allele carriers produced significantly more IL-13 than the combined CT-and TT-allele carriers. Our study shows that serotonin in higher concentrations suppresses IFN-γ and IL-4 production. IL-13 displays a different manner to IL-4. Substance P might modulate the effects of serotonin on cytokine production. Furthermore, genetic differences indicate individual differences, which might account for observed interindividual variances.

Reduced monocyte activation in Tourette's syndrome?

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There is growing evidence that infection and inflammatory mechanisms play a role in the pathogenesis of Tourette's syndrome (TS), at least in a subgroup of TS patients. As monocytes play an important role in the innate recognition of bacterial cell wall components, the aim of this study was to examine monocytes and the levels of monocyte-derived cytokines and -receptors such as tumor necrosis factor alpha (TNF-α) and interleukin 1 receptor antagonist (IL1-ra). Besides we studied the activation products neopterin and soluble CD14 (sCD14) as well as C-reactive protein (CRP). In 46 TS patients and 43 (40 age- and sex-matched) healthy controls the amount of monocytes and levels of CRP were determined by standard methods. TNF-α, sCD14, serum-neopterin and IL1-ra were detected by immunoassays. Although within the normal range, CRP and the amount of monocytes were significantly higher in TS patients. Interestingly, TNF-α, IL1-ra and sCD14 serum concentrations were significantly lower in TS patients compared to healthy controls. The higher levels of CRP and neopterin in TS patients could be a sign of a latent inflammatory process. Low levels of IL1-ra, TNF-α, and sCD14 along with increased monocyte counts could be explained by reduced monocyte activation that cannot be compensated by increased monocyte numbers. The results of this study point to a dysfunction of the innate immune system which could be responsible for insufficient clearing from infectious agents.

Relationship between Kynurenines and S100b in CSF of schizophrenic patients

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A reduced activation of the glutamatergic neurotransmission may be crucially involved in the pathophysiology of schizophrenia. Kynurenic acid, one of the degradation products of the kynurenine pathway, is the only endogenous NMDA receptor antagonist. Kynurenine (KYN) is produced through cleavage of the indole ring of tryptrophan (TRP) and further metabolised either to the neuroprotective kynurenic acid (KYNA), or to the neurotoxic intermediate 3-hydroxykynurenine (3-HK). Pro-inflammatory cytokines induce the formation of the neurotoxic product, while anti-inflammatory cytokines inhibit this metabolic pathway, indirectly inducing the formation of KYNA. Schizophrenia seems to be accompanied by a predominance of anti-inflammatory cytokines. Within the CNS, astrocytes appear to be responsible for the induction of an anti-inflammatory immune response. Moreover, astrocytes play an important role in the glutamatergic neurotransmission. S100b as an astrocytic protein is an indicator of astroglial function and serum and CSF levels of this secretory protein are increased in schizophrenia. We were therefore interested in the relationship between the major intermediates of the kynurenine pathway and S100b in CSF of schizophrenic patients. We investigated CSF samples of 100 patients suffering from an acute episode of schizophrenia. CSF levels of kynurenines were measured by HPLC. CSF levels of S100b were measured by immuno-assay. Mean CSF levels of the analytes were as follows: TRP 363 ± 135 ng/ml; KYN 6.29 ± 3.55 ng/ml; KYNA 2.85 ± 3.05 ng/ml; 3-HK 2.31 ± 1.06 ng/ml; S100b 283.25 ± 162 pg/ml. There was a significant positive correlation between S100b and TRP ($P < 0.001$), KYN ($P < 0.001$), and 3-HK ($P = 0.020$), while S100b was negatively correlated with KYNA ($P < 0.001$). Our data indicate a functional relationship between the kynurenine pathway intermediates and astroglial function. Previous studies of schizophrenia have demonstrated a significant elevation of KYNA in postmortem pre-frontal cortex and in CSF. In Alzheimer's disease, elevated S100b is associated with dystrophic neurites. According to our data,

the neuroprotective KYNA may be involved in the prevention of S100b excretion. In future studies, the combination of S100b and KYNA may therefore serve as a tool to discriminate between distinct schizophrenia subgroups.

NEUROENDOCRINOLOGY AND IMMUNE FUNCTION

Sleep enhances circulating Interleukin-7 concentrations in healthy young men

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IL-7 signals are crucial for the survival of naive and memory T cells, and the IL-7R is expressed on the surface of these cells. Following infection, the IL-7R is expressed on only a subset of effector CD8+ T cells, and has been demonstrated to be important for the survival of these memory precursors. In the present study we aimed to investigate the effect of sleep and wakefulness on the 24-h pattern of circulating interleukin-7 (IL-7) concentrations in 18 healthy young men since sleep is known to support immunity. All subjects were examined twice: during a regular wake-sleep cycle and during a 24 h period of continuous wakefulness. Sleep markedly increased circulating IL-7 concentrations as compared to the wake condition ($P < 0.03$). This difference between sleep/wake was most pronounced during the second part of the night (0330 h–0630 h: $P < 0.02$). The sleep-associated increase in circulating IL-7 concentrations may facilitate the survival and formation of memory T cells following infection.

Progesterone supplementation abrogates stress-triggered foetal rejection in a mouse model via up-regulation of galectin-1, Th2 cytokines and $CD8^+$ cell-dependent pathways

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Problem: One of the most remarkable immunological regulations is the maternal immune tolerance towards the foetal semi-allograft during pregnancy, which has been referred to as immunity's pregnant pause. Cytotoxicity against the semi-allogeneic trophoblast cells must be selectively inhibited and pathways presumably include Th2 cytokines unopposed by Th1 cytokines. Steroid hormones, including progesterone, have similar effects. Low levels of progesterone and Th2 cytokines and high levels of Th1 cytokines are attributable for increased abortions in mammalians, which may be triggered by psychoemotional stress. Thus, the aim of the present study was to provide experimental evidence for the mechanism involved in the endocrine-immune bi-directionality during pregnancy and stress-triggered pregnancy failure.
Method of Study: DBA/J-mated CBA/J female mice were randomized in three groups: (i) control females; (ii) mice exposed to stress on gestation day (Gd) 5.5; and (iii) mice exposed to stress and substituted with the progesterone derivative dydrogesterone (Gd 5.5). On gestation day 7.5 mice of each group were sacrificed, and the frequency of $CD8^+$ cells and cytokine expression (IL-4, IL-12, TNF-α, IFN-γ) in uterus cells was evaluated by flow cytometry. Galectin-1 expression was evaluated by IHC. Additionally, some mice were depleted of CD8 cells by injection of monoclonal antibody.
Results and Conclusions: We observed that progesterone substitution abrogated the abortogenic effects of stress exposure by decreasing the frequency of abortogenic Th1 cytokines. This pathway was exceedingly CD8-dependent, since depletion of CD8 led to a termination of the pregnancy-protective effect of progesterone substitution. Further, galectin-1 and progesterone appear to act synergistically in promoting foetal tolerance.

Circadian rhythm of regulatory T cell distribution and function

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Vaccination is a powerful tool for the prevention of infectious diseases. Relatively little is known about the impact of the time point of vaccination on the ensuing immune response. Previous studies showed that vaccination in the afternoon compared to that applied in the morning hours caused a highly significant approx. four-fold higher mean antibody titer. No information is available on the underlying mechanisms. One possibility could be modulation of anti-vaccine immune responses by regulatory T cells (Treg). Thus, it seems reasonable that number and/or function of Treg might have a circadian rhythm. We investigated 12 healthy young men for 24 h and monitored various parameters in the peripheral blood bi-hourly. We analysed the absolute numbers of Tregs by FACS using CD4-FITC- and CD25-PE-antibodies. Cosinor analysis revealed significant circadian rhythm in this population with highest levels during night time (~95 Treg/μl blood) and lowest levels in the day (~55 Treg/μl blood). In a second study we addressed the analysis of the functional circadian rhythm of Treg by performing proliferation and suppression assays for the measurement of the suppressive

capacity of Treg at different time points during a 24 h period. The first data provide evidence for circadian rhythm of regulatory T cell function with the lowest suppressive capacity of Treg at 7:00 am. At 3:00 pm for example we measured a significant higher suppressive capacity ($P < 0.0005$) compared to 7:00 am. To our knowledge this is the first analysis of Treg distribution and function in the context of circadian rhythm. These findings could provide profound evidence for the essential role of daytime for the development of balanced immune reactions after infections or vaccination. Gefördert durch die DFG: SFB 654, Teilprojekt C3.

Increased chromogranin A serum levels in patients with rheumatoid arthritis and systemic lupus erythematosus: another indication for an activated sympathetic nervous system

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Earlier studies demonstrated an increased sympathetic nervous tone in patients with inflammatory diseases. In this study, we aimed to find another indication for an increased tone of the sympathetic nervous system using chromogranin A (CGA). CGA binds catecholamines and is responsible for biogenesis of secretory granules. CGA is stored and co-released with catecholamines from dense-core secretory granules of neuroendocrine cells. We investigated 107 healthy controls, 24 patients with rheumatoid arthritis (RA), and 24 patients with systemic lupus erythematosus (SLE). In healthy subjects, CGA levels increased during aging ($R = 0.260$, $P = 0.007$), and it was correlated with plasma norepinephrine levels ($R = 0.296$, $P = 0.004$). This indicates that CGA is a marker of the sympathetic nervous system. In patients with RA and SLE, CGA levels were significantly increased as compared to controls (RA vs. Co: 129.9 ± 10.5 vs. 97.3 ± 9.7 ng/ml, $P < 0.001$; and SLE vs. Co: 129.1 ± 16.7 vs. 79.1 ± 4.9 ng/ml, $P < 0.001$). This difference was particularly evident in female patients of both disease groups. In addition, in patients with RA, we detected CGA-positive synovial cells in the inflamed tissue which were significantly increased as compared to control tissue of patients with osteoarthritis. In conclusion, this study demonstrates a marked increase of serum levels of CGA. The origin of CGA is probably the adrenal gland but inflamed tissue CGA-positive cells might also contribute to elevated serum levels. In summary, increased CGA levels are another indication for an activated sympathetic nervous system.

Bacterial muramyl dipeptide differently affects IL-6 and VEGF production in pituitary folliculostellate TtT/GF cells through NOD2

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The folliculostellate (FS) cells of the pituitary share properties with dendritic cells and astrocytes and produce numerous growth factors (e.g. VEGF, bFGF, TGF-beta) and cytokines (e.g. IL-6, LIF), through which they communicate with hormone producing pituitary cells in paracrine manner. We had demonstrated that FS cells express Toll-like receptor 4 (Tlr4), a key component of the innate immune system. Bacterial lipopolysaccharide (LPS), the ligand of Tlr4 stimulated the release of IL-6 from FS cells which then enhanced ACTH secretion by corticotropes. Recently, nucleotide-binding oligomerization domain (NOD) proteins, NOD1 and NOD2 were found to represent an intracellular component of the innate immune system in dendritic cells and macrophages. We have studied, whether the folliculostellate TtT/GF cell line expresses functional active NODs. By RT-PCR and immunohistochemistry we could demonstrate in TtT/GF cells the presence of NOD1/NOD2 mRNA and protein, respectively. Stimulation of TtT/GF cells with the bacterial cell wall component muramyl dipeptide, the ligand of NOD2, induced a rapid translocation of NF-kappaB into the nuclei. Moreover, NOD2 protein expression was increased in FS cells after 24 h stimulation with MDP. We also observed significantly enhanced IL-6 secretion in the supernatant of TtT/GF cell cultures after stimulation with MDP whereas the production of vascular endothelial growth factor (VEGF-A) was strongly suppressed. Our preliminary data suggest that MDP-induced and NOD2-mediated effects on IL-6 and VEGF-A secretion by FS cells may play a role in modulating pituitary function during bacterial infections. The role of diaminopimelic acid, the ligand of NOD1, in FS cells is still under investigation.

Differential circadian regulation of migratory behavior of lymphocyte and monocyte subpopulations via ß2-adrenoceptors

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Distinct subsets of T cells, NK cells and monocytes, i.e. naïve and effector T cells, immunoregulatory $CD16^{-}CD56^{bright}$ and cytotoxic $CD16^{+}CD56^{dim}$ NK cells, $CD14^{+}CD16^{-}$ and proinflammatory $CD14^{dim}CD16^{+}$ monocytes can be identified in the blood. Previous findings indicate that sympathetic activation (during short-term exercise, mental stress and catecholamine infusion) can exert a profound influence on migratory

behavior of such cell subpopulations. Here we investigated in healthy humans circadian changes in blood counts of lymphocyte and monocyte subpopulations. In parallel, changes in blood catecholamine concentrations as well as the expression of ß2-adrenoceptors in the different subpopulations were analysed to specify the role of catecholaminergic activity in the circadian regulation of cell counts. Plasma norepinephrine and epinephrine concentrations showed the expected 24-h maximum during daytime and were generally decreased during nighttime. Two groups of WBC subpopulations were identified with opposite circadian variations: these were, on the one hand, the naïve T cells, immunoregulatory NK cells and $CD14^{+}CD16^{-}$ monocytes, which showed generally increased numbers during nighttime, and on the other hand the effector T cells, cytotoxic NK cells and proinflammatory monocytes, which showed generally increased numbers during daytime. Circadian variations in counts of the latter types of cells were positively correlated with circadian changes in catecholamine concentrations. Importantly, subpopulations of effector T cells, cytotoxic NK cells and proinflammatory monocytes were not only CD62L negative but also expressed distinctly higher levels of β2-adrenergic receptors than their counterpart subpopulations that showed decreased cell numbers during daytime. Effector T cells, cytotoxic NK cells and proinflammatory monocytes all have effector (mainly cytotoxic) functions in the periphery. Their increased numbers during daytime probably reflect increased mobilization upon daytime catecholamine release. The circadian mobilization of these cells during daytime, like that upon stress, presumably acts to strengthen effector immune defense against potential tissue damage and infection encountered during the active phase.

Alpha-and beta-adrenoreceptors mediate opposing effects on CD4+ lymphocyte cytokine secretion in healthy and arthritic DBA/1 mice

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The sympathetic nervous system confers proinflammatory effects in local lymph nodes in the healthy situation and anti-inflammatory effects in the arthritic situation in the model of collagen-induced (CII) arthritis in DBA/1 mice. Thus systemic sympathectomy with 6-hydroxydopamine before the induction of CII-arthritis leads to a protection, with enhanced IL-10, IL-4, and reduced TNF cytokine levels in local lymph nodes. In contrast, sympathectomy during established arthritis leads to aggravation of arthritis with elevated levels of TNF and IFN in local lymph nodes. To this end, we tested whether cytokine secretion of CD4+ cells from local lymph nodes can be directly modulated by selective adrenergic agonists in the healthy and established arthritis in this mode.Local lymph nodes from 6 to 8-weeks-old DBA/1 mice were collected at day 0 (collagen-naive situation) and at day 60 (established arthritis). CD4+ cells were isolated by MACS bead isolation technique and stimulated with plate-bound anti-CD3 antibodies with addition of different concentrations of isoproterenol (beta-agonist), p clonidin (alpha2-agonist), and methoxamin (alpha1-agonist). Supernatant was collected after 72 h of incubation and cytokines determined by luminex multi-cytokine detection assays.The addition of isoproterenol at day 0 caused a reduction of IL-4 secretion at high concentrations (10^{-5} M/l, $P = 0.026$) and methoxamin enhanced secretion of TNF (10^{-6} M/l, $P = 0.015$) thus creating a pro-inflammatory profile. In contrast in established arthritis on day 60 clonidin (10^{-6} to 10^{-4} M/l, $P < 0.001$), methoxamin (10^{-6} to 10^{-4} M/l, $P < 0.02$), and isoproterenol (10^{-7} to 10^{-5} M/l, $P < 0.001$) mediated an inhibition of TNF secretion, creating an anti-inflammatory profile.Similar to the results of the *in vivo* sympathectomy experiments, *in vitro* stimulation of CD4+ cells from local lymph nodes at day 0 generated a proinflammatory profile mediated by adrenergic beta- and alpah1-stimulation but generate an anti-inflammatory profile during established arthritis. We conclude from this data, that CD4+ lymphocytes from local lymph nodes can be differentially modulated in the context of health and disease.

Repeated administrations of ACTH during late gestation in pigs: effects on HPA axis, brain neurotransmitter systems, behaviour and immune responses in the offspring

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Epidemiological and animal studies indicate that environmental factors acting prenatally on the foetus are important determinants for disorders later in life. There is increasing evidence that maternal glucocorticoids play an important role as a programming factor during prenatal development. We investigated the effects of increased maternal cortisol levels in pregnant sows on central and peripheral alterations of the HPA axis, brain neurotransmitter profiles, open-field behaviour and immune responses of their piglets. Increased endogenous cortisol release was induced in pregnant sows by repeated intramuscular administrations of ACTH every second day during late gestation, whereas control sows received injections of saline. The ACTH treatment of sows significantly increased the birth weight of piglets without affecting gestation length or the number of total born piglets or the frequency of stillborn piglets. There was a suppressive effect of prenatal ACTH administration on lymphocyte proliferation of piglets after birth in

response to the T-cell mitogen ConA and, in tendency, on the proliferation in response to the B-cell mitogen LPS. The ACTH administration to sows also significantly decreased the corticosteroid-binding globulin (CBG) levels and increased the noradrenaline levels in plasma after birth. In addition, glucocorticoid receptor binding after birth was in tendency reduced in the hypothalamus and serotonergic activity in the locus coeruleus region was significantly decreased. During an open-field test, piglets of ACTH-treated sows showed significantly more escape behaviour than control piglets. First results indicate that piglets from ACTH sows respond with higher cortisol and noradrenaline levels than control piglets after an acute restraint stress. In conclusion, prenatal exposure of pigs to increased maternal cortisol levels affect their growth, cell-mediated immunity, HPA axis and neurotransmitter systems. These alterations may account for the increased emotional reactivity and the increased stress response observed during challenging situations.

Peripheral immune consequences of striatal dopamine depletion

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The central dopaminergic system seems to actively participate in neuro-immune interactions but experimental data are scarce. This study was aimed to investigate the impact of experimental dopamine (DA) depletion in the rat striatum on peripheral immune measures in the blood and the spleen. Adult male rats were subjected to stereotaxic surgery and dopaminergic neurons in the striatum were selectively destroyed in both brain hemispheres using the neurotoxin 6-hydroxydopamine (6-OHDA). Noradrenergic neurons were protected by intraperitoneal injection of the NA reuptake inhibitor desipramine. Ten days post surgery, a 5-min open-field task was employed to evaluate locomotion and exploratory behavior. Four days later, the animals were sacrificed to determine the location and the dimension of the neurochemical lesion by TH immunohistochemistry. Leukocyte subsets in the blood and the spleen were analysed by flow cytometry and proliferation and cytokine production of the splenocytes were measured after stimulation ex vivo. In addition, plasma corticosterone and splenic neurotransmitter levels were determined. Behavioral analysis showed that the bilateral DA depletion in the striatum induced strong deficits in exploratory behavior, i.e. increased latency to reach the open-field center, reduced permanency in the center and reduced frequency of center crossings, without affecting locomotion parameters. Moreover, 6-OHDA lesioned rats exhibited marked changes in leukocyte subsets compared to sham lesioned animals and untreated controls. The percentage of $CD3^+$ T cells was significantly increased in the blood and decreased in the spleen. This effect was mainly related to alterations in der $CD4^+$ T helper subset. In addition, the surgical procedure caused a significant reduction in circulating monocytes and NK cells. Splenic DA and DOPAC contents were significantly increased in the 6-OHDA lesioned group whereas plasma corticosterone levels were not affected by this treatment. These findings indicate that the central dopaminergic system is involved in the modulation of peripheral immune responses probably by affecting the peripheral neuro-immune interactions. Changes in exploratory behavior seem to reflect the central DA depletion and can be used as a tool to assess such disturbances *in vivo* without the necessity of pharmacological approaches. The clinical and biological relevance of these findings needs to be determined in future experiments.

A role for neuropeptide Y (NPY) and vascular endothelial growth factor (VEGF) on $CD26^+CD4^+CD25^+$ T regulatory (Treg) cells in the rat

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Strong evidence is emerging that immune responsiveness to self- and allo-antigens can be controlled by naturally occurring $CD45RC^{low}CD4^+CD25^+$ regulatory T (T_{reg}) cells. Recently, the G protein-coupled receptor (GPR)83 – a homologue to tachykinin and neuropeptide Y (NPY) receptors – as well as Neuropilin-1 (Nrp1) – a class III semaphorin subfamily and vascular endothelial growth factor (VEGF) receptor have been described as surface markers with potentially suppressive activity, being possibly useful to distinguish T_{reg} from both naïve as well as recently activated $CD4^+CD25^+$ T cells in mice. Here, we characterized the expression of Nrp1, gpr83, CD26 and other Y receptors on rat T_{reg} cells focussing on the species *rattus norvegicus* as these animals a priori represent frequently used animal models e.g. for asthma. Furthermore, we examined the role of the $Nrp1^+$ subpopulation in a rat model of allergic asthma. As CD26 plays a crucial role in T cell activation and is also expressed on T_{regs}, we used wild type F344/Ztm as well as CD26 deficient F344/GER rats in our study. $Nrp1^+$ T cells were characterized in peripheral blood, lung, and the draining lymph node by FACS analysis over 24, 48 and 72 h after OVA challenge, as well as in cell culture experiments for IL-10, IFN-γ and TGF-ß levels.

Composition of T_{regs} in healthy control rats revealed that these cells could further be divided into Nrp1 positive and negative subpopulations. Interestingly, CD26 deficient rats exhibited higher numbers of $Nrp1^+$ T_{regs} than wild type rats. This $Nrp1^+$ subpopulation had a higher capacity to produce IL-10 and IL-4 in both substrains compared to $Nrp1^-$ cells. $CD45RC^{low}CD4^+CD25^+$ cells were increased in lungs of both substrains not until 48 h after OVA challenge. However, a further division of these cells into $Nrp1^+$ and $Nrp1^-$ populations revealed that $Nrp1^+$ cells were significantly increased only in CD26 deficient rats and showed specific expression pattern of NPY, NPY Y1, Y2 and Y5 as well as GPR83 receptors. Analogous, at this time point, supernatants from lungs of CD26 deficient rats showed higher levels of IL-10 and TGF-ß. These finding support the hypothesis that the adrenergic cotransmitter NPY may modulate immune responses of the lungs for example during stress via differential Treg activation. Funded by: grants of the DFG, SFB 587, B11

Acute effects of intranasal oxytocin on endocrine parameters and peripheral immune functions in males

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The neuropeptide Oxytocin (OT) has repeatedly been shown to affect behaviour, including learning and memory and social interactions. However, there has been no investigation of the impact of OT on human immune functions. The purpose of this study was to elucidate the effects of acute intranasal OT administration on cardiovascular and endocrine parameters and peripheral immune functions. In a double blind, placebo controlled, balanced cross-over design study, 10 healthy men were randomly assigned to receive intranasal OT (24 IU) or placebo. Cellular composition of peripheral blood and cytokine production of isolated peripheral mononuclear blood cells (PBMC) after Concavalin A (ConA) stimulation by flow cytometry were continuously assessed for 75 min. OT plasma levels significantly increased after intranasal OT administration. In addition, 50 min after OT-treatment monocyte numbers in peripheral blood were significantly increased. Both, the OT-treated group as well as the placebo control group displayed significant changes in IL-12 and IFN-y secretion over time. However, none of these effects were correlated with the increase in monocyte numbers 50 min after OT administration. Plasma levels of dopamine, epinephrine, nor-epinephrine and cortisol did not significantly differ between subjects that received a single dose of intranasal OT or placebo. These data indicate that intranasally administered OT significantly elevated plasma OT levels and increased monocyte numbers in peripheral blood, however, does neither affect sympathetic activity nor glucocorticoid production. Follow-up studies will analyse monocyte activation and cytokine production after OT administration *in vivo* and *ex-vivo*.

Cardiovascular and pupillary autonomic nervous dysfunction and mortality in patients with rheumatoid arthritis

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Autonomic nervous dysfunction carries an increased risk of mortality in diabetes mellitus. In rheumatoid arthritis (RA) patients, the association between cardiovascular (CAD) or pupillary autonomic dysfunction (PAD) and mortality has never been investigated. Between 1997 and 1998, 33 RA patients were examined for baseline characteristics, and parameters of CAD and PAD. Thirty patients have been re-evaluated 8.3 ± 0.1 year later using a telephone questionnaire (response rate = 91%). During the 8-year observation period, 4/30 RA patients died (13%) due to heart failure ($n = 1$), immunodeficiency / infection ($n = 1$), and sudden deaths ($n = 2$). Non-survivors as compared to survivors had increased heart rate variation in the respiratory arrhythmia test ($P = 0.038$, hyper reflexia) but largely decreased heart rate variation in the lying-to-standing test ($P = 0.009$). Non-survivors as compared to survivors demonstrated more frequent pupillary autonomic dysfunction (100% vs. 42%, $P = 0.035$). Fifteen patients were diagnosed with PAD, and mortality was significantly higher in patients with PAD than without PAD (27% vs. 0%, $P = 0.035$). Six patients were diagnosed with CAD (20%), and mortality tended to be higher as compared to patients without CAD (33% vs. 8%, $P = 0.113$). This study demonstrates that diagnosis of PAD was associated with an increased mortality risk in patients with RA. Patients with a poor test result in the lying-to-standing test are also at increased risk of death. This study in RA patients demonstrates similar results as in patients with diabetes mellitus.

Peripheral neuroimmue interactions

Role of cutaneous opioid receptors in pruritus

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Opioid receptors are key players in induction of chronic itch. This could be confirmed using opiate receptor

knockout mice experiments and clinical studies on patients with chronic itch. We have induced a dry skin dermatitis as a model for chronic itching on μ- (MOR) and κ- (KOR) opioid receptor knockout (KO) mice. MOR KO mice scratched significantly less than wild type (WT). Additionally the epidermal hypertrophy caused by chronic dermatitis and the amount of epidermal nerve endings in MOR KO mice were significantly decreased than in WT mice. KOR KO mice showed similar scratching behavior as MOR KO mice; however the changes were less significant. In addition, we performed a double blind, placebo controlled, cross over study using topically applied opioid receptor antagonist, Naltrexone, on patients with pruritus in atopic dermatitis. The results revealed significant effects of the topical application of Naltrexone in patients with chronic pruritus (45% improvement of pruritus by VAS compared to placebo, $n = 24$), but not in patients with acute pruritus (7%, $n = 15$). These studies establish the clinical relevance of MOR system and the peripheral, epidermal nerve endings in chronic pruritus and warrant further research and therapeutic potential for such research.

Erythropoietin and the hair follicle: a tissue protective cytokine

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In recent years, it has been recognized that the main regulator of the red blood cell production, erythropoietin (EPO) has multiple functions beyond the erythropoiesis and also outside of the bone marrow. EPO serves as a tissue-protective cytokine in several cell populations (endothelial cells, neurons, tubular epithelial cells in the kidney) and it has been shown to inhibit apoptosis in pathological circumstances (ischemic and toxic stress). EPO has been even reported to stimulate wound healing and angiogenesis in mice. Therefore, here we addressed the question, whether EPO and its receptor (EPOR) have any relevance in human skin and hair follicle biology under normal, hypoxic or pathologic conditions. To investigate the expression of EPO/EPO-R in human skin, we have studied whether normal human scalp skin expresses EPO and/or EPO-R transcripts. By real time RT-PCR, we detected specific PCR products for both EPO (132 bp) and EPOR (315 bp) in human scalp skin. Even stronger EPO and EPO-R transcript signals have been found in microdissected human anagen VI hair bulbs. By immunohistology (EnVision-AP method), specific EPO immunoreactivity was confined to the skin epithelium. EPO immunoreactivity was most prominent in the central outer root sheath of anagen VI hair follicles, while epidermal and hair matrix keratinocytes showed only a faint signal. As EPO synthesis is induced by hypoxia via the hypoxia induced factor (HIF) in the kidney, we performed a PCR analysis of hypoxia treated hair follicles. We have found that -just like in the kidney- hypoxia up-regulated the EPO expression, since the amount of EPO and HIF mRNA remained unchanged. In order to identify possible target genes for EPO in human dissected hair follicles, a microarray analysis was performed on EPO treated follicles. Our further data shows that the treatment with recombinant human EPO did not change hair growth and matrix keratinocyte proliferation. However, chemotherapy-induced intrafollicular apoptosis in situ was inhibited by prior and concomitant EPO administration. Here we provide the first evidence that normal human skin expresses EPO and functional EPO-R in situ and that human scalp hair follicles are important sources and targets of EPO/EPO-R signaling and that EPO may serve as an endogenous hair follicle cytoprotectant.

Expression and functional significance of HPA axis components in human basophils

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The basophil is regarded as an important cellular player in a number of allergic and immune-mediated diseases but its regulation by neuropeptides is poorly understood. By using (i) the immature human basophil cell line KU812 as an *in vitro* model; (ii) purified human basophils from normal individuals; and (iii) basophil activation assays of leukocytes derived from normal individuals and patients with type I allergies, we investigated the expression and functional significance of several components of the HPA axis in this cell type. The melanocortin-1 receptor (MC-1R) which mediates the actions of α-melanocyte-stimulating hormone (α-MSH) and adrenocorticotropin (ACTH) could be identified in both KU812 cells and isolated human peripheral blood basophils. The MC-1R expressed by basophils was functional as α-MSH induced intracellular cAMP dose-dependently and also inhibited IL-4 expression. α-MSH, ACTH but not C-terminal peptides of α-MSH blocked basophil activation induced by artificial stimuli or natural allergens as measured by CD63 surface expression. This effect of α-MSH was abrogated by pharmacological blockade of the MC-1R. Expression

analysis of other HPA components in KU812 basophils revealed lack of proopiomelanocortin expression while corticotropin-releasing hormone receptor II and enzymes of the steroid biosynthesis pathway including Cyp11A1, Cyp17 and Cyp21A2 were detectable. Conditioned media from KU812 basophils contained significant amounts of progesterone, 17-OH progesterone but neither cortisone nor cortisol suggesting that the former sex hormones may maintain the Th2 effector milieu of basophils. Our data highlight novel interactions between neuropeptides, sex hormones, and effector functions of basophils which may have novel implications for the pathophysiology of allergic diseases and their future therapeutic intervention.

Immune-regulatory role of PPAR-alpha in the epidermis

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Previously, peroxisome proliferator activated receptor (PPAR)-alpha activation has been shown to exert anti-proliferative, pro-differentiating and anti-inflammatory effects in the epidermis. However its immune-regulatory role in the epidermis was unknown. We here report an exaggerated ear swelling response to contact allergens in mice lacking PPAR-alpha. Whereas PPAR-alpha was detectable in keratinocytes and immature Langerhans cells isolated from wild-type epidermis, there was a decrease in PPAR-alpha during LC maturation. Pharmacologic PPAR-alpha activation delayed LC maturation. In contrast, this effect was not observed in PPAR-alpha knock out mice, indicating receptor specificity. Nevertheless, LC maturation was normal in LC isolated from PPAR-alpha deficient animals, presumably due to compensatory mechanisms. Furthermore, PPAR-alpha activation reduced the migratory capacity of LC, their production of cytokines and their ability to drive T cell proliferation. Moreover, activation of PPAR-alpha inhibited NF-kappaB but not SAPK/JNK, p38MAPK and ERK1/2. NF-kappaB inhibition was abolished in PPAR-alpha deficient mice and decreased when DNA-binding of PPAR-alpha was blocked, indicating a transcriptional effect. We conclude that PPAR-alpha activation by endogenous ligands may provide a molecular signal that allows LC to remain in an immature state within the epidermis for extended periods of time despite minor environmental stimuli. PPAR-alpha could be involved in the counterbalance of ongoing immune responses, by preventing tissue destruction after ligand activation by arachidonic acids and derivatives released from damaged cells.

In vitro adrenergic and glucocorticoid regulation of cytokine production in inflammatory bowel diseases (IBD)

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Psychological stress and depressive symptoms have been found to play a role in disease initiation and exacerbation in inflammatory bowel diseases (IBD); however, the mechanisms still remain unclear. The goal of this project was to test the hypothesis that peripheral neuro-immune interactions are disturbed in patients with IBD. A total of $n = 56$ IBD patients (Ulcerative Colitis UC, $n = 26$ / Crohn's disease MC $n = 30$) in clinical remission or in the acute phase of the disease, and $n = 19$ healthy controls were recruited. Clinical, psychological, and sociodemographic parameters were assessed and blood samples were drawn to analysed basal LPS-stimulated cytokine production, as well as the *in vitro* effects of the β-adrenergic agonist terbutaline and of the glucocorticoid agonist dexamethasone on LPS-stimulated TNF-α and IL-10 production and of the glucocorticoid agonist dexamethasone on LPS-stimulated TNF-α production by peripheral blood cells. The results revealed no differences between Ulcerative Colitis or Crohn's disease or between males and females. Therefore, the data were pooled to address effects of disease status. Interestingly, whereas controls showed a significant and concentration-dependent increase in IL-10 production in response to terbutaline, this response was significantly diminished both in IBD in remission as well as in active IBD. However, the response was significantly more diminished in active IBD compared to IBD in remission. At the same time, concentration-dependent suppression of TNF-α production by both terbutaline and dexamethasone was comparable in both IBD patient groups and controls. These data provide further evidence that adrenergic-immune interactions are disturbed in IBD, irrespective of the type of diagnosis. Given the pivotal role of pro-and anti-inflammatory cytokines in the pathophysiology of IBD, our findings of disturbed adrenergic IL-10 regulation, which was particularly pronounced during disease exacerbation, could be part of the mechanism(s) underlying the modulation of disease activity by psychological factors.

The blockade of neuropilin-2 as a new therapeutic principle in arthritis

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In earlier studies a significant loss of sympathetic nerve fibers in patients with rheumatoid arthritis (RA) was observed. Sympathetic neurotransmitters in high concentrations exert anti-inflammatory effects. Thus, their loss probably aggravates inflammation. In previous work two probable molecules involved in the repulsion of sympathetic nerve fibers were identified. These are semaphoring 3C (SEMA 3C) and placental growth factor (PLGF) which bind the receptor neuropilin-2. It is the main goal in this project to neutralize neuropilin-2 and to study the effects of inhibition of this important receptor *in vitro* and *in vivo*. The blockade of only one particular nerve repellent factor may be without an effect *in vivo* because it is known that different factors like SEMA 3C, PLGF and VEGF bind the neuropilin-2 receptor. Therefore a soluble neuropilin-2 Fc fusion construct was generated and the effect of this Fc-fusion construct will be tested in the neurite outgrowth assay. Immunohistochemistry was used in order to study the presence of neuropilin-2 in synovial tissue of patients with RA and osteoarthritis (OA) and to show that neuropilin-2 plays an important role in the repulsion of sympathetic nerve fibers. Therefore a self-manufactured antibody against neuropilin-2 was used. A neurite outgrowth assay for sympathetic nerve fibers was established to be able to test the neuropilin-2 Fc-fusion construct *in vitro*. Therefore sympathetic ganglia of mice were removed under microscopical control. Ganglia were digested with dispase and they were set in matrigel which was growth factor reduced. To support the growth of nerve fibers on this gel a neurobasal medium which contained mouse ß-NGF (nerve growth factor) was added.It could be shown that neuropilin-2 is expressed on sympathetic nerve fibers. The staining of neuropilin-2 is concordant with earlier studies in which was shown a significant loss of sympathetic tyrosine hydroxylase positive nerve fibers in patients with RA as compared to patients with OA. This result confirms the assumption that a repulsion of sympathetic nerve fibers takes place in inflamed tissue of RA patients and not a downregulation of tyrosine hydroxylase is responsible for the disappearance of tyrosine hydroxylase positive nerve fibers. Furthermore it was possible to establish a neurite outgrowth assay which can be used to test our neuropilin-2 Fc-fusion construct *in vitro*.

The indoleamine melatonin suppresses caspase activation in UV-irradiated human keratinocytes

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The indoleamine melatonin is a hormone with strong antioxidant activities that helps attenuate the effect of oxidative stress in conditions such as neurodegenerative disorders, ischemia/reperfusion, sepsis, aging and UV induced damage. Most recently, a role for melatonin in cutaneous biology has also been uncovered with the skin expressing a full melatoninergic system that appears to provide protective effects against UV-induced damage. This UV-protection may be mediated by melatonin radical scavenging properties and/or its antiapoptotic effects. We have now used human keratinocytes to investigate if melatonin prevents UV-induced apoptosis at the cell morphology level; we tested intrinsic and extrinsic apoptotic pathways as well as mitochondrial membrane potential. Pretreatment with melatonin in UV-irradiated keratinocytes resulted in a higher degree of cell confluency, less cell blebbing, less dysmorphic shape and less nuclear condensation compared to untreated controls. Melatonin also reduced the UV-induced decrease of mitochondrial membrane potential ($\Delta\psi$) and, consequently, activation of initiator caspase 9 and effector caspases 3 and 7 was suppressed. Furthermore, activation of poly (ADP-ribose) polymerase (PARP) was also attenuated. Keratinocytes as the constitutive cell population of a peripheral organ are extensively exposed to UV-irradiation. Thus, melatonin, a neuroendocrine substance, does act on keratinocytes where it prevents UV-induced damage through actions at different cellular levels. Since human skin expresses a fully developed melatoninergic system, melatonin may represent an important local protective agent against UV-induced damage.

Interactions between nerve growth factor, immune-derived products and sympathetic innervation in an animal model of autoimmune lymphoproliferative disease

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Fas-deficient, lpr/lpr mice are often used as a model of the human autoimmune disease systemic lupus erythematosus. We have recently shown that the sympathetic innervation is gradually lost in the spleen of C57Bl/6

lpr/lpr mice as the disease progresses. Since nerve growth factor (NGF) is essential for survival, differentiation and functional activity of sympathetic neurons and it has been shown that circulating levels of NGF are altered in lupus patients, we started to investigate whether this neurotrophin plays a role in the sympathetic alterations that parallel the development of the disease in lpr/lpr mice. NGF concentration in the spleen of lpr/lpr and normal C57Bl/6 mice was determined by ELISA. While no significant differences between control and lpr/lpr mice were observed in 2-week-old animals, we have found that NGF concentration is decreased in the spleen of 27 and 40-week-old lpr/lpr mice, i.e. when clinical symptoms are overtly manifested. These results suggest a link between decreased NGF levels and the stage of the disease. We are at present evaluating NGF concentration in other organs (thymus, lymphnodes, heart and adrenal glands) during ontogeny. We also studied whether lymphoid cells from C57Bl/6 and lpr/lpr mice produce factors that can affect neurite outgrowth. For this purpose, the well-established model of NGF-induced differentiation of the rat pheochromocytoma PC12 cell line was used as a test system. Cells obtained from the spleen of 9 and 27-week old control and lpr/lpr mice were stimulated with concanavalin A or lipopolysaccharide and the supernatants were collected 48 h later. Additional supernatants from non-stimulated cell were prepared in parallel. The different supernatants were added to PC12 cells in the presence of NGF and neurite outgrowth was evaluated stereologically (grid-intersection analysis). So far, the most interesting observation was that NGF-induced neurite outgrowth was completely inhibited when PC-12 cells were cultured in non-diluted supernatants, independently of their source. A clear decrease of neurite outgrowth was still observed when supernatants were diluted 10-fold. Further studies are needed to identify the immune-derived products responsible for this effect.

The nerve repellent factor semaphorin 3 C and the distribution of sympathetic and sensory nerves in the colon in Crohn's disease

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Sympathetic neurotransmitters in micromolar concentrations exert anti-inflammatory effects, whereas substance P (SP) is proinflammatory. Loss of sympathetic and sprouting of SP+ nerve fibers is unfavorable in inflammation. This study investigated the behavior of these nerve fibers in Crohn's disease (CD). The expression of the sympathetic nerve repellent semaphorin 3C (SEMA3C) was studied in addition. Thirteen patients with CD and 22 control subjects were included. A immunofluorescent histomorphological analysis of nerve fibers was carried out in different colon layers. An antibody to SEMA3C was developed and SEMA3C expression was studied. In all layers of the colon, CD patients demonstrated a loss of sympathetic nerve fibers, which was found in macroscopically inflamed and non-inflamed areas. In the submucosa, the number of vessels positive for sympathetic nerve fibers was reduced in CD compared to controls. Neutrophil infiltration was related to loss of sympathetic vessels. SEMA3C was detected in epithelial cells, and there was a marked increase of SEMA3C – positive crypts in the mucosa of CD compared to controls. The number of SEMA3C – positive crypts negatively correlated with density of mucosal sympathetic nerve fibers. In contrast, there was marked sprouting of SP+ nerve fibers in the mucosa. This study demonstrated a loss of sympathetic and an increase of SP+ nerve fibers in CD. SEMA3C, a sympathetic nerve repellent factor, is highly expressed in the epithelium of CD patients. The loss of sympathetic nerve fibers and the increase of SP+ nerve fibers is probably a proinflammatory signal.

Involvement of the non-neuronal cholinergic system in acne inversa pathogenesis

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Acne inversa (AI) is a chronic inflammatory skin disorder emerging from the follicular infundibulum. Since smoking is a well known risk factor of AI and nicotine is the main toxin in cigarette smoke, we wanted to determine the role of nicotinic acetylcholine receptors (AChR) in AI. We have shown previously that stimulation of nicotinic AChR causes epidermal hyperplasia *in vitro*. In the intertriginous areas of smokers, nicotine reaches higher concentrations than elsewhere on the skin, because it is secreted in apocrine and eccrine sweat and applied occlusively. We compared the epithelial thickness at representative points of healthy intertriginous skin and AI patients. Healthy skin showed a mean of 50 μm while AI epidermis was significantly thicker with a mean of 149 μm. Using double label immunofluorescence we could demonstrate specific patterns for epidermal acetylcholine receptors in different patterns of both healthy and lesional skin with strongest AChR density around the AI infundibulum. In lesional epidermis the choline-acetyltransferase (ChAT)-reactivity was particularly pronounced in the epidermal basal layer, while in the sinus tracts, ChAT reactivity was extended to all epithelial layers. ChAT could not be detected in macrophages *in vitro* or *in vivo* indicating that macrophages do not actively contribute to ACh signa-

ling in AI but may rather be targets of ACh and hence nicotine induced immunomodulation. We found alpha5, alpha7 and alpha10 nAChR subunits present in blood monocytes of both healthy and AI patients while other subunits showed a scarce and variable expression. For the first time, we could demonstrate the presence of alpha3, alpha5 and alpha7 nAChR immunoreactivity in variable amounts on differentiated macrophages in lesional skin. Altogether in the pathogenetic center of AI, the follicular infundibulum, we found the highest concentration of AChR and the highest endogenous ACh production that may act synergistically with tobacco delivered nicotine in aggravating follicular hyperkeratosis and follicular plugging and thus initiating AI lesions. In addition, cholinergic modulation of macrophage functions is a plausible explanation for the observed hitherto unexplained chronicity of AI.

Stress activated cutaneous mast cells hold a central switchboard position in skin inflammation

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A prominent 'brain-skin connection' as well as a local neuro-endocrine-immune circuitry promote the pathogenesis of inflammatory skin diseases that are triggered or aggravated by stress. In stressed C57Bl/6 mice, nerve growth factor (NGF), substance P (SP) and mast cells are recruited hierarchically to induce neurogenic skin inflammation. Strikingly, in mast cell deficient or neurokinin-1 receptor knock out mice, stress exposure fails to induce skin inflammation. In the present study, the technique of retrograde tracing has been employed in a murine model to unveil that stress-triggered skin inflammation of the skin results in neuronal plasticity, mirrored by alterations of substance P $(SP)^+$ and calcitonin gene related peptide $(CGRP)^+$ neurons in dorsal root ganglia. We observed that stress exposure perpetuates the secretion of murine skin mast cell derived tryptase *in vivo*, which could be mimicked *in vitro* by co-culture of mast cells with SP or CGRP. Hence, we substantiate the central switchboard position of skin mast cells within the 'brain-skin connection', since mast cells evidently serve as target as well as effector cells within the pathology of skin inflammation in response to stress. We further propose that cutaneous stress research in mice and men serves as a model pivotal for studying the cross-talk between peripheral and systemic responses to psychological stress, since the skin's stress response patterns are easily discerned by the naked eye and tissue sampling for in-depth analysis is reasonably simple.

The neuropeptide calcitonin-gene related peptide (CGRP) reduces hair shaft elongation and induces catagen development in isolated human hair follicles *in vitro*

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The perifollicular network of sensory nerve fibers contains neuropeptides such as vasointestinal peptide (VIP), substance P (SP) and calcitonin-gene related peptide (CGRP) and is subject to hair-cycle-associated changes. Here we have investigated whether and how CGRP influences the human hair follicle *in vitro*. Human isolated hair follicles were cultured in the presence of CGRP (10^{-7}M) alone or with the neutral endopeptidases (NEP)-inhibitor phosphoramidon and hair shaft elongation, hair cycle stages, hair matrix keratinocyte proliferation and MHC class I expression was assessed. Hair shafts grew significantly less upon addition of CGRP and phosphoramidon compared to controls. Quantitative histomorphometry revealed induction of mid and late catagen upon supplementation with CGRP plus phosphoramidon. This was accompanied by regression of the pigment producing follicular melanocytes, whereas this was not the case when the NEP inhibitor was omitted. The number of proliferating (Ki67 positive) matrix keratinocytes in CGRP treated hair follicles was significantly elevated compared to untreated hair follicles, while intrafollicular apoptosis (TUNEL positive cells) was unaffected. When CGRP degradation was inhibited by phosphoramidon, however, the number of proliferating matrix keratinocytes dropped significantly. In an additional set of experiments we tested whether CGRP exerts immune modulatory effects in the human hair follicle. As detected by quantitative immunohistochemistry, CGRP did not affect the MHC class I expression in the immune privileged hair follicle epithelium. Intriguingly, pre-incubation with CGRP significantly reduced IFNγ-induced up-regulation of MHC class I expression in the epithelial compartment. In summary, our results agree with earlier findings in murine skin organ culture where CGRP inhibited the anagen progression and development. Moreover, they hint towards a dose-dependent effect of CGRP which should be the subject of future studies. This *in vitro* model gives a promising example how the neuropeptide CGRP can directly promote catagen induction in human hair follicles and may lead to hair loss without direct deterioration of the immune privilege.

Murine mast cells prime the development of cytotoxic T cell responses by epicutaneous peptide vaccination with TLR7 ligand as adjuvant

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Until recently, IgE-activated mast cells have been regarded merely as effector cells of adaptive immune responses, involved in allergic reactions and mucosal immunity to some parasites. Herein we report that murine dermal mast cells, activated by local administration of a creme containing the synthetic TLR7 ligand imiquimod, are essential to initiate an early inflammatory reaction. The mast cell-derived cytokines TNF-α and IL-1β play an important role in this process. Furthermore, TLR7 activated mast cells are also able to promote the emigration of Langerhans cells, which partly depends on the expression of mast cell-derived IL-1β. We have previously shown that TLR7 ligation enhances transcutaneous immunization evoked by topical application of vaccine antigens to the skin, a procedure which directly targets skin-resident antigen presenting cells. Consequently, we now demonstrate here that the capacity to mount a full-blown peptide-specific cytotoxic T lymphocyte response following transcutaneous immunization employing imiquimod as adjuvant is severely impaired in mast cell-deficient mice. Thus, these findings demonstrate the potent versability of alternatively activated mast cells at the interface of innate and adaptive immunity. This work was supported by the Deutsche Forschungsgemeinschaft grant SFB548 A10 and STA 984/1-1 (M. Stassen)

Stress-triggered skin inflammation and maturation of dendritic cells can be abrogated by blockade of ICAM-1/LFA-1 cross talk

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The skin is an active immunological organ, continuously serving as a biosensor of multiple exogenous stressors. Superimposed on this is the impact of endogenous stressors such as perceived stress, which challenges the skin immune integrity and can induce or aggravate immune dermatoses. We previously reported that the neuropeptide substance P (SP) is involved in stress-triggered neurogenic inflammation in murine skin. Interestingly, a modulation of antigen presentation by the autonomic nervous system can be deduced from spatial adjacencies of SP^+ nerve fibers and antigen presenting dendritic cells (DC) in various tissues. Thus, we now aimed to unveil whether exposure to stress (sound) in C57Bl/6 mice challenges the cardinal role of skin DC in orchestrating immune responses. We observed a skew towards maturation of skin DC in response to stress, mirrored by the up-regulated expression of MHC class II and intercellular adhesion molecule-1 (ICAM-1), accompanied by an altered barrier function. Since the cross talk between costimulatory molecules on DC, such as ICAM-1, and the respective ligand leukocyte function-associated antigen-1 (LFA-1) on T cells is crucial for T cell activation and inflammation, we blocked ICAM-1/LFA-1 mediated intercellular adhesion events in our murine model, which significantly abrogated stress-induced skin inflammation. The relevance of the above findings was further corroborated by showing that blocking of LFA-1/ICAM-1 also significantly reduced stress associated keratinocyte apoptosis and endothelial expression of ICAM-1. In conclusion, skin DC mature within the adaptation cascade to perceived stress. However, it remains elusive whether such adaptation processes to stress are still physiological responses, which may promote the generation of DC-dependent pathogen-specific immune responses at antigenic portals such as the skin. On the other hand, chronic adaptation to stress may induce immune dermatoses, and a cardinal cell subset such as skin DC could aggravate autoimmunity or chronic inflammation.

Evidence of a modulatory effect of melanocortin peptides on bleomycin-induced collagen synthesis in human dermal fibroblasts

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The melanocortins α-, β-, and γ-melanocyte-stimulating hormone (α-, β-, γ-MSH) as well as adrenocorticotropic hormone (ACTH) elicit their biologic effects via binding to melanocortin receptors (MC-Rs). Previously, we demonstrated that not only melanocytes but also fibroblastic cell types of the skin including human dermal fibroblasts (HDF), dermal papilla cells and periadnexal fibroblasts express MC-1Rs. Besides eliciting anti-inflammatory effects, α-MSH was shown in HDF to suppress transforming growth factor-β_1 (TGF-β_1)-induced collagen synthesis. To consolidate the antifibrogenic potential of melanocortins we set out to investigate the effect of α-MSH, its superpotent analogue NDP-α-MSH, and ACTH on bleomycin-induced collagen turnover in HDF *in vitro*. All melanocortin peptides significantly reduced the steady-state levels of collagen type I and III in cells exposed to

non-cytotoxic bleomycin doses. The effect of α-MSH and related peptides on collagen synthesis was mimicked by the artificial cAMP inducer forskolin suggesting that the cAMP pathway is pivotal for the observed effect of the various melanocortins. The upregulating effect of bleomycin on collagen transcription was not mediated via activation of the smad signalling pathway which is typically induced by TGF-β_1. Actinomycin D profoundly attenuated the inductive effect of bleomycin on collagen synthesis. Assuming that bleomycin acts as an electrophilic agent and oxidant we next performed coincubation studies with various anti-oxidants including ascorbic acid, melatonin and dithiotreitol which uniformly blocked the upregulating effect of bleomycin on collagen transcription. In summary, these findings expand our current knowledge on the modulatory effect of melanocortins on collagen turnover and implicate an anti-oxidative mechanism being responsible for the suppressive effect of these peptides in bleomycin-induced collagen synthesis.

PolyI:C-induced maternal immune challenge during pregnancy affects basal cytokine levels in the adult offspring

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Epidemiological and experimental evidence links abnormal priming of the immature immune system to a higher incidence of immunological dysfunctions in adulthood, including allergy and parasite infections. It has been suggested that antigen exposure in early life may bias adult cytokine responses towards a particular phenotype, thereby increasing the risk for immunopathology in later life. However, it is unknown to date whether adult cytokine imbalances can also result from the prenatal exposure to an unspecific cytokine-associated immune challenge. Here we demonstrate in mice that the maternal exposure to the cytokine-releasing agent polyriboinosinic–polyribocytidilic acid (PolyI:C) on gestation day (GD) 9 or 17 reduces the serum levels of interleukin (IL)-2 and interferon (IFN)-gamma in the adult offspring. In contrast, granulocyte macrophage-colony stimulating factor (GM-CSF) was exclusively decreased in adult offspring subjected to prenatal PolyI:C exposure on GD17, whereas IL-4, IL-6, IL-7, IL-10 and tumor necrosis factor (TNF)-alpha remained unaffected after both interventions. These results highlight for the first time that the induction of pro-inflammatory cytokine responses in the maternal and/or fetal systems leads to long-lasting changes in basal serum cytokine levels of the adult offspring. Moreover, the specificity of this effect is influenced by the precise times of prenatal immune activation.

Perspectives in hair follicle neuroendocrinology

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Here, we summarize recent neuroendocrinological findings from our own and collaborating laboratories that extend endocrine research in hair follicle biology 'beyond the androgen horizon'. We focus on the role of human hair follicles as sources of various neuroendocrine mediators. Recently, we have shown that normal human scalp hair follicles in anagen VI (i) synthesize CRH, ACTH, alpha-MSH and cortisol and display a fully functional equivalent of the hypothalamus-pituitary-adrenal axis; (ii) synthesize melatonin, up-regulate melatonin synthesis and secretion upon stimulation with noradrenaline (just like in the pineal gland), and express melatonin receptors in a hair cycle-dependent manner; and (iii) synthesize prolactin, express functional prolactin receptors, and respond to their stimulation by premature catagen induction. In addition, evidence is presented that human scalp hair follicles may have established yet another hypothalamus-pituitary axis equivalent, the TRH-TSH system. Studying the effects of calcitonin gene-related peptide (CGRP), a key skin neuropeptide associated e.g. with stress responses and neurogenic inflammation, on organ-cultured human hair follicles, we also noted that CGRP inhibits both hair matrix keratinocyte proliferation in situ and hair follicle pigmentation, while the prototypic stress-associated neuropeptide, substance P, induces a collapse of the immune privilege of human anagen hair follicles. In turn, alpha-MSH is portrayed as a neuroendocrine 'guardian of immune privilege'. These recent findings are discussed in the context of how the organ-culture of human scalp hair follicles can be exploited for addressing questions of general importance in human dermatoendocrinology.

The eosinophil – a novel target for the neuropeptide alpha-melanocyte-stimulating hormone

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There is increasing evidence that melanocortins such as alpha-melanocyte stimulating hormone (alpha-MSH) can elicit potent modulatory effects on various cells of the peripheral blood thus emphasizing interconnections between the neuroendocrine and the immune system. Although modulatory effects of alpha-MSH have been previously reported on human and rat neutrophils however nothing is known on the impact of this melanocortin peptide in human eosinophils. In order to check if eosinophils are

target cells for melanocortins we purified peripheral blood eosinophils from non-atopic healthy subjects and patients with atopic dermatitis (AD, extrinsic type, defined by the criteria of Hanifin and Rajka) by CD16-negative selection (>98% purity). RNA expression of the precursor protein for melanocortin peptides, proopiomelanocortin (POMC), as well as expression of the melanocortin-1 receptor (MC-1R) was subsequently assessed by conventional RT-PCR and real-time PCR. Surface expression of MC-1R on eosinophils was also determined by flow cytometry using an antibody against the amino acids 2–18 of the N-terminal domain of human MC-1R. Release of superoxide anions was assessed by lucigenin-dependent chemiluminescence (Hamamatsu) and expression of beta2-integrin CD11beta, a cell adhesion molecule and marker of eosinophil activation, was examined by flow cytometry. Peripheral blood eosinophils from both healthy individuals ($n = 8$) and patients with AD ($n = 7$) expressed MC-1R as well as POMC at similar RNA levels. However, MC-1R expression at protein levels was significantly lower in patients with AD ($n = 5$) compared with those from healthy individuals ($n = 5$). Functional studies revealed a dramatic release of superoxide anions by stimulation with complement factor C5a ($P < 0.001$) which was significantly inhibited by alpha-MSH ($P < 0.001$). Preincubation with agouti signalling peptide (ASP), a natural MC-1R antagonist, completely reversed this effect of alpha-MSH ($P < 0.01$). Further, alpha-MSH significantly inhibited IL-3 induced up-regulation of CD11beta in both non-atopic and atopic dermatitis eosinophils ($P < 0.05$). These data show a novel anti-inflammatory activity of alpha-MSH as demonstrated by inhibition of eosinophil activation and function by the peptide. Endogenously produced melanocortins may thus function as modulators of eosinophilic inflammation. Moreover, exogenously applied melanocortins may be useful for future immunointervention of eosinophilic disorders.

In vitro characterization of the vitamin D endocrine system in human sebocytes

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Extrarenal local synthesis of biologically active vitamin D metabolites has been shown to be of critical importance for regulation of growth, differentiation and other cellular functions in a broad variety of tissues. In this study, we have investigated whether sebocytes are target cells for biologically active vitamin D metabolites, and whether they possess the enzymatic machinery for the local synthesis and metabolism of the biologically active vitamin D metabolite $1,25(OH)_2D_3$. Using real time PCR we detected VDR expression in SZ95 sebocytes. Incubation with $1,25(OH)_2D_3$ resulted in a dose-dependent regulation of cell proliferation (crystal violet dye uptake and MUH techniques), whereas inhibition of SZ95 sebocyte proliferation (up to 30%) occurred under serum-supplemented and stimulation under serum-free conditions. Moreover, modulation of cell cycle regulation and of apoptosis was detected by flow cytometry. In addition, minor changes on SZ95 sebocyte lipids (nile red fluorescence assay) but marked inhibition of IL6 and IL8 secretion (ELISA) resulted after incubation of SZ95 sebocytes with $1,25(OH)_2D_3$. RNA for vitamin D-25-hydroxylase, 25-hydroxyvitamin D-1α-hydroxylase (1αOHase) and 1,25-dihydroxyvitamin D-24-hydroxylase (24OHase) was detected in SZ95 sebocytes by real time PCR. Expression of VDR and 24OHase was up-regulated along with vitamin D analog treatment. Although several other splice variants of 1αOHase were detected by nested touchdown PCR, our findings indicate that the full length product represents the major 1αOHase gene product in SZ95 sebocytes. In conclusion, the vitamin D endocrine system is of high importance for sebocyte function and physiology. SZ95 sebocytes express the VDR and the enzymatic machinery to synthesize and metabolize biologically active vitamin D analogs and represent target cells for biologically active metabolites. Our findings indicate that sebaceous glands represent potential targets for therapy with vitamin D analogs or for pharmacological modulation of $1,25(OH)_2D_3$ synthesis/metabolism.

Behaviourally conditioned suppression of peripheral T lymphocyte function and signalling

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Behavioral conditioning of immune functions is one of the most fascinating examples of the bidirectional communication between the central nervous system and the immune system. The aim of this study was to elucidate the specificity of the conditioned immune response. Our model implemented a conditioned taste avoidance (CTA) paradigm, by pairing an injection of the immunosuppressive drug cyclosporine A (CsA, 20 mg/kg) as unconditioned stimulus (UCS) with saccharin taste (0.2% saccharin solution) as conditioned stimulus (CS). CsA exerts its immunosuppressive actions specifically on T cells via inhibition of the calcineurin-mediated signal transduction pathway. In this

study, male Dark Agouti rats were conditioned by conducting 3 association trials (CS/UCS) and three evocation trials (CS only). One hour after the third evocation trial, the cellular composition of peripheral blood and spleen were analyzed by flow cytometry, the proliferative response as well as the Th1- cytokine production of splenic T cells, following stimulation with anti-CD3, were measured *ex-vivo* and the cellular calcineurin (CaN) activity in the spleen was assessed. The physiological changes in the conditioned animals were compared to non-contingently conditioned rats, conditioned-not evoked rats, CsA-treated rats, a pharmacological control group and an untreated control group. The CsA-treated group as well as the pharmacological control group showed a strong suppression in both T cell proliferation and Th1- cytokine production. More importantly, however, also the conditioned animals exhibited a substantial reduction in the *ex-vivo* T cell response. Additionally, the conditioned as well as the CsA-treated group and the pharmacological control group displayed a significant reduction in cellular CaN activity in the spleen demonstrating that rats seem to be capable of associating the gustatory stimulus of saccharin with the T cell specific immunosuppressive effects of CsA. These data indicate that the mechanisms of conditioned immunosuppression by CsA seem to be mediated on a cellular level specifically via calcineurin-sensitive T cell signalling. Ongoing studies on the calcineurin pathway will give a more detailed insight in the peripheral mechanisms of behaviourally conditioned immunosuppression.

Endothelin-converting-enzyme 1 degrades substance P in endosomes to regulate resensitization of neurokinin-receptor-1

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The neurokinin receptor 1 (NK1R) along with other G protein-coupled receptors is characterized by prolonged desensitization after challenge with its cognate peptide agonist. Here, we demonstrate a novel mechanism with fast recovery of substance P sensitivity. Internalized NK1R was found to be co-localized with the endopeptidase, endothelin-converting-enzyme-1 (ECE-1) in acidic early endosomes. ECE-1 inactivates endocytosed substance P by cleavage of the peptide amino-terminal from position Phe7 and Leu10. This intracellular located degradation of substance P allowed the dissociation of b-arrestin from the internalized NK1R and rapid resensitization of substance P sensitivity. These data indicate a new biological function function of ECE-1-isoforms distributed in the endocytotic pathway and suggest a novel mechanism of peptide receptors regulation

Mechanisms of internalization and recycling of somatostatin-receptor

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Endocytosis of somatostatin receptors regulate cellular responses to the two natural peptides, somatostatin-14 (SST-14) and somatostatin-28 (SST-28), and to synthetic ligands used in the clinical diagnosis and symptomatic therapy of neuroendocrine tumours. Thus it is of importance to understand the mechanism and function of receptor-subtype specific internalization and trafficking. We investigated SST-14 induced internalization and trafficking of stably expressed rat somatostatin-receptor 1 (sst1) in rat insulinoma 1046-38 cells and determined the localization and function of intracellular resident sst1. During the first 2–5 min of stimulation, 50% of surface located receptors are internalized, cotransporting 50% of surface-bound SST-14 into acidic wash resistant cell compartments. Chronic stimulation with SST-14 induced a sst1-mediated accumulation of SST-14. After 75 min of stimulation, 2.2 times the amount specifically bound SST-14 were found cell-associated. The amount of accumulated SST-14 directly depends on the KD-value of sst1. Refreshing the medium induced recycling of intact and biological active SST-14. The biochemical data indicate a dynamic process of sst1-mediated endocytosis, recycling, and re-endocytosis of SST-14. Receptor and FITC-SST-14 were initially internalized within superficial cell compartments. Intracellular resident sst1 was located within Rab5a- and Rab11a-positive compartments, indicating receptor distribution within the endocytoic and recycling pathway of the cell. SST-14 activated translocation of resident sst1 to the cell surface. In summary, our results identified the pathway of SST-14 induced trafficking of sst1 and demonstrate that sst1 mediates dynamic process of endocytosis, recycling and re-endocytosis of SST-14

In vitro analysis of expression and function of splicing variants of the CYP27B1 (25-hydroxyvitamin D-1α-hydroxylase) gene in human skin

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1,25-Dihydroxyvitamin D3 (1,25(OH)2D3), the biologically active metabolite of vitamin D, has been shown to regulate the growth of various cell types, including human keratinocytes. 25-hydroxyvitamin D3-1α -hydroxylase (CYP27B1) catalyses the synthesis of 1,25(OH)2D3 in the kidney. Extrarenal activity of CYP27B1 has been reported in

various cell types including macrophages, keratinocytes, prostate and colon cancer cells and local production of 1,25(OH)2D3 has been postulated to play an autocrine or paracrine role in vitamin D–mediated growth control. Alternative splicing can play a role in regulating the enzyme level and may cause tissue-specific variations in healthy cells. We detected CYP27B1 splicing variants in various normal and malignant skin cells, including melanocytes and keratinocytes. Using western blot analysis, we characterized UV-B-induced changes in the CYP27B1 splicing variant pattern of primary cultured normal human melanocytes, spontaneously immortalized keratinocytes (HaCaT), melanoma cells (SkMel5), and squamous cell carcinoma cells (SCL-1). We identified a characteristic splicing variant pattern in each cell type. Following UV-B-exposure, we show time and dose dependent changes in the splicing pattern in confluent HaCaT but not in melanoma or squamous cell carcinoma cells. In conclusion, our findings indicate that biological effects of UV-B-radiation on keratinocytes may be in part due to changes in the CYP27B1 splice variant pattern.

Present concepts and future outlook: Function of peroxisome proliferator-activated receptors (PPARs) for pathogenesis, progression, and therapy of malignant melanoma

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Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily of transcriptional regulators that regulate lipid, glucose, and amino acid metabolism. In recent studies it also has been shown that these receptors are implicated in tumor progression, cellular differentiation, and apoptosis and modulation of their function is therefore considered as a potential target for cancer prevention and treatment. Using real time PCR, we have characterized expression of PPARα, δ and γ in primary cultured normal melanocytes and in melanoma cell lines. We show that PPARδ is the strongest expressed PPAR in these cells. PPAR ligands and other agents influencing PPAR signalling pathways have been shown to reveal chemopreventive potential by mediating tumor suppressive activities in a variety of human cancers and could represent a potential novel strategy to inhibit tumor carcinogenesis and progression. In addition, transcription of PPARs has been shown to be directly regulated by 1,25(OH)2D3. We now demonstrate antiproliferative effects of various PPAR-ligands and/or 1,25(OH)2D3 on melanoma cell lines. In conclusion, our data support the concept that PPARs may be of importance for pathogenesis, progression, and therapy of malignant melanoma.

Oxidative stress via hydrogen peroxide modulates the pro-opiomelanocortin peptide response in a dose dependent manner

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The human skin holds the capacity for autocrine processing of the pro-opiomelanocortin (POMC) derived peptides. The presence and functionality of ACTH, α- and β-MSH and β-endorphin in the regulation of skin pigmentation has been well established and a role has been put forward for α-MSH as an effective antioxidant with potent anti-inflammatory activities. Patients with the depigmentation disorder vitiligo accumulate hydrogen peroxide (H_2O_2) in the 10^{-3} M range in their epidermis and decreased epidermal POMC-processing and low α-MSH levels were documented previously. Therefore we examined the effect of H_2O_2 on POMC-derived peptides as possible targets for oxidation by this reactive oxygen species (ROS). To address this we employed immunofluorescence labelling, dot blot analysis, Fourier-Transform Raman spectroscopy, functionality studies and computer simulation of the peptide structures. We demonstrate a dose-dependent H_2O_2 mediated oxidation of epidermal ACTH, α- MSH and β-endorphin in vitiligo due to oxidation of methionine residues in the sequences of these peptides. Moreover, we show that both β-endorphin and α-MSH loose their functionality when oxidised whereas these changes are reversed upon reduction of epidermal H_2O_2 levels by a pseudocatalase PC-KUS. Importantly, oxidation of α-MSH can be prevented by formation of a 1:1 complex with the abundant cofactor 6-tetrahydrobiopterin ($6BH_4$). Taken together we demonstrate that epidermal oxidative stress via H_2O_2 can affect POMC peptide redox homeostasis in a dose-dependent manner.

Angiotensin II and inflammation: studies on AT1- and AT2-receptor coupled signalling using the novel non-peptide AT2-receptor agonist Compound 21

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It is well established that the AT1-receptor (AT1-R) mediates pro-inflammatory actions of angiotensin II (Ang II), while the role of the AT2-receptor (AT2-R) in inflammation is far less understood. In this study we investigated the potential role of the cytochrome P450 dependent arachidonic acid

metabolites 20-hydroxyeicosatetraenoic acid (20-HETE) or epoxyeicosatrienoic acid (EET) as second messengers for the pro- or anti-inflammatory effects of angiotensin II (Ang II), respectively, using IL-6 mRNA expression (real-time PCR) as read-out. Furthermore, NF-kappaB activity was determined in the context of this signalling cascade by immunohistochemical detection of NF-kappaB-p50-subunit nuclear translocation. All studies were performed in human primary dermal fibroblasts. Stimulation with Ang II (10-7M) for 12 h led to a five-fold increase in IL-6 mRNA expression. This could be totally blocked by co-incubation with the AT1-R antagonist irbesartan (10-5M) and partially blocked by the inhibitor of 20-HETE-synthesis, HET (10-7M). IL-6 synthesis was also stimulated by incubation with 20-HETE (10-7M) itself. In a second approach, cells were treated with TNF alpha (10 ng/ml) in order to stimulate IL-6 expression and to determine anti-inflammatory effects of Ang II via the AT2-R. Co-incubation of cells with either Ang II (10-7 M) under concomitant AT1-R blockade (irbesartan 10-5M) or with the specific AT2-R-agonist Compound 21 (10-6M) led to a significant reduction of elevated IL-6 levels. This reduction was attenuated by addition of the inhibitor of EET-synthesis, PPOH (10-7M). Ang II and 20-HETE induced NF-kappaB nuclear translocation, while Compound 21 had an inhibitory effect on TNF alpha-induced NF-kappaB activation, which could again be attenuated by blockade of EET-synthesis. We conclude, that 20-HETE and NF-kappaB activation act as second messengers of Ang II leading to the stimulation of IL-6 synthesis via the AT1-R. In contrast, the AT2-R mediates a reduction of IL-6 levels, which is mediated by an inhibition of NF-kappa B activity and by EET. Preliminary data indicate that AT2-R stimulation by Compound 21 acts anti-inflammatory and anti-fibrotic *in vivo* in the mouse model of bleomycin induced scleroderma.

Low baseline cortisol and low cortisol in relation to ACTH predict clinical improvement during 12 weeks of anti-TNF antibody therapy in rheumatoid arthritis

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Some patients with chronic inflammatory diseases such as rheumatoid arthritis rapidly profit from anti-TNF antibody therapy whereas others show no or little benefit. No markers are available which might predict outcome of prolonged anti-TNF antibody therapy. This study investigated the predictive value of hypothalamic – pituitary - adrenal (HPA) axis hormones for clinical improvement during anti-TNF antibody therapy. In this study in 23 patients with rheumatoid arthritis without glucocorticoid treatment, we measured at baseline serum levels of adrenocorticotropic hormone (ACTH), cortisol, and 17 hydroxy–progesterone. Improvement during anti-TNF antibody treatment was judged by the disease activity score (DAS28 at baseline, week 12), and serum cortisol was measured at week 12. Improvement of the DAS28 negatively correlated with baseline serum cortisol ($R = –0.500$, $P = 0.015$) and the cortisol/ACTH ratio ($R = –0.810$, $P < 0.00001$). Using this ratio as a marker of HPA axis integrity in a ROC analysis, we determined cut-off values below which DAS28 improvement can be predicted in up to 93%. In the longitudinal study over 12 weeks, those patients with good improvement and initially low serum cortisol levels demonstrated an increase of serum cortisol, which was opposite in patients with no or little improvement. This is the first study in a human chronic inflammatory disease which demonstrates that inflammation-induced TNF disrupts the HPA axis with low cortisol production, the consequence of which is increased disease activity. These findings position the HPA axis at a central place in the vicious circle of perpetuation of chronic inflammation.

Higher fitness levels are associated with increased adrenal androgens and decreased plasma IL-6 in peripheral obese but not in lean, central obese, and general obese elderly women

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Presence of peripheral fat mass appears to counteract the diabetogenic/atherogenic trends of central or general obesity through mechanisms presently poorly understood. In elderly women with distinct forms of body fat distribution, we investigated whether the fitness level is related to plasma levels of cortisol, adrenal androgens, and IL-6 accomplishing an anti-atherogenic milieu. In this study, a total of 286 patients were included with different forms of body fat distribution (lean: 83, peripheral obese: 43, central obese: 41, general obese: 109, this is a total of 276). Fat distribution was measured by dual-energy X-ray absorptiometry. These women reported four distinct fitness levels (no exercise, 1x/week 1–1.5 h, 2x/week 1–1.5 h, >2x/week 1–1.5 h). Main outcome measures: Plasma levels of cortisol,

17-hydroxyprogesterone (17OHP), dehydroepiandrosterone (DHEA), androstenedione (ASD), and IL-6 were measured. In all groups, plasma levels of cortisol and 17OHP were not related to fitness levels. However, only in peripheral obese women, plasma DHEA and ASD increased with the degree of fitness. This is also mirrored in the ratios of cortisol/DHEA and cortisol/17OHP. Only in peripheral obese women, a higher level of fitness was associated with markedly lower levels of plasma IL-6. This study demonstrates that fitness in peripheral obese women relates to higher adrenal androgens relative to cortisol which was not observed in lean, central obese, or general obese women. Since adrenal androgens such as DHEA and ASD inhibit leukocyte IL-6 secretion, the fitness-induced increase of these hormones is probably an anti-atherogenic and anti-diabetogenic signal.

Apoptotic effect of noradrenaline on T regulatory cells

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We have previously shown that noradrenaline (NA), the main sympathetic neurotransmitter, can induce apoptosis in T and B cells obtained from the spleen of normal mice. This effect is independent of Fas-FasL interactions. We have also recently reported that the sympathetic nervous system plays a relevant role for the course of the autoimmune, lymphoproliferative disease that Fas-deficient lpr/lpr mice develop. Interestingly, the degree of splenic sympathetic innervation changes with the progression of the disease. On the other hand, regulatory T cells (Tregs) are considered at present important modulators of autoimmune processes. On this basis, we wanted to study whether Tregs from normal and lpr/lpr mice are sensitive to the pro-apoptotic effects of the neurotransmitter. Since we were not able to find information on the percentage of Tregs in C57Bl/6 lpr/lpr mice, we first determined this parameter. We found that the percentage of Tregs (as evaluated by flow cytometric analysis of CD4 and CD25 expression) is elevated in lymphoid cells obtained from the spleen of 30-week old lpr/lpr male compared to that of the normal, age-matched C57Bl/6 littermates. At this age, symptoms of the autoimmune disease, such as splenomegaly and lymphoadenopathy, are clearly observed in lpr/lpr mice. The transcription factor Foxp3 is considered at present the most specific marker to characterize Tregs. Thus, further experiments were performed to detect intracellular expression of Foxp3 in live-gated $CD4^+$ lymphoid cells obtained from animals of different ages. The results confirmed our previous observation of increased percentage of Tregs in the spleen of both female and male lpr/lpr mice. Similar results were obtained using Tregs harvested from lymphnodes. Only few cells expressing Treg markers can be detected by flow cytometry in the thymus of both control and lpr/lpr mice. Preliminary studies indicate that, *in vitro*, NA can induce apoptosis of Tregs obtained from wildtype and Fas-deficient mice. However, Tregs seem to be less sensitive to dexamethasone-induced apoptosis than non-Treg cells, as also reported by other authors. We are investigating at present whether the sensitivity of Tregs to NA-induced apoptosis changes during ontogeny and as the disease progresses.

Corticotropin-releasing hormone is an endocrine and intracrine modifier of human skin functions and is involved in inflammatory skin diseases

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A pathway similar to that of the hypothalamic-pituitary-adrenal (HPA) axis has been described in human skin. Several skin compartments, and particularly the pilosebaceous unit, express neuropeptide receptors. Corticotropin-releasing hormone (CRH) is the most proximal element of the HPA axis and it acts as central coordinator for neuroendocrine and behavioral responses to stress. To further examine the significance of CRH function on the human skin we investigated the expression of CRH, CRH-binding protein (CRH-BP) and CRH receptors (CRH-R) 1 and 2 by immunohistochemistry in biopsies from facial skin of 33 patients with acne, non-involved thigh skin of the same patients and normal skin of eight age-matched healthy volunteers. Moreover the expression and regulation of the CRH system in SZ95 sebocytes *in vitro* was investigated. Very strong positive reaction for CRH was observed in acne-involved skin in all types of sebaceous gland cells, irrespective their differentiation stage, whereas in non-involved and normal skin sebaceous glands exhibited a weaker CRH staining depending upon the differentiation stage of sebocytes. The strongest reaction for CRH-BP in acne-involved sebaceous glands was in differentiating sebocytes. CRH-R1 and CRH-R2 exhibited the strongest expression in sweat glands and sebaceous glands, respectively. CRH, CRH-BP, CRH-R1 and CRH-R2 were detectable in SZ95 sebocytes at the mRNA and protein levels. Like

in vivo, CRH-R1 was the predominant receptor type (CRH-R1/CRH-R2 = 2). CRH and urocortin, a peptide structurally related and sharing a 45% homology with CRH, inhibited SZ95 sebocyte proliferation. CRH also stimulating interleukin (IL)-6 and IL-8 release from SZ95 sebocytes but had no effect on IL-1α and IL-1β production or IL1β-induced IL-8 release in these cells. α–helical-CRF, a CRH antagonist, annulled the CRH effect on SZ95 sebocyte proliferation and IL secretion. Moreover, CRH induced a biphasic increase in synthesis of sebaceous lipids with a maximum stimulation at 10^{-7} M and up-regulated mRNA levels of 3α-hydroxysteroid dehydrogenase/α^{5-4} isomerase, while the non-peptidic CRH-R1 selective antagonist antalarmin inhibited the increased production of neutral lipids caused by CRH. CRH markedly stimulated, dexamethasone inhibited and arachidonic acid did not affect the intracellular CRH synthesis by SZ95 sebocytes, but no CRH secretion could be detected by intact SZ95 sebocytes. On the other hand, CRH, dehydroepiandrosterone and 17β-estradiol did not modulate CRH-R expression but testosterone at 10^{-7} M down-regulated CRH-R1 and CRH-R2 mRNA expression at 6 to 24 h and GH switched CRH-R1 mRNA expression to CRH-R2 at 24 h. In conclusion, expression of the complete CRH system is abundant in human skin, especially in the sebaceous glands. CRH is an intracrine hormone for human sebocytes exerting homeostatic lipogenic activity but its activation affects inflammatory processes probably leading to the development and stress-induced exacerbation of acne. Paracrine CRH activity may be exhibited through the unique holocrine type of sebocyte secretion. Moreover, testosterone, GH and corticosteroids induce a CRH negative feedback through different mechanisms.

