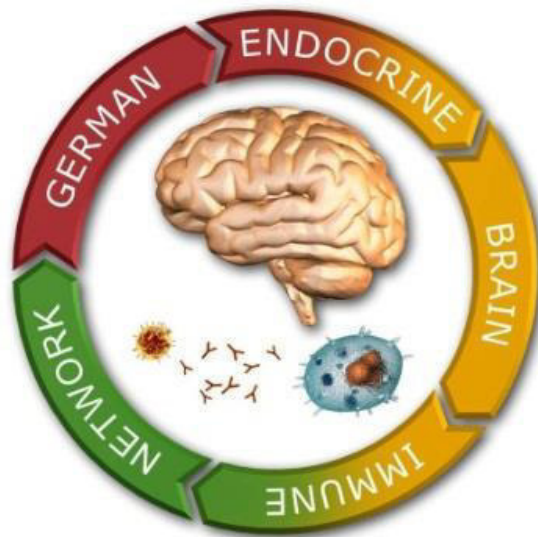


11th Scientific Meeting

April 23-25, 2015 - Munich, Germany

GEBIN 2015



www.gebin-2015.de

Dear colleagues,

On behalf of the Steering Committee of the **German Endocrine-Brain-Immune-Network (GEBIN)** it is our great pleasure to cordially invite you to the 11th Meeting of the GEBIN in Munich 2015.

GEBIN was founded in November 1997 in Munich during the Volkswagen Foundation-Symposium “Neuroimmunologie, Verhalten und Befinden”, a funding initiative which started in 1990, 25 years ago. A historical symposium will celebrate this **25th anniversary of interdisciplinary research** in the fields of anatomy, dermatology, endocrinology, ethology, gynecology, immunology, neurology, pharmacology, psychiatry, psychology and zoology.

The GEBIN meeting will be divided into several thematic sessions. Each session consisting of short oral presentations will be opened by an introductory key lecture of an internationally renowned expert in the respective field.

As in previous years, GEBIN will also offer an Educational Short Course for graduate and PhD students on April 22-23, 2015.

We are very much looking forward to welcoming you in the wonderful city of Munich in spring for the GEBIN 2015.

Harald Engler and Volker Stefanski
Coordinators of the GEBIN

Doris Grillitsch, Karin Koelbert, Bianka Leitner,
Norbert Müller, Gregor Schütze, Markus Schwarz
Local Organizing Committee

Contact:

For details please follow:

www.gebin-2015.de

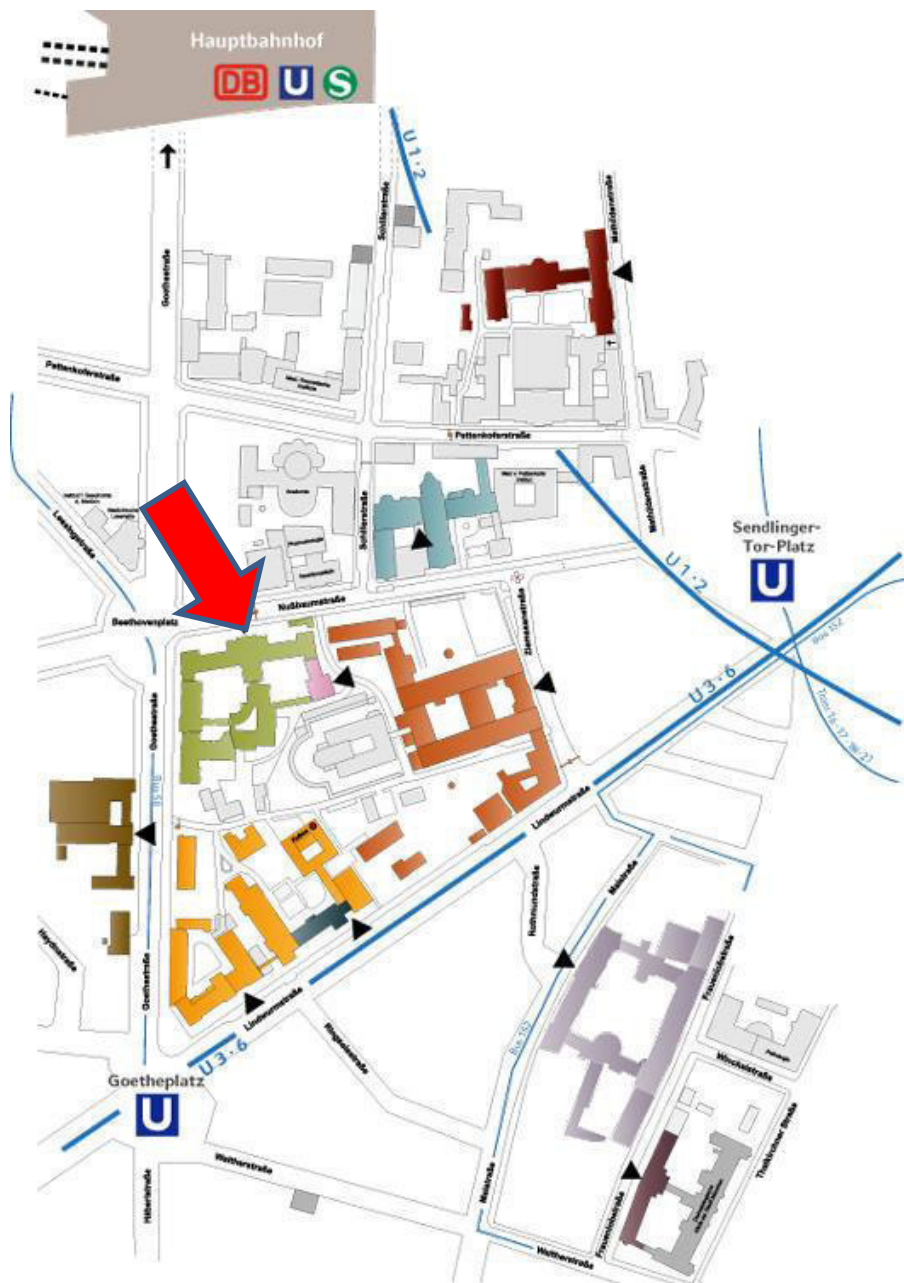
For more details please contact us:

info@gebin-2015.de

Meeting Site:

Klinik für Psychiatrie und Psychotherapie
Lecture Hall

Nußbaumstr. 7
80336 München
Germany



Program:

Wednesday, April 22

- 12:00 - 18:00 Educational short course

Thursday, April 23

- 8:30 - 12:30 Educational short course

- 13:30 Welcome reception 11th scientific meeting

- 14:30 - 17:30 Scientific meeting - **Jahrestreffen des AK NeuroEndokrinImmunologie**



- 14:30 – 15.15 Key note: Peripheral Neuroimmune Interactions

Chairs: U. Gimsa, E. Peters

K. A. Radek, Loyola University, Chicago, USA:

The role of cholinergic neurotransmission in wound healing

- 15.30 - 17:00 Session 1: Peripheral Neuroimmune Interactions

Chairs: U. Gimsa, E. Peters

T. Lowin, Regensburg:

Pro-inflammatory cytokines up-regulate and sensitize metabotropic and ionotropic cannabinoid receptors in rheumatoid arthritis and osteoarthritis synovial fibroblasts

S. Benson, Essen:

Inflammation-induced pain sensitization in men and women: Does sex matter in experimental endotoxemia?

G. Pongratz, Regensburg:

Beta-2 adrenoceptor signal is augmented in B cells in the course of arthritis to increase IL-10

A. Stegemann / M. Böhm, Münster:

Tropisetron – an emerging anti-inflammatory and antifibrotic agent

M. Böhm, Münster:

Beta-endorphin suppresses collagen synthesis in human dermal fibroblasts and attenuates skin fibrosis in a mouse model of scleroderma

S. Dimitrov, Tübingen:

Beta2-adrenergic receptor signaling down-regulates the avidity of beta2-integrins activated through T- or natural killer-cell receptors in humans

- **17.00 – 17.30 Awards ceremony**

Chairs: N. Müller, A. del Rey

♦ *17:30 - 18:00 GEBIN steering committee meeting*

• **18:00 - 19:30 Get together and poster session**

Poster titles see below

Friday, April 24

• **8:30 - 18:30 Scientific meeting**

- **8:30 – 9.15 key note: Stress, Behaviour and Immune Function**

Chairs: H. Engler, V. Stefanski

M.T. Bailey, The Ohio State University, Columbus, USA

Bidirectional interactions between the brain and the gut microbiota in health and disease

- **9.15 – 9.30 Break**

- **9.30 – 10.45 Session 2: Stress, Behaviour and Immune Function**

Chairs: H. Engler, V. Stefanski

L. Lückemann, Essen:

Pre-exposure to the unconditioned stimulus or conditioned stimulus does not affect learned immunosuppression in rats

M. Hadamitzky, Essen:

Acute and chronic immunosuppressive treatment with rapamycin affects brain and behavior

N. Havenstein, Hohenheim:

Seasonal impairments in immune competence in an obligate hibernator, the edible dormouse

B. Yi, München:

520-d isolation and confinement simulating a flight to Mars reveals heightened immune responses and alterations of leukocyte phenotype

K. Bösche, Essen:

Reconsolidation abrogates extinction of learned immunosuppression and prolongs heart allograft survival in rats

- **10.45 – 11.00 Break**

- **11.00 – 11.45 key note: Neuroendocrinology and immune Function**

Chairs: M. Böhm, T. Lange

C. Scheiermann, University of Munich, Germany

Rhythmic control of leukocyte migration

- **11.45 – 13.15 Lunch**

- **13.15 – 13.45 Late breaking news**

Chair: M. Schwarz

I. Sommer, University of Utrecht, Netherlands

Severe chronic psychosis after allogeneic SCT from a schizophrenic sibling

- **13.45 – 15.15 Session 3: Neuroendocrinology and Immune Function**

Chairs: M. Böhm, T. Lange

L. Engert, Hohenheim:

The pig (*Sus scrofa domestica*) as suitable non-rodent model for diurnal immunity:
Porcine immune cells exhibit circadian rhythms

D. Blömker, Essen:

The role of the sympathetic nervous system during acute Friend retrovirus infection in mice

M. L. Barcena de Arellano, Berlin:

Sex differences in oestrogen-dependent macrophage-fibroblast interaction in cardiac inflammation

H. Engler, Essen:

Men and women differ in inflammatory and neuroendocrine responses to endotoxin but not in the severity of sickness symptoms

T. Lange, Lübeck:

Differential effects of sleep on inflammatory responses to pathogen- and damage-associated molecular patterns

H. Johar, München:

Association of salivary cortisol levels and type 2 diabetes in the KORA-Age study

- **15.15 – 15.30 Break**

- **15.30 – 16.15 Key note: Neuroimmunology**

Chairs: E. Weihe, A. del Rey

B. Engelhardt, University of Bern, Switzerland

How brain barriers control immune cell entry into the CNS

- **16.15 – 17.30 Session 4: Neuroimmunology**

Chairs: E. Weihe, U. Meier

S.V. Ramagopalan, London

Co-associations of multiple sclerosis with schizophrenia and bipolar disorder: record linkage studies

U.-C. Meier, London:

The brain and the environment in multiple sclerosis

P. Dua, London:

The role of enolase as a potential target antigen in Tourette's Syndrome and other neuropsychiatric disorders associated with streptococcal infection

K. Berer, Munich:

The intestinal microbiota and its role in CNS-specific autoimmunity

E. M. Schmidt, Tübingen:

Effects of minocycline on sleep, memory, and immune parameters in humans

- **17.30 – 18.00 Walk to the restaurant 'Weisses Bräuhaus'**

- **18.30 – 19.30 25 years anniversary symposium**
"25 years of PNI research, induced by Volkswagenstiftung"
Chairs: H. Engler, V. Stefanski



H. Besedovsky, Marburg: More than 25 years of PNI research

E. Weihe, Marburg: 25 years of neuroimmune anatomy in PNI research

M. Schedlowski, Essen: 25 years of experimental PNI research

R. Straub, Regensburg: 25 years of peripheral neuro-immune interactions research

N. Müller, München: 25 years of PNI research in Psychiatry

•19:30 congress dinner

Saturday, April 25

•9:00 - 12:00 Scientific meeting

- **9.00 – 9.45 key note:**
Neuroendocrine Immune Network in Psychiatric Disease and Mental Health
Chairs: N. Müller, M. Schwarz

F. Benedetti, Ospedale San Raffaele, Milano, Italy
The impact of cytokines for grey-matter changes in psychiatric disorders

- **9.45-10.00 Break**

- **10.00 – 11.30 Session 5:**
Neuroendocrine Immune Network in Psychiatric Disease and Mental Health
Chairs: N. Müller, M. Schwarz

Ch. Böck, Ulm:
The effects of child maltreatment on mitochondrial functionality of peripheral immune cells

M. Elwenspoek, Luxemburg
Early life adversity alters the activation status of T cells

J. Hahn, Tübingen:

Impact of sleep on innate immune cells

R. Emeny, München:

Attachment style determines the association of oxytocin with anabolic and immune factors in older adults; findings from the cross-sectional KORA Age Study

E. Weidinger, München:

The Inflammation Hypothesis of Tourette's Syndrome

K. Bechter, Günzburg:

CSF findings support the mild encephalitis hypothesis of severe mental illness

- **11.30 – 12.00 Poster award ceremony**

Chairs: M. Schedlowski, R. Straub

•**12:00 Farewell**

Posters:

Stress, behavior and immune function

Frank Risto Rommel, Gießen:

Stress affects mast cell protease expression: potential role of cholinergic signaling

Tilman Otto Kleine, Marburg:

Behaviour modifies cellular immune surveillance (CIS) of human central nervous system (CNS); reviewed with Marburg Cerebrospinal-Fluid (CSF) Model

Liubov Petrakova, Essen:

Psychosocial Stress increases salivary alpha-amylase activity independently from plasma noradrenalin levels

Luciana Besedovsky, Tübingen:

Sleep compared to continuous wakefulness reduces the number of various human T cell subsets in blood

Ulrike Kübler, Zürich:

Dark chocolate attenuates intracellular pro-inflammatory reactivity to acute psychosocial stress in men

Charlotte Heyer, Hohenheim:

Effects of dietary phosphorus and calcium on the adaptive immune response following immunization in pigs

Julia Kirchhof, Essen:

Adrenoceptor-dependent interaction between A kinase anchor proteins and the protein phosphatase calcineurin in human CD4⁺ T cells

Anna Lena Kahl, Essen:

Cyclosporine A-induced immune suppression does not impair attention or memory performance in humans

Andrea Fuchs, Ulm:

Effects of stress coping and bite wounds on the time course of splenic GC resistance during chronic subordinate colony housing

Dominik Langgartner, Ulm:

Environmental factors substantially affect the outcome of an established murine model for chronic psychosocial stress

Neuroendocrinology and peripheral neuroimmune interactions

Susanne Klatt, Regensburg:

Early Sympathectomy Inhibits Egress of Lymphocytes in Control and Arthritic Animals and Ameliorates Arthritic Disease

Claudia Zuccarella, Bern:

Higher macrophage superoxide anion production in essential hypertension

Hubert Stangl, Regensburg:

Catecholaminergic-to-cholinergic transition of sympathetic nerve fibers is stimulated under healthy but not under inflammatory arthritic conditions

Matthias Ebbinghaus, Jena:

IL-17A does not contribute to the impact of the sympathetic nervous system on experimental arthritis

Zsuzsa Jenei-Lanzl, Regensburg:

Interaction of PDE4 and β -arrestin reverses anti-inflammatory effects of catecholamine-producing cells in chronic arthritis via adrenoceptor switching from $G_{\alpha s}$ to $G_{\alpha i}$ signalling

Neuroendocrine immune network in psychiatric disease and mental health

Karlijn Becking, Groningen:

Innate immune responsiveness differentiating between unipolar and bipolar depressive episodes

Laura Große, Münster:

Cellular immune activation and suppression co-occurring in major depressive disorder

Nilay Hepgul, London / Gregor Schütze, München :

Interferon-alpha-induced depression: the involvement of the kynurenine and tryptophan pathway

Bianka Leitner, München:

COX-2 inhibition as an antidepressant therapy- new results

Martin Schäfer, Marburg:

Microglia express the vesicular nucleotide transporter (VNUT) essential for exocytotic

ATP release

Abstracts:

Sex differences in oestrogen-dependent macrophage-fibroblast interaction in cardiac inflammation

M. L. Barcena de Arellano, T. Haschler, G. Kararigas, V. Regitz-Zagrosek

Institute for Gender in Medicine, Center for Cardiovascular Research, Charité – Universitätsmedizin Berlin, Berlin Germany

Sex hormones directly act on the immune system via hormone receptors. Most of the autoimmune diseases are more prevalent in women than in men, which is attributed to the immune stimulatory effects of oestrogen (E2). However, gastritis, ankylosing spondylitis and myocarditis are more prevalent in men than in women and are characterized by acute inflammation and pro-inflammatory immune reactions.

We propose sex-differences in the macrophage-fibroblast interactions in myocarditis. And that macrophages, fibroblasts and adaptive immune cells cross-talk is involved in the impaired wound healing, which leads to fibrosis.

To verify the E2 involvement in the sex-differences in the macrophages-fibroblast interactions in the heart, rat male and female macrophages were differentiated into M1/M2 macrophage and rat cardiac fibroblast (RCF) were used to analyse the influence of E2 and oestrogen receptors (ERs). RCF were incubated with M1/M2 supernatant to evaluate fibroblast activation and chemotaxis.

Male and female M1/M2 macrophages showed different morphology, which was directly modulated under E2 treatment. M1/M2 macrophages were strongly modulated by E2 and ERs. We saw differences in the activation of RCF under E2 influence. We demonstrated gender-specific differences in the fibroblast-phenotype after incubation with male and female M1/M2 supernatant.

This study confirmed gender-differences in the morphology and polarization of macrophages as well as in the activation of RCF, leading to sex-specific phenotypes. Furthermore, E2 via ERs significantly influence the differential activation of macrophages and fibroblasts, promoting the modulation of the immune response in the heart during inflammation, which seems to be directly involved in cardiac tissue remodelling.

CSF findings support the mild encephalitis hypothesis of severe mental illness

K. Bechter, H. Maxeiner, L. Kühne, H. Reiber, D. Fuchs, M. Schneider

The etiology and pathogenesis of severe mental illness, the core represented by the affective and schizophrenic spectrum, remains widely unknown, though many researchers assume some biological basis. CSF analysis is a key bodily fluid to assess in vivo the pathogenetic processes involved in brain diseases. We demonstrated in several research projects, focused on therapy resistant cases of severe mental illness, in about 40 % of patients some CSF abnormalities by conventional sophisticated CSF analysis, in addition increased CSF neopterin in about 30 %, together indicating immune pathological responses within the intrathecal space in more than 50 % of cases, in addition blood CSF barrier dysfunction was found, summing up to 70 % of cases with CSF pathology. Beyond in 100 % of these cases we demonstrated CSF cytokine abnormalities, often paralleled by corresponding though not identical cytokine abnormalities in blood. Together our results reveal immune inflammation pathology in a large subgroup of patients of the affective and schizophrenic spectrum, focused within the intrathecal spaces, and thus informative about the brain, supporting the mild encephalitis hypothesis.

Maxeiner H. G., et al. Cerebrospinal fluid and serum cytokine profiling to detect immune control of infectious and inflammatory neurological and psychiatric diseases. *Cytokine* 2014;69:62-67

Bechter K. Updating the mild encephalitis hypothesis of schizophrenia. *Progress NBPB* 2013; 42: 71-91

Bechter K et al. Cerebrospinal fluid analysis in affective and schizophrenic spectrum disorders: identification of subgroups with immune responses and blood–CSF barrier dysfunction. *J Psychiatr Res* 2010;44(5):321–30.

Innate immune responsiveness differentiating between unipolar and bipolar depressive episodes'

K. Beeking

Recent studies suggest the use of inflammatory biomarkers in the staging of bipolar disorder (BD) and to discover depressed patients who are at risk of developing BD. However, peripheral inflammatory markers are strongly influenced by lifestyle and disease status. The expression of cytokines in response to ex-vivo stimulation by lipopolysaccharide (LPS) may give more insight, since it measures the innate production capacity of cytokines, which is under strong genetic control. Therefore this study aimed to examine if the stimulated innate immune response could predict the onset of BD during 6-years of follow-up in depressed patients. Depressed patients were selected from the Netherlands Study of Depression and Anxiety and divided into patients without and with onset of bipolar disorder (resp. N=253 and N=53) established with the Composite International Diagnostic Interview. Using Multi-Analyte Profiling technology, plasma levels of 13 cytokines were assayed after whole blood stimulation by addition of LPS. LPS-stimulated inflammatory markers were overall higher in patients with onset of BD, but after adjustment for covariates, only monocyte chemoattractant protein-1 (MCP-1) remained a significant predictor for BD. However, we found that the onset of BD was particularly high in patients with multiple elevated levels of stimulated inflammatory markers. Different markers of the innate immune response are increased in depressed patients who develop bipolar disorder. If our results are replicated in larger samples, LPS-stimulation might be used as a tool to discover patients at risk of developing bipolar disorder.

Sleep compared to continuous wakefulness reduces the number of various human T cell subsets in blood

L. Besedovsky^a, S. Dimitrov^a, J. Born^a, T. Lange^b

^a Department of Medical Psychology and Behavioral Neurobiology, University of Tübingen, Tübingen, Germany,

^b Department of Internal Medicine, University of Lübeck, Lübeck, Germany

Circulating T cells show circadian rhythms, the direction and extent of which depend on the specific subset. Naïve T cells show the most pronounced rhythm with peak numbers during nocturnal sleep. Memory T cells display a comparable rhythm, although with a smaller amplitude, whereas cytotoxic effector T cells show an opposite rhythm with highest numbers during daytime. In the current study, we were interested in how sleep affects these different circadian rhythms and therefore measured 8 T cell subsets (naïve, central memory, effector memory, and effector T-helper and cytotoxic T cells) over a 24-h period under conditions of sustained wakefulness compared to a normal sleep-wake cycle in 14 healthy males. Sleep significantly reduced the number of all T cell subsets during nighttime, except from effector T-helper cells. Sleep was furthermore associated with an increase in growth hormone, prolactin, and aldosterone levels, whereas concentrations of catecholamines tended to be lower than during nocturnal wakefulness.

We propose that the decrease in catecholamine levels during sleep is responsible for the reduction of effector cytotoxic T cells, which were shown in previous studies to selectively respond to epinephrine administration with a demargination. In contrast, the decrease of the less differentiated subsets during sleep is more likely to be a consequence of high growth hormone and aldosterone levels, two hormones that are suggested to support the extravasation and subsequent migration of naïve and memory T cells to lymph nodes.

The role of the sympathetic nervous system during acute Friend retrovirus infection in mice

D. Bloemker¹, K. Gibbert², A. del Rey³, U. Dittmer², M. Schedlowski¹, H. Engler¹

1 Institute of Medical Psychology and Behavioral Immunobiology, University Hospital Essen, University of Duisburg-Essen, Germany;

2 Institute for Virology, University Hospital Essen, University of Duisburg-Essen, Germany; ³ Institute of Physiology and Pathophysiology, Philipps University of Marburg, Germany

The sympathetic nervous system (SNS) plays an important role in immune regulation. Primary and secondary lymphoid organs are richly innervated by sympathetic nerve fibers and noradrenaline (NA), the main sympathetic neurotransmitter, has been shown to influence a wide spectrum of immune responses via β_2 -adrenoceptor-dependent mechanisms. However, only little is known about the role of the SNS during viral infections. Here, we demonstrate that experimental infection of C57BL/6 mice with murine Friend retrovirus (FV) led to a massive (-90%) but transient depletion of NA in the spleen, one of the main reservoirs of FV replication. At the same time, expression of the catecholamine-degrading enzymes monoamine oxidase A and catechol-O-methyltransferase was markedly upregulated. Peripheral chemical sympathectomy with 6-hydroxydopamine two days prior to FV infection enhanced the severity of the infection as evident from higher viral loads and a more pronounced enlargement of the spleen. In contrast, pharmacological treatment with the β_2 -adrenoceptor agonist terbutaline around the peak of the infection (5 to 9 days post infection) had no influence on the course of the disease. These findings suggest a complex and potentially clinically relevant interplay between the SNS and retroviral infections.

The effects of child maltreatment on mitochondrial functionality of peripheral immune cells

Böck, C.^a; König, A.^a; Schury, K.^a; Calzia, E.^b; Karabatsiakis, A.^a; Kolassa, I-T.^a

a Clinical & Biological Psychology, Institute of Psychology and Education, Ulm University

b Department of Anesthesia, Section of Anesthesiological Pathophysiology and Process Development, Ulm University

The experience of maltreatment during childhood detrimentally influences the development of a child and often leads to a chronic state of stress with long-lasting biological consequences. Due to the close interactions within the psycho-neuro-immunological networks, the cells of the peripheral immune system are especially susceptible to the effects of prolonged psychological stress. The cellular immune system is crucially dependent on the functionality of its mitochondria, which are the main suppliers of biochemical energy in form of ATP fueling the immune response. There is a body of evidence showing that chronic stress is associated with a blunted functionality of the immune system leading to a higher incidence of secondary health problems throughout life.

In a cohort of 30 women, we investigated the effects of a history of child maltreatment on mitochondrial activity in peripheral blood mononuclear cells. Interestingly, maltreatment load, as assessed by the Childhood Trauma Questionnaire, was associated with an increased level of physiological mitochondrial activity and increased ATP-production-related mitochondrial respiration. At the same time, maltreatment load showed an influence on non-mitochondrial oxygen consumption, which indicates an increased formation of reactive oxygen species. This implies an increased level of oxidative stress in women with a history of child maltreatment.

Maltreatment during childhood might lead to changes in the basal energy demand of the cellular immune system with further possible consequences for an altered immune reactivity when facing immune challenges.

Beta-endorphin suppresses collagen synthesis in human dermal fibroblasts and attenuates skin fibrosis in a mouse model of scleroderma

M. Böhm, M. Apel, T. Luger, A. Stegemann

Dept. of Dermatology, University of Münster, Münster, Germany

The opioid beta-endorphin (beta-ED) is an endogenous peptide derived from proopiomelanocortin (POMC). It belongs to the group of neuroendocrine mediators acting via opioid receptors (ORs). Previously, we showed that POMC-derived α -melanocyte stimulating hormone suppresses collagen synthesis in human dermal fibroblasts (HDFs) and attenuates skin fibrosis induced by bleomycin (BLM) in a mouse model of scleroderma. Other neuroendocrine mediators such as endocannabinoids and activators of α 7 nicotinic acetylcholine receptors likewise modulate collagen metabolism and tissue fibrosis. In this study, we investigated whether beta-ED could act as a novel neuropeptide with antifibrotic properties. Beta-ED dose-dependently suppressed TGF-beta1-induced collagen type I secretion in HDF. This effect occurred at protein as well as at mRNA level of collagen type I expression. Interestingly, this suppressive effect of beta-ED did not alter TGF-beta1-mediated SMAD3 signaling. Moreover, we could not detect the expression of the classical (μ -, δ - and κ -) ORs in HDF suggesting an OR-independent action of beta-ED. In accordance with this beta-ED neither suppressed forskolin-mediated increase of intracellular cAMP nor induced intracellular Ca^{2+} mobilization as reported for canonical OR-mediated signaling. To define the in vivo effect of beta-ED we finally utilized the BLM model of scleroderma. Here, beta-ED significantly attenuated skin fibrosis induced by subcutaneous injections of BLM. The antifibrogenic effect of beta-ED was present at RNA level as well as at protein level as shown by quantitative RT-PCR and SDS-PAGE of the collagen content in murine skin. In summary, we present beta-ED as a novel neuropeptide that modulates collagen synthesis in vitro and in vivo.

Reconsolidation abrogates extinction of learned immunosuppression and prolongs heart allograft survival in rats

K. Bösche¹, M. Hadamitzky¹, L. Lückemann¹, H. Engler¹, M. Schedlowski¹

1 Institute of Medical Psychology and Behavioral Immunobiology, University Hospital Essen, University of Duisburg-Essen, 45122 Essen, Germany

In an established paradigm of behaviorally conditioned taste aversion (CTA) in rats, contingent pairings of the novel taste saccharin (CS) and the immunosuppressant cyclosporine A (US) lead to CTA and a suppression of immune functions when animals are subsequently re-exposed to the CS alone during evocation. Like every other learning process however, behaviorally conditioned immunosuppression is subject to extinction, a progressive decrease in the conditioned response over time. This is a considerable problem for the systematic application of conditioning paradigms as a supportive treatment option in immuno-pharmacological regimens. Thus, in the present study we aimed to interfere with the extinction of behaviorally conditioned immunosuppression. Interestingly, when combining sub-therapeutic doses of cyclosporine A together with the CS during evocation, extinction of learned immunosuppression is abrogated. In contrast, extinction of conditioned immunosuppression could not be inhibited by administering sub-therapeutic CsA 8 hours after exposure to the CS. Furthermore, we could show that this updated or reconsolidated learned immunosuppression is able to prolong the survival time of a cardiac allograft in rats. Our findings might pave the way for the systematic integration of associative learning paradigms as supportive therapy in immunopharmacological regimens.

Beta2-adrenergic receptor signaling down-regulates the avidity of beta2-integrins activated through T- or natural killer-cell receptors in humans

S. Dimitrov^{1,2,3}, L. Besedovsky¹, E.-M. Schmidt¹, A. T. Ramstedt Jensen⁴, C. Gouttefangeas⁵, J. Born^{1,2,3}, H.-G. Rammensee⁵, T. Lange⁶

1 Department of Medical Psychology and Behavioral Neurobiology, University of Tübingen, Tübingen, Germany

2 German Center for Diabetes Research (DZD), Tübingen, Germany

3 Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at the University of Tübingen (IDM), Tübingen, Germany

4 Department of International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark

5 Department of Immunology, Institute for Cell Biology, University of Tübingen, Tübingen, Germany

6 Department of Internal Medicine, University of Lübeck, Lübeck, Germany

Activation of β_2 -integrins in response to inside-out signaling plays a major role for leukocyte adhesion to endothelium, antigen-presenting cells or target cells. Chemoattractants stimulate their cognate receptors on leukocytes, leading to rapid integrin activation and clustering, which together result in avidity modulation allowing leukocyte attachment to endothelium. $G\alpha_s$ -coupled receptor signaling, such as via β_2 -adrenergic receptors (ARs) has been previously shown to rapidly down-modulate this integrin activation, supposedly explaining the leukocyte de-adhesion from endothelium and mobilization into the peripheral blood caused by β_2 -AR agonists. In lymphocytes, integrin avidity is also rapidly induced by stimulation of T-cell and activatory natural killer (NK)-cell receptors. The resultant adhesion of T- or NK-cells to target cells with formation of cytotoxic synapses is required for lysis of, e.g., virus-infected cells. The goal of the current report was to study the effect of $G\alpha_s$ -coupled receptor signaling on integrin avidity by T-cell and activatory NK-cell receptors in humans. Using ligand-complex-based adhesion assay to assess β_2 -integrin activation and clustering we show that β_2 -AR agonists can rapidly down-modulate the avidity of β_2 -integrins, which were activated through anti-CD3 or anti-CD16 in T and NK blood cells, respectively. For T cells, the decrease in integrin avidity was most pronounced for terminally differentiated cytotoxic CD8 T-cells and was also observed for cytomegalovirus-specific CD8 T-cells activated through soluble peptide-major histocompatibility complex class I tetramers. This active down-regulation of integrin avidity through $G\alpha_s$ -coupled receptors might be an essential mechanism how β_2 -AR agonists such as epinephrine can regulate cytotoxic immune defense during acute stress.

To Investigate the Role of Enolase as a Target Antigen in Tourette Syndrome and Other Neuropsychiatric Disorders Associated with Streptococcal Infection

P. Dua*, D. Martino*, A. Church, S. Sisay*, D. Baker*, G. Giovannoni*, U. C. Meier***

*Centre for Neuroscience and Trauma, Queen Mary University of London. ** Institute of Neurology, University College London.

Background:

Anti-neuronal autoantibodies have been associated with neuropsychiatric disturbances like Tourette syndrome and Sydenham chorea. These antibodies have been shown to bind to common neural autoantigens of molecular weight 40, 45 and 60 kDa identified as glycolytic enzymes aldolase C, neuronal enolase and pyruvate kinase M1 respectively. Proteins that are structurally similar to human enolase have also been observed on the the surface of bacterium like *S. pyogenes* where it appears to function as an efficient plasmin(ogen) binding protein which influences tissue invasiveness and pathogenicity. Hence an autoimmune mechanism involving molecular mimicry has been proposed.

Aim:

To analyse the effect of anti-neuronal antibodies from patients with neuropsychiatric disorders on the enzymatic activity enolase a putative autoantigen.

Method:

An assay developed for measuring the enzymatic activity of enolase was carried out on PC12 neuronal cells after probing with anti-neuronal antibodies purified from patient sera. The absorbance measured was directly proportional to the levels of NADH which in turn was inversely proportional to enolase activity.

Results:

A significant inhibition of enolase activity in both PC12 whole neuronal cells and its membrane fraction after the addition of sera from patients as compared to healthy controls was observed confirming its role as a putative autoantigen in these disorders.

Conclusion:

The above findings point towards a possible autoimmune origin of neuropsychiatric disorders that will have implications on our understanding of the pathophysiology of these disorders and future therapeutic strategies.

IL-17A does not contribute to the impact of the sympathetic nervous system on experimental arthritis

M. Ebbinghaus¹, S. Hensellek¹, R. Bräuer², H.-G. Schaible¹

¹ Institute of Physiology 1/Neurophysiology – Friedrich Schiller University of Jena

² Institute of Pathology, Jena University Hospital – Friedrich Schiller University of Jena

Interleukin 17 (IL-17), a current target for the therapy of several inflammatory diseases, is also assumed to be a mediator of inflammation in experimental models of arthritis. In murine antigen-induced arthritis (AIA) a strong anti-inflammatory effect was achieved by systemic sympathectomy in C57BL/6 mice. This effect was accompanied by a significant reduction of Th17 responses. In addition, lymphocytes produce less IL-17 after antagonistic beta-adrenergic treatment in vitro. This raised the question whether IL-17 is causally involved in the proinflammatory role of the sympathetic nervous system in AIA. Therefore we performed experiments using a popular strain of IL-17 knockout (IL-17^{-/-}) mice (Nakae et al., *Immunity*. 2002;17(3):375-87). First we found that IL-17^{-/-} mice express all subtypes of IL-17 (B, C, D, E, F) except subtype “A”. In AIA IL-17^{-/-} mice showed severe signs of inflammation similar as wild type mice, and sympathectomy in the absence of IL-17A still acted anti-inflammatory. Furthermore, in IL-17^{-/-} mice, significantly less IL-2, IL-6 and IFN γ was measured at the inflamed joint after sympathectomy and the number of splenic T helper 1 and T regulatory cells in IL-17^{-/-} mice was decreased after sympathectomy. But in contrast to wild-type mice, lymphocytes derived from arthritic IL-17^{-/-} mice did not produce less proinflammatory cytokines following previous sympathectomy. Taken together we found no evidence that IL-17A is essential for sympathetic neuro-immune interactions influencing the severity of AIA. Further studies have to clarify the role of both sympathectomy and IL-17A in the local effects in the joint and in the general immune response.

Early life adversity alters the activation status of T cells

MMC Elwenspoek^{1,2}, FAD Leenen^{1,2}, X Hengesch³, H Schächinger³, C Vögele⁴, JD Turner², CP Muller^{1,2}

1 Institute of Immunology, Luxembourg Institute of Health, Luxembourg

2 Department of Immunology, Institute of Psychobiology, University of Trier, Germany

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Early life adversity (ELA) has been associated with an altered response to stress, as well as an increased susceptibility to infections. Since the stress and immune system are strongly interconnected, changes in stress response could negatively influence immune function, leading to a higher risk of infection.

20 healthy participants, of which 8 post-institutionalized (PI) and 12 age-matched controls, underwent a 3-minute socially evaluated cold pressor test. The expression of two immune activation markers, HLA-DR and CD69, were measured on T cells at baseline and in response to stress.

At baseline, HLA-DR expression was higher in PI subjects compared to the controls on CD8+ cytotoxic T cells (CTLs, $P=0.01$) and CD4+ T-helper (Th) cells ($P=0.09$), whereas CD69 expression was similar in both groups. Acute stress did not affect HLA-DR expression within 4 hours post-stress. CD69, on the other hand, was down-regulated in both groups immediately after stress and did not return to baseline levels within the measured time frame. Interestingly, the decrease in CD69+ CTLs was significantly stronger in the PI group ($P=0.05$).

In the absence of any specific infection, the higher numbers of baseline HLA-DR+ T cells and the stress associated decrease in CD69+ T cells in PI individuals, suggest that adversity in early life programs cell-mediated immune activation. The increased sensitivity to stress related immune suppression could be part of the explanation why ELA or PI individuals are more susceptible to infections.

Attachment style determines the association of oxytocin with anabolic and immune factors in older adults; findings from the cross-sectional KORA Age Study

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Background

Despite a rapid increase in knowledge of oxytocin's central role in social stress and stress perception over the past decade, there is a paucity of studies that have investigated the role of oxytocin in socioemotional functioning in healthy, elderly individuals. We recently provided evidence for a stress-associated role of oxytocin even in the last three decades of life (Emeny et al 2015 PNE in review). Higher plasma oxytocin was associated with the experience of a stressful life event, yet only in participants who reported a secure attachment style. We have extended these analyses to investigate whether anabolic hormones (IGF-I, IGFBP-3, DHEAS) and immune factors (IL-6, TNF α , white blood cell count (wbc), platelet count (plt)) associate with this stress-induced oxytocin response.

Methods

Attachment style was assessed in participants (n=952) in the clinical interview of the population-based KORA Age study (2008/2009) by the Relationship-Specific Attachment Scales for Adults. Non fasting blood was obtained in the morning hours for biomarker assessment.

Results and Outlook

Age and sex adjusted regression models indicate a positive association between oxytocin levels and wbc in the total population ($\beta=0.101$, $SE=0.04$, $p=0.004$), yet only among securely attached participants (n=542, 57%) was oxytocin positively associated with serum levels of IGFBP3 ($\beta=0.109$, $SE=0.05$, $p=0.03$), IL-6 ($\beta=0.124$, $SE=0.05$, $p=0.009$) and TNF α ($\beta=0.110$, $SE=0.05$, $p=0.04$). These data support the polyvagal theory of social engagement and stress responsiveness. Ongoing analyses will consider mediating roles of these oxytocin associated factors that may contribute to improved mental health after adverse life experiences in older age.

The pig (*Sus scrofa domestica*) as suitable non-rodent model for diurnal immunity: Porcine immune cells exhibit circadian rhythms

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Diurnal rhythms regulate various immune parameters in vertebrate species, which are studied mostly in nocturnal rodents. Besides conducting experiments in humans, the domestic pig might be a suitable model organism to study rhythms in immune function as well as underlying molecular mediators in a diurnal active mammal. Since no studies investigated rhythms in porcine immune cells to date, the aim of the present study was to characterize the number and distribution of various peripheral blood immune cells as well as hematological parameters in domestic pigs throughout the day.

Castrated male pigs were held under an artificial light regimen (12:12), were fed concentrate twice daily and had ad libitum access to water and hay. Blood samples were taken every two hours within one day via Vena cephalica catheters.

Total leukocyte counts showed a diurnal distribution with two troughs. Whereas granulocytes, erythrocytes and NK cells were found in higher numbers in peripheral blood during the day, T cells and dendritic cells were found to show maxima during the night. Compared to memory T helper cells, naive T helper cells showed stronger diurnal amplitudes.

In summary, pigs show diurnal changes in the number of various peripheral blood leukocyte populations, which mostly resemble those found in humans. The domestic pig, therefore, seems to be a suitable model organism for investigating immune rhythms and underlying mediators in diurnal mammalian species.

Men and women differ in inflammatory and neuroendocrine responses to endotoxin but not in the severity of sickness symptoms

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Depressed mood and increased anxiety represent two core symptoms of sickness behavior that are thought to be mediated by pro-inflammatory cytokines. Moreover, excessive inflammation is implicated in the development of mood/affective disorders. Although women are known to mount stronger pro-inflammatory responses during infections and are at a higher risk to develop depressive and anxiety disorders, experimental studies on sex differences in sickness-related behavioral symptoms are scarce. The present study aimed at comparing physiological and psychological responses to endotoxin administration between men and women. Twenty-eight healthy volunteers (14 men, 14 women) were injected with a low dose (0.4 ng/kg) of lipopolysaccharide (LPS) and plasma concentrations of cytokines and neuroendocrine factors as well as negative state emotions were measured before and until six hours after endotoxin administration. Women exhibited a more profound pro-inflammatory response with significantly higher increases in plasma concentrations of TNF- α and IL-6. Plasma levels of the anti-inflammatory cytokine IL-1ra were also significantly higher in women. In contrast, the LPS-induced increase in plasma IL-10 concentration was significantly higher in men. The cytokine alterations were accompanied by changes in neuroendocrine factors known to be involved in inflammation regulation. Endotoxin administration induced a significant increase in plasma noradrenaline, but without evidence for sex differences. The LPS-induced increase in plasma cortisol was significantly higher in woman, whereas plasma changes in dehydroepiandrosterone were more pronounced in men. Endotoxin administration also resulted in increased secretion of prolactin, but only in women. Despite these profound sex differences in inflammatory and neuroendocrine responses, men and women did not differ in endotoxin-induced alterations in mood and state anxiety. This suggests that compensatory mechanisms exist that counteract the more pronounced inflammatory response in women, preventing an exaggerated sickness response. Disturbance of these compensatory mechanisms by environmental factors such as stress may promote the development of affective disorders in women.

Effects of stress coping and bite wounds on the time course of splenic GC resistance during chronic subordinate colony housing

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Chronic subordinate colony housing (CSC, 19d) is an established mouse model for chronic psychosocial stress and causes glucocorticoid (GC) resistance in splenocytes and IL-4 producing peripheral lymph node cells. The aim of this study was to unravel the time course of CSC-induced GC resistance in splenocytes and if these alterations are dependent on the individual stress coping strategy (active vs. passive) and/or the severity of received bite wounds during CSC.

Therefore, splenocytes from CSC and single-housed control (SHC) mice were isolated at different time points (d9, d15, d16, d20) during CSC and stimulated in vitro with lipopolysaccharide in the absence or presence of different corticosterone concentrations. Moreover, behaviour during CSC was recorded and spleen weight, number of isolated splenocytes, as well as severity of dermal and subdermal bite wounds were quantified. For the latter we developed a detailed score considering both size and intensity of wounds.

Our results indicate that splenocytes of CSC mice show GC resistance at all time points assessed. Moreover, spleen weight and splenic GC resistance in CSC mice were highly correlated (positive) with the severity of bite wounds. Currently we are analyzing whether the latter is related to differences in the individual stress coping strategy during CSC and whether the GC resistance is mediated by changes in GC receptor expression and/or functionality. So far, our results indicate that GC resistance in splenocytes already develops at an early stage (d9) of CSC exposure and that it is strongly dependent on the severity of received bite wounds.

Cellular immune activation and suppression co-occurring in major depressive disorder

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Background:

There is accumulating evidence that cell-mediated immunity plays an important role in the pathogenesis of major depressive disorder (MDD). In support of this view, we recently described an up-regulation of a coherent set of immune activation genes in circulating monocytes of MDD patients. However, apart from immune activation, there are studies indicating cellular immune suppression in MDD, such as reduced proliferative responsiveness of T cells and reduced levels and functions of natural killer (NK) cells. In this study, we aim to examine whether immune activation and immune suppression are part of a single pathophysiological pathway within the same individuals, or whether they represent different processes occurring in different individuals.

Methods:

Using the same sample of MDD patients and controls in which monocyte activation was previously determined ¹, we analyzed various leukocyte subset percentages and serum growth factor concentrations in N = 71 MDD patients and N = 71 age- and gender-matched controls.

Results:

We found significantly reduced percentages of NK cells and a reduced capability of T helper cells to turn into T helper-2 cells and T helper-17 cells in MDD patients vs. controls. Except for percentages of natural T regulatory (Treg) cells, which were decreased in patients with elevated monocyte activation only, cellular immune suppression was largely unrelated to the previously determined monocyte immune gene expression.

Conclusion:

Cellular immune activation and suppression might co-occur within the same MDD patients. Further studies should investigate the potential role of Treg cells in the activation of other leukocyte populations.

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Acute and chronic immunosuppressive treatment with rapamycin affects brain and behavior

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Rapamycin (RAPA) is a drug widely used for prevention of acute graft rejection and cancer therapy because of its antiproliferative and immunosuppressive properties. It specifically inhibits the activity of the mammalian target of rapamycin (mTOR), a protein kinase responsible for cell growth, proliferation and antibody production. Clinical observations show that patients undergoing therapy with immunosuppressive drugs frequently suffer from affective symptoms such as anxiety. Whether these symptoms are attributed to the action of the immunosuppressive compounds remains unclear. Thus, we investigated in naive healthy rats the consequences of RAPA-induced mTOR inhibition on brain neuronal activity and behavior. Intraperitoneal administration of acute (3 mg/kg) and chronic (1, 3, and 5 mg/kg) RAPA treatment led to enhanced neuronal activity in the amygdala analyzed by intracerebral electroencephalography and/or c-fos protein expression. This enhanced amygdaloid activation correlated with a drug-induced increase in anxiety-related behaviors in the elevated plus-maze. Moreover, acute RAPA induced amygdaloid expression of KLK8 and FKBP51, proteins that have been implicated in the development of anxiety and depression. Together, our findings demonstrate that acute and chronic mTOR inhibition due to RAPA treatment causes behavioral alterations that may be related to altered amygdaloid neuronal activity.

Impact of sleep on innate immune cells

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Recent studies have shown that sleep has a strong impact on different components of the immune system. Sleep deprivation has been described to affect both humoral as well as cellular immunity. Previously, the NLR ligand muramyl dipeptide (MDP), chemically unique cell wall component of all bacteria, has been identified as a sleep-promoting factor in humans and animals, which accumulates during the active period to eventually induce slow wave sleep (SWS). On the other hand, sleep deprivation leads to a lethal sepsis in rats. Here we addressed whether sleep has an impact on the cellular components in blood and spleen and on the outcome of infection. Our results show that sleep deprivation (SD) in mice for up to 6 hours led to a significant reduction of cell numbers in the blood in particular of monocytes whereas no effect was seen for PMNs. Further, we observed a reduction of monocytes in the spleen. After intravenous infection of mice with the model pathogen *Yersinia enterocolitica*, to mimic sepsis, the bacterial load in the spleen of SD-mice was significantly higher one and three days post infection. Also the survival of the SD-Mice was significantly reduced compared to the control group. These data suggest that sleep regulates homeostasis and function of innate immune cells, which are important in the early defence against pathogens.

Seasonal impairments in immune competence in an obligate hibernator, the edible dormouse

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Hibernation represents the most extreme physiological adaptations of small mammals to reduce energy expenditure during harsh environmental conditions. During hibernation heart and metabolic rate are drastically reduced and body temperature can reach values around the freezing point. Circulating leukocytes decrease enormously (~ 90%) and a variety of immune functions are impaired severely during torpor, but so far were shown to recover immediately afterwards.

The edible dormouse is an obligate hibernator, inhabiting deciduous European woodlands. The aim of this study was to investigate, whether hibernation affects immune competence.

We therefore examined the blood picture (pocH-100iV Diff, Sysmex) and lymphocyte proliferation (LTT) in 103 female and 145 male edible dormice, inhabiting five different study sites in South-Western Germany during their active period.

Our previous studies on edible dormice have already revealed a delay in restoration of innate immune cell counts after emergence from hibernation. The LTT now furthermore proved that proliferative capacity of lymphocytes was severely reduced during this period. A resurgence of innate immune cell numbers as well as of lymphocyte proliferation capacity took place only after a period of approximately 4 to 6 weeks. These results indicate that edible dormice experience a hibernation-associated deprivation of both, the innate as well as the adaptive arm of the immune system. This phenomenon may make edible dormice susceptible to pathogens in early summer and explain their low survival rates during this time period.

Interferon-alpha-induced depression: the involvement of the kynurenine and tryptophan pathway

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Background: Interferon-alpha (IFN- α) is the standard treatment for chronic hepatitis C virus infection. This treatment clears the virus, but induces major depression and other neuropsychiatric adverse effects in 30-50% of patients. Although the biological mechanisms through which IFN- α treatment causes depression are still not clear, it has been hypothesised that the serotonergic system may be involved. Tryptophan is the primary precursor of serotonin and upon activation of inflammatory pathways, is broken down into kynurenine, and other neurotoxic and neuroprotective metabolites. The aim of this study is to investigate changes in tryptophan and kynurenine pathway metabolites during IFN- α treatment, and their contribution to the development of IFN- α -induced depression.

Methods: 27 patients with chronic hepatitis C virus infection (mean \pm SEM age: 44.5 \pm 2.2 years; gender: 77.8% males) were assessed using a prospective cohort design; at baseline and at treatment weeks 8 and 24 (TW8 and TW24) of IFN- α therapy. Severity of depressive symptoms was assessed using the Inventory of Depressive Symptomatology. Plasma levels of tryptophan, kynurenine and kynurenic acid were measured at the same time points using high performance liquid chromatography (HPLC). Data were analysed using SPSS.

Results: Depression scores were significantly higher at TW8 and TW24 when compared to baseline ($p < 0.001$). Tryptophan levels ($\mu\text{g/ml}$) were significantly lower at TW8 and TW24 when compared to baseline ($p < 0.001$). Kynurenine levels (ng/ml) were significantly higher TW8 and TW24 when compared to baseline ($p < 0.05$). Finally, kynurenic acid levels (ng/ml) were also lower at TW24 when compared to baseline however, this was not significant ($p = 0.1$). A partial correlation, controlling for baseline depression scores showed baseline levels of kynurenic acid were negatively correlated with depression scores at both TW8 and TW24 ($r = -0.36$, $p = 0.08$ and $r = -0.39$, $p < 0.05$).

Conclusion: IFN- α significantly affects tryptophan metabolism leading to a decrease in levels of tryptophan and decreased levels of the potentially neuroprotective kynurenic acid. We also find an increased production of kynurenine. We conclude that the elevated IDO activity might also lead to decreased levels of serotonin and increased levels of the neurotoxic metabolite 3-hydroxykynurenine. The combination might be part of the observed IFN- α -induced depression.

Effects of dietary phosphorus and calcium on the adaptive immune response following immunization in pigs

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There is increasing interest in dietary ingredients that are appropriate to support immune functions and animal health. Although phosphorus (P) is an essential nutrient, there is little information on the impact of dietary P on the immune system of pigs. Furthermore, dietary macronutrient composition may affect immune response. The present study aimed to assess the effects of diets with varying calcium-phosphorus (CaP) levels and different protein sources on the adaptive immune response after immunization in pigs.

In 2 consecutive experiments, growing pigs (N=31) were fed either a corn-soybean meal- or a corn-pea meal-based diet, each supplemented with two different CaP levels (low vs. high). After 3 weeks of adaptation to the diets, all pigs were immunized 2 times with the neoantigen keyhole limpet hemocyanin (KLH) (week 4 and 6) and blood samples were taken 2 weeks after the second immunization. In week 8, the amount of anti-KLH IgG, KLH-specific lymphocyte proliferation and cell numbers of lymphocyte subpopulations were analysed in blood samples. Irrespective of the protein source, pigs fed the low-CaP diets showed lower anti-KLH IgG titers compared to pigs fed the high-CaP diets ($P < 0.05$). Both the KLH-specific lymphocyte proliferation and the numbers of T and B cells were not affected by the protein source or the CaP level.

In conclusion, dietary CaP seems to have modulating effects on the adaptive immune system such as the differentiation of naive antigen-specific B cells into potent antibody-producing plasma cells, and sufficiently high amounts of CaP may be required to support animal's health.

Interaction of PDE4 and β -arrestin reverses anti-inflammatory effects of catecholamine-producing cells in chronic arthritis via adrenoceptor switching from $G_{\alpha s}$ to $G_{\alpha i}$ signalling

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In chronic inflammation, prevention of cAMP degradation by phosphodiesterase 4 (PDE4) inhibition can be anti-inflammatory therapy. However, PDE4 inhibition was not effective in rheumatoid arthritis (RA). Recent studies demonstrated that PDE4/ β -arrestin interaction at β adrenoceptors resulted in switching from $G_{\alpha s}$ to $G_{\alpha i}$ signaling and ERK1/2 activation. Such a switch in signaling might elicit proinflammatory effects. We aimed to investigate this possible $G_{\alpha s}$ to $G_{\alpha i}$ signaling switch in RA and osteoarthritis (OA) mixed synoviocytes.

Synoviocytes were treated alone or with combinations of adrenergic, dopaminergic, and adenosinergic drugs, rolipram (PDE4 inhibitor), inhibitors of $G_{\alpha i}$ signaling (pertussis toxin), and blockers of protein kinase A (PKA). Under normoxic or hypoxic conditions, proinflammatory TNF was the readout-parameter from mixed synoviocytes. We investigated co-expression and interaction of PDE4 and β -arrestin by imaging techniques. Expression of pERK1/2 was analyzed by western blotting.

Mixed synoviocytes in RA and OA possessed all major $G_{\alpha s}$ -coupled neurotransmitter receptors. Under hypoxia, particularly in RA cells, $G_{\alpha s}$ -coupled receptor agonists unexpectedly increased TNF and respective antagonists decreased TNF. Under hypoxia, rolipram alone or rolipram plus $G_{\alpha s}$ agonists increased TNF, which was reversed by pertussis toxin or PKA inhibition. In synovial tissue and cells, PDE4 and β -arrestin were in close apposition as detected by proximity ligation assay. $G_{\alpha s}$ agonists or rolipram plus $G_{\alpha s}$ agonists increased pERK1/2 expression.

This study in human arthritic synovial tissue presents an unexpected proinflammatory switch from $G_{\alpha s}$ to $G_{\alpha i}$ signaling, which depends on PDE4/ β -arrestin interaction. This phenomenon is most probably responsible for reduced efficacy of PDE4 inhibitors and $G_{\alpha s}$ agonists in RA.

Association of salivary cortisol levels and type 2 diabetes in the KORA-Age study

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Background

Dysregulation in the hypothalamic pituitary adrenal (HPA) axis, specifically in cortisol secretion (a marker of HPA axis activity), has been suggested to play a role in the development of Type 2 Diabetes (T2D). However, conflicting and limited epidemiological evidence on the association of cortisol and diabetes, demands further investigations.

Aim

To examine the association of salivary cortisol levels and T2D in a representative sample of older men and women.

Methods

A cross-sectional analysis was conducted among 757 study participants (mean=75±6, 65 - 90 years old,) of the population-based KORA (Cooperative Health Research in the Region of Augsburg)-Age study. Associations were examined between T2D status and salivary cortisol measured upon waking (M1), 30 min after awakening (M2), and in the late evening (E). Multivariate regression analyses were used to examine the association of cortisol levels and T2D.

Result

After adjustment for relevant covariates, T2D status was significantly associated with higher cortisol awakening response (CAR) in men ($\beta=0.30$, CI=0.32-0.42, $P=0.04$) and greater evening level in women ($\beta=0.30$, CI=0.34-0.46, $P=0.03$).

Conclusion

Our findings suggest sex-specific associations between T2D and dysregulated cortisol secretion.

Cyclosporine A-induced immune suppression does not impair attention or memory performance in humans

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Clinical and experimental evidence document that treatment with immunosuppressive and anti-proliferative drugs like the calcineurin inhibitor cyclosporine A (CsA) is associated with mental health problems and neuro-psychological disturbances in patients. However it remains unclear whether and to what extent cognitive functions like memory and attention processes are affected by the pharmacological treatment. This is partly due to the fact that it is difficult to refer the observed neuropsychological disturbances to the drug itself, to drug-induced immune suppression or to interaction with other medication. Thus, in a double-blind study with healthy male participants (n=18) we investigated if short-term intake of CsA (4 x 2.5mg/kg) affects attention, working memory performance and anxiety levels. So far, our preliminary data of this ongoing study indicates that CsA administration and the resulting decrease in TH₁ cytokines (interleukin 2 and interferon- γ) production are neither accompanied by a decrease in attention or memory performance nor by increased anxiety levels.

Adrenoceptor-dependent interaction between A kinase anchor proteins and the protein phosphatase calcineurin in human CD4⁺ T cells

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It is well documented that immune functions can be modulated by associative learning processes such as classical conditioning. Employing the calcineurin inhibitor and immunosuppressant cyclosporine A as an unconditioned stimulus in a taste aversion paradigm in rats, the learned suppression of the production of T cell cytokines interleukin (IL)-2 and interferon (IFN)- γ is mediated via sympathetic-adrenal mechanisms, the release of noradrenaline and β_2 -adrenoceptor-dependent mechanisms. In an attempt to understand the intracellular processes of this learned immunosuppression, we found in previous experiments in rodents that a stimulation of CD4⁺ T cells with the β -adrenoceptor agonist terbutaline, leads to a decrease in IL-2 expression due to the inhibition of calcineurin (CaN) activity. The exact intracellular mechanisms of this β -adrenoceptor induced inhibition of calcineurin activity are yet unknown. Thus, in our current experiments we are investigating the interactions of the β_2 -adrenoceptor and calcineurin activity in human CD4⁺ T cells. Our data so far show that terbutaline is dose-dependently inhibiting IL-2 production in human CD4⁺ T lymphocytes. In our ongoing experiments we are analyzing if this inhibition of the IL-2 production is also accounted by a decreased CaN activity and will determine the intracellular pathways responsible for this interaction between the T cell and β -adrenoceptor. These will be important in order to understand the cellular pathways responsible for the learned inhibition of t cell functioning.

Early Sympathectomy Inhibits Egress of Lymphocytes in Control and Arthritic Animals and Ameliorates Arthritic Disease

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Background:

The sympathetic nervous system (SNS) plays an important role in course and development of autoimmune diseases like arthritis. Early sympathectomy (SYX) prior to immunization ameliorates disease severity, but beneficial mechanisms are not completely understood. The aim of this study was to determine how the SNS influences energy expenditure in lymph nodes / spleen and egress of lymphocytes from draining lymph nodes / spleen of control and arthritic animals.

Methods:

The spatial energy expenditure configuration (SEEC) technique is based on removal of tissue during the course of arthritis and determination of oxygen consumption in vitro. SEEC was applied to healthy control animals, arthritic animals and animals that underwent early SYX. We evaluated homing of lymphocytes, CCR7 expression on lymphocytes, CCL21 concentration in cell culture supernatants and sphingosine-1-phosphate (S1P) serum levels of arthritic, SYX arthritic, and control animals.

Results:

We observed a marked increase in oxygen consumption in draining lymph nodes and spleens of arthritic animals during the course of arthritis. Early SYX increased energy consumption in arthritic, but also in control lymph nodes. After SYX, enhanced migration to lymph nodes and spleen, elevated expression of CCR7, and higher levels of CCL21 in supernatants were observed. Importantly, early SYX decreased S1P serum concentration in CIA animals to control levels.

Conclusions:

Using the SEEC technique, we identified draining lymph nodes as target organs of the SNS. SYX-induced disease amelioration is probably exerted by sequestration of lymphocytes in secondary lymphoid organs. This might prevent recirculation of immune cells to peripheral sites of inflammation.

Behaviour modifies cellular immune surveillance (CIS) of human central nervous system (CNS); reviewed with Marburg Cerebrospinal-Fluid (CSF) Model

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In CNS of healthy humans, CIS is carried out with lymphocytes which are produced e. g. in bone marrow and bursa, to migrate into blood. Lymphocytes can not traverse from blood into CNS: Blood-brain-barrier (bbb) locks CNS blood capillaries with tight junctions and basement membrane (bm); blood-CSF-barrier (bCSFb) locks blood lymphocyte transfer with epithelial tight junctions and bm in choroid plexus, where CSF is produced. - CIS is investigated with flow cytometry (FC) of lymphocytes in ventricular CSF (V-CSF), suboccipital CSF (SOP-CSF), lumbar CSF (L-CSF), thoracic duct lymph (TDL) and corresponding peripheral venous blood (PVB). In V-CSF, SOP-CSF and L-CSF, FC detects CD3+, CD3+HLADR+, CD4+, CD8+, CD16+56+3–, CD19+3– subsets: These lymphocytes are pressed from blood through fenestrated capillaries into matrix of 3 circum ventricular organs (CVO capillaries without bbb); to migrate through ependymal cell lacks into V-CSF: Blood pressure and human posture modify extravasation of blood lymphocytes into V-CSF; blood pressure is influenced by affective feeling and excitement. – V-CSF with the lymphocytes flow into SOP CSF, subarachnoidal CSF or spinal CSF: Human posture (erect / lying) and breathing modify lymphocyte numbers in lumbar and V-CSF. - When spinal CSF flows out along nerve roots e.g. in ductus thoracicus, some lymph with lymphocytes and 5 subsets (without CD3+HLADR+) reflux; thus increasing spinal lymphocyte numbers. Human posture (erect / horizontal) and deep breathing modify reflux of lymph lymphocytes into spinal space. Thus, in healthy humans behavior (emotional states / somatic conditions) influences cellular CIS of CNS with lymphocytes and 6 subsets.

Dark chocolate attenuates intracellular pro-inflammatory reactivity to acute psychosocial stress in men

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Background:

Flavanoid-rich dark chocolate consumption has beneficial effects on cardiovascular health, but underlying mechanisms are not completely understood. We investigated the effect of acute dark chocolate intake on inflammatory stress responses in humans.

Methods:

Healthy men aged 20-50 years were randomly assigned to a single intake of either 50g of flavonoid-rich dark chocolate (n=31) or 50 g of optical identical flavonoid-free placebo chocolate (n=34). Two hours after chocolate intake, both groups underwent the Trier Social Stress Test. We measured DNA binding activity of the pro-inflammatory transcription factor NF- κ B (NF- κ B-BA) in peripheral blood mononuclear cells, as well as plasma and whole blood mRNA levels of the pro-inflammatory cytokines interleukin (IL)-1 β and IL-6, and the anti-inflammatory cytokine IL-10 prior to chocolate intake, before and several times after stress cessation. We also repeatedly measured the flavonoid epicatechin and the stress hormones epinephrine and cortisol.

Results:

Compared to the placebo chocolate group, the dark chocolate group revealed a significantly blunted stress reactivity of NF- κ B-BA, IL-1 β mRNA, and IL-6 mRNA ($p \leq .036$) with higher epicatechin levels relating to lower inflammatory stress reactivity ($p \leq .033$). Stress hormone changes were controlled. There were no group differences in stress reactivity of IL-10 mRNA and the plasma cytokines IL-1 β , IL-6, and IL-10 ($p \geq .23$).

Conclusion:

Our data suggest a buffering effect of acute flavonoid-rich dark chocolate intake on the intracellular pro-inflammatory stress response that seems to be directly mediated by chocolate flavonoids. This mechanism may add to beneficial effects of dark chocolate on cardiovascular health.

Differential effects of sleep on inflammatory responses to pathogen- and damage-associated molecular patterns

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Sleep regulates immune functions. By facilitating the release of pro-inflammatory hormones at a time when anti-inflammatory hormones are low, slow-wave sleep (SWS) can promote inflammation and thus foster immune responses to microbial challenges. On the other hand, sleep deprivation can lead to an increase in blood levels of pro-inflammatory cytokines. These at a first glance contradictory findings might in part stem from differences in experimental designs across sleep studies in terms of the time point of measurement (e.g., night or day), the type of sleep manipulation (e.g., total or partial sleep deprivation) or the duration of insufficient sleep (acute or prolonged). However, the impact of sleep on inflammatory processes might also depend on the type of immune activation, i.e., whether the immune system was stimulated by pathogen- (PAMPs) or damage-associated molecular patterns (DAMPs). Against the background of the present literature we posit the conceptual view, that during an infectious challenge PAMPs, by stimulating the release of pro-inflammatory cytokines, induce SWS as an integral part of sickness behavior. SWS in turn supports host defense mechanisms as it promotes the further release of pro-inflammatory mediators and thus helps the organism to overcome the infection. On the other hand, under non-infectious conditions information processing, food intake and physical activity during wakefulness induce an accumulation of DAMPs, which likewise induce SWS by stimulating the release of pro-inflammatory cytokines. SWS then serves the metabolic clearance of DAMPs and therefore the down-regulation of the inflammatory response. Conversely, when sleep is lacking sterile inflammation will arise.

Environmental factors substantially affect the outcome of an established murine model for chronic psychosocial stress

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Chronic subordinate colony housing (CSC, 19 days, 12h/12h light/dark cycle (LDC), normal drinking water (NW)), an established model for chronic psychosocial stress in male mice, has been repeatedly shown to cause basal evening hypocorticism, spontaneous colitis and increased anxiety-related behavior. However, running this model at a novel facility under slightly different environmental conditions (14h/10h LDC, acidified drinking water (AW)) revealed that both, the physiology of single housed control (SHC) mice as well as the extremely robust CSC effects are strongly dependent on the prevailing environmental conditions. Notably, a 14h/10h LDC and AW represent standard housing conditions in many animal facilities, whereas commercial animal suppliers (e.g. Charles River, Sulzfeld, Germany) often employ a 12h/12h LDC and NW.

In detail, our data indicate that SHC mice kept under a 14h/10h LDC and AW, even 4 weeks after delivery from Charles River show increased basal morning plasma corticosterone (CORT) levels. This effect was even more pronounced two weeks after arrival and could be completely prevented by employing a 12h/12h LDC. Importantly, using a 12h/12h LDC, but keeping an AW, did not bring back any of the above described CSC effects. However, the combination of a 12h/12h LDC and NW instead of AW at least brought back the well-known effects of CSC on HPA axis functionality, namely basal evening hypocorticism and a reduced adrenal in vitro ACTH sensitivity. Given that CSC exposure under these conditions still does not cause anxiety and colitis, we are currently investigating other environmental factors that might play a role.

COX-2 inhibition as an antidepressant therapy- new results

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Introduction

A proinflammatory state in a subgroup of depressed patients has been reported repeatedly. Treatment with COX-2 inhibitors downregulate increased inflammatory markers. Therefore an adjunctive treatment of depression with COX-2 in combination with an antidepressant might lead to a better clinical outcome.

M & M

This is a dual-center, randomized, double-blind, placebo-controlled, parallel group phase IIa study. It investigates the mean change in clinical outcome and in serum cytokine levels from baseline to endpoint in patients with major depression treated with sertraline plus celecoxib versus sertraline plus placebo for six weeks. 49 depressed patients (18-60yrs) without any recent inflammatory related disease were randomized.

Results

Repeated measure ANOVA showed a significant effect for time. Symptomatic reduction from baseline was greater in celecoxib group at week 2, 3 and 6. More patients in the celecoxib group responded to treatment at week 3 and 6. Furthermore 42% of the celecoxib group experienced remission by week 6 compared to only 26% in the placebo group.

Conclusion

Although differences in the HAMD change, remission and response rate between the celecoxib and placebo group were detected, there was no significant difference. Since only two of all centers contributed to the clinical study, the expected number of patients by far could not be reached, a higher number of cases might have led to a significant outcome. Furthermore both groups of patients have shown a very quick reduction in the HAMD score. The relationship of clinical outcome and immune markers such as neopterin and macrophage inhibition factor will be discussed.

Pro-inflammatory cytokines up-regulate and sensitize metabotropic and ionotropic cannabinoid receptors in rheumatoid arthritis and osteoarthritis synovial fibroblasts

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Background:

In collagen-induced arthritis, elevation of endocannabinoid levels improves clinical parameters and decreases synovial inflammation. Joint pain and inflammation are driven by pro-inflammatory cytokines like TNF, which is also involved in sensitizing transient receptor potential channels (TRP). Besides activating cannabinoid receptors type I and II (CB₁ and CB₂), endocannabinoids also bind several TRPs with TRPV1 and TRPA1 being the most important. In this study we demonstrate the influence of TNF, IL-1 β and IFN- γ on the expression and function of metabotropic (CB₁ and CB₂) and ionotropic (TRPs) cannabinoid receptors.

Methods:

Cannabinoid receptor expression was analyzed by western blotting. MMP-3 and cytokines were detected by ELISA. ERK 1/2 and p38 phosphorylation was assessed cell-based ELISA and western blotting. Calcium response was analyzed with Fura-2 staining.

Results:

Prolonged incubation with TNF (10ng/ml, 7 days) significantly increased CB₁, CB₂, TRPV1, TRPA1 and FAAH protein (fatty acid amide hydrolase, important endocannabinoid metabolizing enzyme). Similar results were obtained with IL-1 β and IFN- γ (1ng/ml and 10ng/ml, respectively). TNF-induced sensitization and up-regulation of TRPA1 was confirmed by an increase in intracellular calcium in response to TRPA1 agonist polygodial. While synovial fibroblasts only responded to high doses of TRPA1 agonist (50 μ M) without TNF pretreatment, TNF incubation (72h, 10ng/ml) not only increased E_{max}, but also lowered the activation threshold of the receptor to 1 μ M. Furthermore, TNF sensitized synovial fibroblasts to the action of the endocannabinoid anandamide.

Conclusion:

The observed up-regulation of metabotropic and ionotropic cannabinoid receptors by TNF might explain lack of effects under healthy/basal conditions. Activation of the cannabinoid receptor system might be an adaptation to the pro-inflammatory environment in rheumatoid arthritis and might help to resolve inflammation.

Pre-exposure to the unconditioned stimulus or conditioned stimulus does not affect learned immunosuppression in rats

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In order to analyze the effects of pre-exposure to the unconditioned (US) or conditioned stimulus (CS), respectively, on learned immunosuppression, we employed an established conditioned taste avoidance paradigm (CTA) in rats. A sweet drinking solution (saccharin) served as CS, an i.p. injection of the immunosuppressive drug cyclosporine A (CsA) as US. In this model, the learned immunosuppression is reflected by a significantly diminished cytokine production of interleukin (IL)-2 and interferon (IFN)- γ as well as impaired T-cell proliferation. In the present approach, male Dark Aguti rats were exposed to the CS (saccharine) three days before conditioning (latent inhibition). A second experiment analyzed the effects of pre-treatment with the US (CsA) prior to conditioning. We could show that presentation of both, the UC, and CS, respectively, prior to conditioning resulted in an accelerated extinction process on the behavioral level (CTA). In contrast however, pre-exposure of saccharine (CS) or CsA (US) did not affect the learned suppression of anti-CD3 stimulated IL-2 production. Extrapolating these approaches to clinical conditions, these data indicate that learned immunosuppression could be induced in patients that are already on immunosuppressive therapy or have had previous contact to the gustatory stimulus. Importantly, these findings might pave the road for employing learning protocols as supportive therapy to pharmacological regimens in immunosuppressed patients, the aim being to reduce the amount of drugs and toxic side effects and to maximize the therapeutic outcome for the patient's benefit.

Psychosocial Stress increases salivary alpha-amylase activity independently from plasma noradrenalin levels

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Salivary alpha-amylase activity (sAA) and plasma noradrenaline (NA) concentrations are often considered as surrogate markers of sympathetic activation in response to stress. However, despite accumulating evidence for a close association between sAA and noradrenaline and other indicators of sympathetic activity, reliability and generality of this relation remains unclear. We employed the Trier Social Stress Test (TSST) in order to directly compare the responses in sAA and NA to psychological stress in healthy female (n=9) and male (n=14) volunteers. The TSST significantly increased sAA and NA plasma levels with no significant differences in females and males. However, when subjects were divided according to their NA responses into low versus high responders, both groups did not significantly differ in their sAA before, during or after stress exposure. These data suggest that in response to acute psychological stress both, plasma NA levels and sAA reflect sympathetic activity, however seemed to increase independently from each other.

β₂ adrenoceptor signal is augmented in B cells in the course of arthritis to increase IL-10

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Background:

Splenic B cells from collagen-induced arthritis (CIA) mice react to a β₂-adrenoceptor (AR) stimulus with increased IL-10 production and adoptive transfer of these cells decreases disease activity. However, B cells from unimmunized mice do not adequately increase IL-10. Therefore, we test the hypothesis that sensitivity to catecholamines changes during CIA. Furthermore, we wanted to test if human peripheral blood B cells from osteoarthritis (OA) and rheumatoid arthritis (RA) patients also increase IL-10 following a β₂-adrenergic stimulus.

Methods:

FACS, ELISA, human and mouse B cell culture, CIA

Results:

In the course of CIA the percentage of β₂-AR⁺ B cells increased (ANOVA p<0.05). Mean fluorescence intensity (MFI) for G-protein coupled receptor kinase (GRK2) decreased from day 6 p.i. (ANOVA p<0.0001). The relative increase in phosphorylation of p38 (ANOVA p<0.001) and cAMP responsive element binding protein (CREB, ANOVA p<0.001) following a β₂-AR stimulus is augmented in late CIA. In human B cells, similar mechanisms are in place, because β₂-AR stimulation of RA but not OA B cells increased IL-10.

Conclusion:

The current data show that B cells become more sensitive to β₂-AR stimuli in the course of CIA, possibly due to a decrease in GRK2 and increase in the percentage of β₂AR expressing splenic B cells. Increased catecholamine sensitivity might support B cell and IL-10 mediated anti-inflammatory mechanisms in the late phase of CIA. A similar mechanism is observed in human peripheral B cells and might be used to improve treatment of autoimmune arthritis.

Stress affects mast cell protease expression: potential role of cholinergic signaling

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Mast cells (MC) are prominent targets of stress-induced worsening in chronic inflammatory diseases such as atopic dermatitis. MCs are target and source of a variety of stress mediators (SM), like nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), substance P (SP) and express cholinergic systems (CS) receptor acetylcholine receptor alpha 7 (Chrna7). Furthermore they are capable to cleave SM with specific murine MC proteases (mMCP). In this study, we analyzed MCs MCP expression in mice under inflammatory stress (experimental allergic dermatitis [AID]) and under psychosocial stress (24h noise-stress) and their interaction with SP and endogenous Chrna7 ligand Secreted Ly-6/uPAR-related protein 1 (SLURP1) in peritoneal MC culture (PCMC). Analysis of mRNA expression in full thickness back skin showed downregulation of the mMCP4 in noise-stressed and inflamed skin, whereas additional blocking of NGF reverses this effect. Under stress or AID mMCP6 is downregulated, additional stress upregulates it and blocking of BDNF amplifies this effect. By immunohistomorphometry, expression of mMCPs was found in skin MCs. In AID skin compared to control mMCP4+ mast cell number was upregulated, additional stress enhanced it. Blocking NGF downregulated positive MCs back to control. Treatments had no effect on mMCP6+ MCs in skin. In PCMC rtPCR revealed no effect of SP stimulation in wild type (Wt) MCs, but upregulation of mMCP4 and mMCP6 in Chrna7 knockout (KO). Additional SLURP1 stimulation upregulated mMCPs in Wt, while this effect was extinguished in KO. In the light of these results we conclude that cholinergic signaling is involved mMCP expression of MCs.

Microglia express the vesicular nucleotide transporter (VNUT) essential for exocytotic ATP release

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One of the early symptoms of most brain diseases is the activation of neuroglia which release so-called gliotransmitters such as glutamate and ATP to modulate synaptic transmission. In response to neuronal injury ATP has been shown to be released from neurons, astrocytes and more recently from microglia. However, the mechanisms of its transport and rapid release in the different cell types are still unclear. Sawada and coworkers identified in 2008 the solute carrier protein SLC17A9 as a vesicular transporter for ATP and other nucleotides (VNUT). Since then VNUT expression has been reported in neurons of the CNS and PNS and in immune cells. The aim of this study was to compare gene expression levels of VNUT in microglia, astrocytes and neurons of adult mouse brain using laser microdissection assisted RT-PCR analysis, qPCR and in situ hybridization. Using RT-qPCR VNUT expression could be detected in many brain regions. Surprisingly, VNUT mRNA levels in primary microglial cultures and in the BV2 cell line were between 10 to 100 fold higher than in neuronal extracts of any brain region. In addition, VNUT RNA transcripts were also more than 10 fold higher in cultured microglia than in primary astrocyte cultures. Our results of high VNUT transcript levels in microglia as compared to astrocytes or neurons provide further evidence for purinergic gliotransmission from microglia to astrocytes and/or neurons via rapid exocytotic release.

Effects of minocycline on sleep, memory, and immune parameters in humans

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Pro-inflammatory molecules are known to mediate interactions between the immune system and the central nervous system. Sleep and especially the slow-wave activity (SWA) characterizing non-rapid eye movement (NonREM) sleep have been shown to be important for the formation of long-term memory. Pro-inflammatory cytokines can alter sleep patterns and neuronal processes underlying memory formation. To more closely characterize this immune influence, we investigated the impact of the anti-inflammatory antibiotic minocycline on sleep, memory consolidation and monocyte function in healthy human subjects.

In a double-blind within-subject crossover design 20 young men received either 200 mg minocycline (orally at 22:30 h) or placebo after learning different memory tasks and before nocturnal sleep. Overnight consolidation of declarative memory, sleep parameters, and monocytic cytokine production were measured. Contrary to our expectation, minocycline enhanced SWA during NonREM sleep stage 2, an effect similarly observed in a previous study of ours after administration of the IL-1 receptor antagonist anakinra. Minocycline concurrently increased sleep spindles, and also tended to increase total sleep time. There were no strong effects of the substance on the retention of memories (tested in the morning 48 hours after learning), except for a slightly improved retention of episodic memory. This improvement can be well explained by the enhancing effect of minocycline on SWA and spindle activity. Minocycline also altered cytokine expression of circulating monocytes. Taken together, the changes observed after minocycline substantiate the relevance of endogenous pro-inflammatory cytokines for sleep and memory consolidation.

Compartmentalisation of inflammatory biomarker signatures in untreated relapsing-remitting multiple sclerosis patients

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Background:

Multiple sclerosis (MS) is the most common disabling neurological disease in young adults in the western world. It is an inflammatory and neurodegenerative disorder of the central nervous system. Recent findings suggest a role for innate immune activation and elevated latent Epstein-Barr virus-nuclear antigen-1 (EBNA-1) titers as risk factors for MS.

Objective:

To analyse the anti-EBNA-1 reactivity and inflammation status in matched serum and cerebrospinal fluid (CSF) samples from untreated relapsing-remitting MS patients (RRMS).

Material and Methods:

Anti-EBNA-1 titers and IL-1 β , IL6, IL-8, IL-10, TNF and IL-12p70 cytokine levels were measured by ELISA in 23 untreated RRMS and 17 healthy controls.

Results:

We found elevated anti-EBNA-1 titers in RRMS compared to healthy controls. Augmented IL-8 levels were found in the serum in RRMS compared to healthy controls however, no other cytokines were detectable. Furthermore, anti-EBNA-1 titers and IL-8 levels were significantly increased in serum compared to CSF in matched RRMS samples. We also detected raised anti-EBNA-1 titers and IL-8 levels in serum compared to CSF in matched RRMS samples.

Conclusion:

Our data shows augmented anti-EBNA-1 titers and IL-8 levels in the periphery in RRMS. Interestingly, we detected higher IL-8 and anti-EBNA-1 titers in serum than CSF in matched RRMS samples. These findings highlight the need for further studies into the compartmentalisation of inflammatory responses RRMS.

Catecholaminergic-to-cholinergic transition of sympathetic nerve fibers is stimulated under healthy but not under inflammatory arthritic conditions

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Objective.

Density of sympathetic nerve fibers decreases in inflamed arthritic tissue tested by immunoreactivity towards tyrosine-hydroxylase (TH, catecholaminergic key enzyme). Since sympathetic nerve fibers may change phenotype from catecholaminergic to cholinergic (example: sweat glands), loss of nerve fibers may relate to undetectable TH. We aimed to investigate possible catecholaminergic-to-cholinergic transition of sympathetic nerve fibers in synovial tissue of animals with arthritis, and patients with rheumatoid arthritis (RA) and osteoarthritis (OA), and we wanted to find a possible transition factor.

Methods.

Nerve fibers were detected by immunofluorescence towards TH (catecholaminergic) and vesicular acetylcholine transporter (cholinergic). Co-culture experiments with sympathetic ganglia and lymphocytes or osteoclast progenitors were designed to find stimulators of catecholaminergic-to-cholinergic transition (including gene expression profiling).

Results.

In mouse joints, an increased density of cholinergic relative to catecholaminergic nerve fibers appeared towards day 35 after immunization, but most nerve fibers were located in healthy joint-adjacent skin or muscle and almost none in inflamed synovial tissue. In humans, cholinergic fibers are more prevalent in OA synovial tissue than in RA. Co-culture of sympathetic ganglia with osteoclast progenitors obtained from healthy but not from arthritic animals induced catecholaminergic-to-cholinergic transition. Osteoclast mRNA microarray data indicated that leukemia inhibitory factor (LIF) is a candidate transition factor, which was confirmed in ganglia experiments, particularly, in the presence of progesterone.

Conclusion.

In humans and mice, catecholaminergic-to-cholinergic sympathetic transition happens in less inflamed tissue but not in inflamed arthritic tissue. Under healthy conditions, presence of cholinergic sympathetic nerve fibers may support the cholinergic anti-inflammatory influence recently described.

Tropisetron – an emerging anti-inflammatory and antifibrotic agent

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Tropisetron is an approved antiemetic for patients undergoing chemotherapy. Recently we reported that tropisetron, originally characterized as a serotonin (5-HT) receptor modulator, suppressed transforming growth factor-beta1-mediated collagen synthesis in normal human dermal fibroblasts (HDFs) as well as in dermal fibroblasts from patients with systemic sclerosis (SSc). This effect of tropisetron was independent of the 5-HT₃ and 5-HT₄ receptor but mediated via the α 7 nicotinic acetylcholine receptor (α 7nAChR) in HDFs. Importantly, tropisetron had antifibrogenic and antifibrotic effects in experimentally induced skin fibrosis of mice. Since lung fibrosis is a common complication in patients with SSc we tested whether tropisetron has antifibrotic effects in extracutaneous organs. In a mouse model of lung fibrosis experimentally induced by a single pharyngeal aspiration of bleomycin (BLM) tropisetron likewise significantly reduced collagen type I and III mRNA expression and protein amounts in the lungs compared with BLM-treated mice. To assess the relevance of these findings in the human system we performed an expression analysis of the putative tropisetron receptors in human lung fibroblasts. Neither 5-HT₃ nor 5-HT₄ receptors were detected while these cells expressed the previously identified off-target receptor of tropisetron, α 7nAChR. Since the BLM mouse models of skin and lung fibrosis are inflammation-driven models we further investigated whether tropisetron can counteract inflammatory cell responses in non-fibroblast cutaneous cell types. Accordingly, we examined the impact of tropisetron on tumor necrosis factor (TNF)- α -mediated expression of interleukin (IL)-6 and 8 as well as on ultraviolet B (UVB)-induced cytokine expression in human epidermal keratinocytes (NHK). Tropisetron suppressed both TNF- α - and UVB-induced expression and secretion of these proinflammatory cytokines in these cells. This effect of tropisetron was independent of canonical p65/NF- κ B signaling. In analogy to human dermal and lung fibroblasts, neither 5-HT₃R nor 5-HT₄R was detectable in NHK. In contrast, α 7nAChR were present and mediated the anti-inflammatory effect of tropisetron in these cells. The in vivo relevance of these in vitro findings was confirmed in the imiquimod mouse model of psoriasis in which injections of tropisetron resulted in a significant reduction of cutaneous inflammation. In summary, our data show that tropisetron has antifibrogenic potential not only in the skin but also in the lung and acts anti-inflammatory in other cutaneous cell type beyond fibroblasts. Further studies on the α 7nAChRs in inflammatory and fibrotic skin diseases will clarify the therapeutic utility of drugs specifically targeting these receptors.

The brain and the environment in multiple sclerosis

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The exact mechanisms underlying neuroinflammation and neuropathology in multiple sclerosis (MS) are still unknown, but susceptibility depends on a combination of genetic and environmental factors and their interactions. There is mounting evidence implicating both late EBV-infection and hypovitaminosis-D as key environmental risk factors in MS. We have previously shown that active white matter lesions in the MS brain show signs of innate immune activation, and that latently EBV-infected cells can be found in these areas. We then investigated whether there is an interdependence between EBV-viral load and vitamin-D levels and found that hypovitaminosis-D did not impact on EBV-status in healthy individuals. More recently, we studied the compartmentalisation of EBV-status and inflammatory signatures in untreated relapsing-remitting MS patients. With little influence on genetic predisposition, the importance of modulating environmental risk factors is becoming an area of great interest.

Inflammation-induced pain sensitization in men and women:

Does sex matter in experimental endotoxemia?

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A role of the innate immune system is increasingly recognized as a mechanism contributing to pain sensitization. Experimental administration of the bacterial endotoxin lipopolysaccharide (LPS) constitutes a model to study inflammation-induced pain sensitization, but all existing human evidence comes from male participants. We assessed visceral and musculoskeletal pain sensitivity following low-dose LPS administration for the first time in healthy men and women to test the hypothesis that women show greater LPS-induced hyperalgesia compared to men. In this randomized, double-blind, placebo-controlled crossover study, healthy men (n=20) and women (n=20) received an intravenous injection of 0.4 ng/kg body weight LPS or placebo. Pain sensitivity was assessed with established visceral and musculoskeletal pain models (i.e., rectal pain thresholds; pressure pain thresholds for different muscle groups). Plasma cytokines (TNF-alpha, Interleukin-6) were measured along with state anxiety at baseline, and 1, 2, 3, 4, and 6h post-injection. LPS application led to significant increases in plasma cytokines and state anxiety in men and women ($p < 0.001$, condition effect), with more pronounced LPS-induced cytokine increases in women ($p < 0.05$, interaction effects). While both rectal and pressure pain thresholds were significantly decreased in the LPS condition (all $p < 0.05$, condition effect), no sex differences in endotoxin-induced sensitization were observed. In summary, LPS-induced systemic immune activation leads to visceral and musculoskeletal hyperalgesia, irrespective of biological sex. These findings support the broad applicability of experimental endotoxin administration as a translational, pre-clinical model of inflammation-induced pain sensitization in both sexes.

520-d isolation and confinement simulating a flight to Mars reveals heightened immune responses and alterations of leukocyte phenotype

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During interplanetary exploration, chronic stress caused by long term isolation and confinement in the spacecraft is one of the major concerns of physical and psychological health of space travelers. And for human on Earth, more and more people live in an isolated condition, which has become a common social problem in modern western society. Collective evidences have indicated prolonged chronic stress could bring big influence to human immune function, which may lead to a variety of health problems. However, to what extent long-term isolation can affect the immune system and immune response? It is still largely unknown. A simulated 520-d Mars mission provided an extraordinary chance to study the effect of prolonged isolation. Six healthy males participated in this mission and their active neuroendocrine and immune conditions were studied with saliva and blood samples from all participants on chosen time points during the isolation period. As a typical neuroendocrine parameter, stress hormone cortisol was measured in the morning saliva samples. Immune phenotype changes were monitored through peripheral leukocyte phenotype analysis. Using an ex-vivo viral infection simulation assay we assessed the immune response changes characterized by the ability to produce representative endogenous pro-inflammatory cytokines. The results of this study revealed elevated cortisol levels, increased lymphocyte amount, heightened immune responses, suggesting that prolonged isolation acting as chronic stressors are able to trigger leukocyte phenotype changes and poorly controlled immune responses.

Higher macrophage superoxide anion production in essential hypertension

C. Zuccarella-Hackl

Background:

Essential hypertension is an important risk factor for coronary artery disease and its underlying process atherosclerosis but involved mechanisms are not fully understood. Both macrophages and superoxide anions have been proposed to play a major role in the pathogenesis of atherosclerosis. In the present study we investigated whether macrophages of hypertensives show higher NADPH oxidase-derived superoxide anion production compared to normotensives.

Methods and Results:

We studied 30 hypertensive (M: 48.7 ± 2.4 years) and 30 age-matched normotensive men (M: 48.6 ± 2.4 years). We assessed macrophage superoxide anion production using the WST-1 assay. The assay bases on the chemical reduction of the cell-impermeative tetrazolium salt WST-1 by superoxide anions that are produced by activated human ex vivo isolated monocyte-derived macrophages. All analyses were controlled for potential confounders. Hypertensives showed higher superoxide anion production compared to normotensives ($F(1,58) = 11.56$, $p = .001$). Complementary analyses using mean arterial blood pressure as a continuous measure revealed that higher mean arterial pressure correlated significantly with higher WST-1 reduction ($\beta = .38$, $p = .003$, $\Delta R^2 = .145$). These results remained significant when controlling for potential confounders.

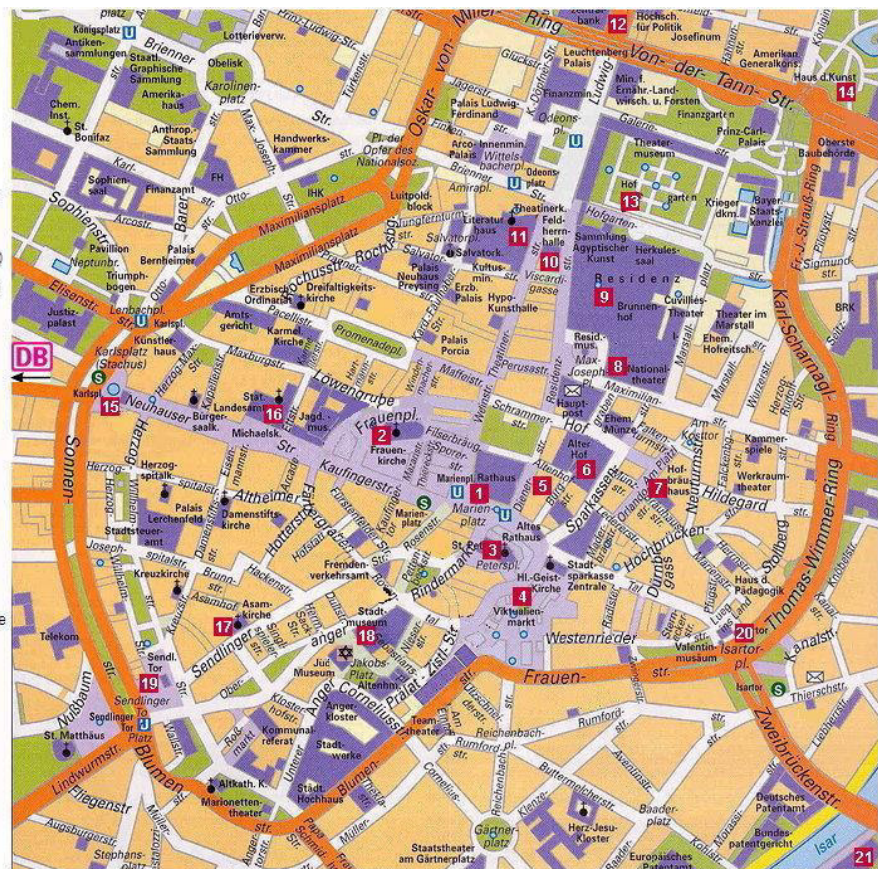
Conclusions:

Our results indicate higher macrophage superoxide anion production in hypertensives compared to normotensives. This may suggest a mechanism underlying cardiovascular risk with hypertension.

Top attractions and sights in Munich

Munich City Map

- 1 Marienplatz with Old- and New Townhall
- 2 Frauenkirche - Cathedral of Our Lady
- 3 St. Peter's Church (Oldest parish church)
- 4 Viktualienmarkt (Traditional food market)
- 5 Medieval Town Clerk House
- 6 Alter Hof (First ducal residence)
- 7 Hofbräuhaus
- 8 National Theatre (Opera House)
- 9 Royal Residence incl. Cuvillies Theatre
- 10 Feldhermhalle - Hall of Generals
- 11 Theatine Church - St. Kajetan
- 12 Ludwig Str. (Boulevard King Ludwig I.)
- 13 Royal Garden
- 14 Entrance to the English Garden
- 15 Karl's Square ("Stachus") with Karl's Gate
- 16 St. Michael's Church
- 17 Asam Church (Rococo style jewel)
- 18 Municipal Museum
- 19 Sendlinger Tor (Medieval city gate)
- 20 Isartor (Medieval city gate)
- 21 River Isar - Deutsches Museum



Marienplatz - Mary's Square - is the heart of Munich and the best place to start your Munich sightseeing tour. It houses the Mariensäule, the Marian Column topped with the golden statue of Virgin Mary, and it is also home to the Old and the New Town Hall of Munich. The tower of the New Town Hall houses the Glockenspiel, a beautiful carillon that is over 100 years old. Come here at 11 a.m. or noon to hear the Glockenspiel chime and watch the 32 life-sized figures reenact historical Bavarian events. Dating back to the 12th century, Marienplatz used to be home to medieval markets, celebrations, and tournaments; today, the square is a popular meeting place.

Frauenkirche - The Catholic Cathedral of Our Blessed Lady is the landmark of Munich and the city's largest church; it holds up to 20,000 people. Together with the Town Hall, the sturdy twin towers of the Cathedral shape Munich's skyline and make it a great point of orientation. You can also climb the steps of the towers - the view of Munich's cityscape and the Bavarian Alps is breathtaking. The architectural style of the brick-built cathedral is late Gothic from the 15th century. Its famous domes atop each tower were modeled on the Dome of the Rock in Jerusalem.

Englischer Garten - Just a few blocks northeast of the Munich Residence is the English Garden, Munich's largest park. Bigger than Central Park in New York, this green oasis is a wonderful place to explore: Rent a paddle boat, stroll along the wooded paths, visit one of its traditional beer gardens, and watch the German answer to surfing on the currents of the waterway called Eisbach. Points of interest in the English Garden: Chinese Tower and its huge beer garden. Japanese Teahouse, where you can take part in a traditional tea ceremony on the weekend. Monopteros, a Greek style temple, which offers great views of Munich's cityscape. Lake Kleinhesseloher See, where you can rent a paddle boat, and its idyllic beer garden Seehaus. Schönfeldwiese, the lawn where nude sunbathing is allowed since the 1960's.

Hofbräuhaus - No trip to Munich is complete without visiting the Hofbräuhaus. The most famous beer hall in the world is located in the heart of Munich's old town, just a few steps from the central square Marienplatz. Established in 1589 as the Royal Brewery of the Kingdom of Bavaria, the Hofbräuhaus, just like Oktoberfest, is an essential part of Munich's history, culture and cuisine.

Deutsches Museum - The German Museum is located on an island in the river Isar that runs through Munich's city center. It is one of the oldest and largest science and technology museums in the world and boasts an impressive collection of historic artifacts. You can see the first electric dynamo, the first automobile, and the laboratory bench where the atom was first split. Other highlights of the museum include exhibitions on astronomy, transportation, mining, printing, and photography.

Olympia Park - The Olympic Park in northern Munich is well known beyond the borders of the capital city. The unique tent architecture of the buildings and the Olympic Tower are some of Munich's well known landmarks. After the Olympic Games in 1972, a 300-hectare-sized park was developed into a recreation center for the entire city. Joggers, cyclists, and walkers take their laps here, and swimmers do lengths in the Olympic swimming facility. At over 50 meters (150 feet) high, the Olympic Hill towers over the park grounds and is an ideal spot to enjoy a view of the roofs of Munich and to the mountains beyond.

BMW-Welt - *BMW Welt* (BMW World) is a space dedicated to one of Germany's most famous exports. Many travelers said it was worth visiting - if not for the cars than for the building's contemporary architecture. You can view the company's latest concept cars, motorcycles and more here. Then head over to the nearby BMW Museum to learn a bit more. Both sites are located on the east side of the Olympiapark.

Residenz - At the edge of Munich's old town lies the Residence, the former royal palace of the Bavarian monarchs. Today the Residence houses one of the best European museums of interior decoration. The Residence, whose first buildings were constructed in 1385, consists of ten courtyards and beautiful historical gardens. The museum itself displays 130 rooms

with antique furniture, art, porcelain, and tapestries that span the Renaissance, Baroque, Rococo, and the neoclassical era.

Viktualienmarkt - Only a few steps away from Marienplatz, you'll find the bustling Viktualienmarkt, Munich's daily outdoor farmers market. Stroll past the 140 colorful booths and enjoy the unique flavor of this market that boasts a great variety of fresh and regional food.

The Viktualienmarkt, whose beginnings date back to the early 19th century, offers everything from flowers, honey, and spices, to meat, cheese, eggs, and pastries. Take in the garlands of sausages, mountains of fresh vegetables, and pyramids of fruits, and let your senses be seduced.



Schnellbahnnetz

