

Pteridines
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Abstracts

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"Mild Encephalitis" - Brain-organic Pathologies Between Neurologic and Psychiatric Disease?

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The term 'mild encephalitis' (ME) (1) was proposed some years ago to address a new approach to severe psychiatric disorders with unknown etiology, the pathogenesis of which appeared not appropriately understood. ME means a type of low level CNS inflammation possibly causing a spectrum of psychiatric disorders in interaction with other contributing factors. In the meanwhile more and more findings support such view including recent own studies by modern CSF diagnostics, considered the diagnostic gold standard in CNS-inflammation, especially acute meningoencephalitis: we found in 41% of patients with affective (n=24) or schizophrenic (n=39) spectrum disorders definite CSF abnormalities; in 14% intrathecal humoral immune responses clearly interpreted as inflammatory; in 10% slightly increased CSF cell counts; in 29% moderate blood CSF barrier dysfunction, in 24% as the only pathological sign; in 20% of affective spectrum patients systemic inflammation, in none of the schizophrenic spectrum patients. In 6% (n=4) we refined the previous differential diagnosis by the results of CSF investigation and diagnosed probable ME due to various specific causes:

streptococcal infection associated autoimmune disorder; HSV 1,2 infection associated autoimmune disorder respectively early state of systemic lupus erythematoses; undefined systemic autoimmune disorder associated with Sjögren syndrom; suggestive chronic Borna-Disease-Virus-infection.

Despite some recent progress, the definition of ME remains preliminary, on the other hand within the general problems of definition and categorization of inflammation: the established definition dates back to Celsus 40 BC, modern definitions depend from the options to investigate the many aspects and factors involved. A recent definition (2) "inflammation is a complex set of interactions among soluble factors and cells that can arise in any tissue in response to traumatic, infectious, post-ischaemic, toxic or autoimmune injury" appears perfect, but is not applied in clinical practice. Conventional histopathology defines inflammation by "visible inflammatory cellular infiltrates within tissue", a limited view. It is known, that CNS-inflammation begins with an accumulation of immune competent cells within defined brain areas yet without visible cellular infiltrates and such may last for longer time. An important difference exists between acute and chronic inflammation, chronic not being clearly defined nor in histopathology nor in the clinic, in the latter by simple time cuts. In neurology it is accepted to consider encephalitis to be chronic after four weeks. In psychiatry there is little agreement about which disorders have to be termed chronic. When adopting the neurological time frame probably most psychiatric disorders

should be considered chronic. In the case of a neurobiological basis such as ME, we would deal nearly exclusively with chronic low level neuroinflammation then in psychiatry.

What is about the symptoms of psychoses and encephalitis? Classical encephalitis can cause various psychiatric syndromes, rare single cases observed may go through the range of psychiatric disorders within weeks, e.g. beginning with personality disturbance, progressing to affective spectrum and later schizophrenic spectrum disorder and only finally to organic psychoses and possibly backwards. The rule of non-specificity of psychiatric symptoms holds for various organic causes including encephalitis. Non-classical inflammation (classical i. fits acute but does not fully cover chronic i.) is now more and more recognized to exist though yet lacking clear definitions. Just recently one new term was proposed that is 'parainflammation', describing chronic inflammation with preferential fibrosis (3). Low level CNS- respectively neuro-inflammation (LLCI) is now often mentioned but lacks clear definition. One good exception is the categorization of microgliaactivation in four grades, representing one important aspect of neuro-inflammation though not always indicating inflammation (4); the lower grades proposed (1+2) would well match with what here is termed LLCI and ME respectively.

Refined definitions are not an academic point but are important for clinic and research. The unmet problem is demonstrated for example with the many open questions regarding the role of HIV-brain-infection for related psychiatric disturbances. When taking a strict definition of HIV-Encephalitis (HIV-E), HIV-E includes the post-mortem finding of three classical inflammatory foci; then postmortem diagnosis matches relatively well with clinical HIV-E-diagnosis (5). However, LLCI is present in non-HIV-E cases postmortem frequently and associated with various psychiatric syndromes, including cognitive decline or psychosis. Even more critical in such definition of HIV-E, not only from a practical but especially from a theoretical point of view is, that non-HIV-E cases may present up to two classical inflammatory foci within the brain which remain non-diagnostic for HIV-E. Such examples

demonstrate the dilemma in the approach to CNS inflammation in general, and even more to LLCI situations. The relationship between brain pathology and clinical psychiatric symptoms is known to be weak. To better understanding causal relationships and finding better definitions we need more parameters and more refined criteria. *In vivo* detection of LLCI is especially difficult. Adopting modern CSF-diagnostics from neurology with its broad background knowledge is an important but only a first step to diagnose ME as a possible psychiatric disease.

Summing up, the present definition of ME remains preliminary, but appears advantageous to the alternative of further neglecting the prevalence of LLCI situations, which may not rarely causally underlie psychiatric disorders. We propose to diagnose in severe psychiatric disorders (schizophrenic or affective spectrum) with the following CSF-findings: probable ME with oligoclonal bands exclusively detected within CSF not in parallel in blood, or with intrathecal production of specific immunoglobulin subclasses (IgA or IgG or IgM or combined); possible ME with exclusive blood-CSF-barrier-dysfunction (for details see (6)).

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Oxidative and Antioxidative Mechanisms in Juvenile and Adult Rats after Application of Haloperidol

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Tardive dyskinesia which is presumed to be connected with elevated oxidative stress, is described as a typical side effect of Haloperidol. However, this neurotoxic effect of Haloperidol is discussed controversially. In this context, previous studies gave little evidence for differences between adults and children as well as adolescents. The purpose of this study is to examine both oxidative mechanisms, substantiated by reactive oxygen species (ROS), and antioxidative mechanisms in juvenile and adult rats after application of Haloperidol.

Measurement of reactive oxygen species (ROS) was performed using radical scavenger CMH by means of microdialysis and subsequent electron spin resonance (ESR) spectroscopy. Organ extraction was carried out 3 hours after an acute application of Haloperidol (2 mg / kg i. p.). The organ parts were homogenized and their amount of ATP, reduced GSH, oxidized glutathione (GSSG), glutathione reductase (GR), glutathione peroxidase (GPX), glutathione-S-transferase (GST), superoxide dismutase (SOD) as well as NADPH oxidase (NOX) was tested by means of various kits.

No elevated radical formation could be measured *in vivo* following an acute dose of Haloperidol - neither in young nor in old animals. Juvenile rats opposed to adult animals showed a

higher ATP content in various brain areas suggesting that their antioxidative mechanisms seem to be less pronounced (GR, GPX, GST) than those of adult rats, while the GSSG content is higher in juvenile rats. The application of Haloperidol to adult rats seems to lead to a reduction of antioxidative mechanisms in various brain areas. There is also evidence that Haloperidol affects ATP. In juvenile rats, Haloperidol seems to have rather little influence on antioxidative mechanisms.

While our results show that Haloperidol does not directly influence radical concentration in both young and old rats, there is evidence that Haloperidol clearly has noticeable, age-related effects on radical formation occurring on the level of radical detoxification mechanisms.

Expression of Indoleamine 2,3-dioxygenase-2 (IDO2) at the Human Feto-Maternal Interface

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Tryptophan catabolism has been implicated in playing a decisive role in feto-maternal immunotolerance. Application of 1-methyl-DL-tryptophan which is able to block Indoleamine 2,3-dioxygenase (IDO) induced rejection of hemiallogeneic but not syngeneic embryos (Munn et al., 1998). The later finding that allogeneic matings of mice in which both parents were genetically IDO-deficient do not lead to higher abortion rates was enigmatic in this context (1). For this reason, our attention has been drawn to potential compensatory or redundant immunosuppressive mechanisms. Until recently the ability to convert tryptophan to N-formylkynurenine was regarded as being confined to IDO and tryptophan 2,3-dioxygenase (TDO, which is not blocked by 1-methyl-DL-tryptophan). However, we now know there is a third enzyme, indoleamine 2,3-dioxyge-

nase 2 (IDO2) with high homology to IDO (now named IDO1) and whose tryptophan-degrading activity is comparatively low (2).

By immunohistochemistry we localized IDO2 protein to the trophoblast of placental villi, with clear preponderance of the syncytiotrophoblast to the cytotrophoblast in first trimester samples. In placenta villi at term, the syncytiotrophoblast was positive, the staining was patchy. Expression of IDO2 in extravillous cytotrophoblast of first trimester decidua was comparatively minor and irregular, in the basal plate of second trimester and term placenta IDO2 is clearly positive in invasive extravillous cytotrophoblast. Other than that, IDO2 was found to some extent in glandular epithelium of first trimester decidua. Non-pregnant endometrium showed hardly any staining. RT-PCR showed expression of IDO2 mRNA in placental villi that increased with gestational age. IDO2 mRNA was also present in first trimester decidua.

In contrast to our previous finding regarding IDO1, the localization of IDO2 at the feto-maternal interface is more suggestive of a potential role in feto-maternal immunotolerance.

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Tetrahydrobiopterin Saves Murine Pancreatic Grafts from Ischemia Reperfusion Injury: Application in an eNOS^{-/-} Model

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Ischemia-reperfusion-injury (IRI)-related graft pancreatitis is a severe complication following pancreas transplantation (PTX). Tetrahydrobiopterin (H₄B), an essential cofactor of nitric oxide synthases (NOS), was shown to significantly attenuate IRI and protect from graft pancreatitis if injected to the donors prior to organ retrieval. Since the underlying mechanism is still under debate, we herein investigated if eNOS is the main target of H₄B concerning the observed protective effects using an eNOS^{-/-} model.

Male syngeneic C57Bl6 (H-2b) mice were used as size-matched donor and recipient pairs. Donors were either C57Bl6 based eNOS^{-/-} or eNOS wt mice. Murine cervical pancreas transplantation was performed with a modified no-touch technique. To induce pancreatitis grafts were subjected to 16 h prolonged cold ischemia time (CIT) as well as to 45 min warm ischemia time. Donors were either pre-treated with 50mg/kg body weight H₄B or were untreated. Non-transplanted animals served as controls. Microcirculation was analyzed by intravital fluorescence microscopy evaluating functional capillary density (FCD). Parenchymal damage was assessed by H&E histology. Intra-graft H₄B levels were assessed by HPLC. Since ischemia reperfusion injury of the pancreatic graft in this model was found to be lethal, survival analysis of the different groups was assessed.

Prolonged CIT resulted in markedly impaired microcirculation compared to non-transplanted controls. Independently of their genotype, 2 h following reperfusion grafts treated with H₄B displayed improved FCD compared to untreated animals ($p = 0.09$). As early as 15 min after reperfusion, there were no differences between treated and untreated animals. In both genotypes, reduction of IRI-related tissue damage like parenchymal edema and acinar and fat necrosis was decreased following H₄B-treatment ($p < 0.05$), however the difference was more pronounced in wt grafts. Following donor pre-treatment with

H₄B, intragraft H₄B levels were significantly higher compared to non-treated controls ($p < 0.05$). Finally, H₄B-treated pancreatic grafts resulted in significantly prolonged recipient survival, independently of the donor genotype ($p < 0.001$).

H₄B attenuates graft pancreatitis and hence significantly improves recipient and graft survival. However, eNOS is not the main target of tetrahydrobiopterin action concerning its protective effects during IRI.

Role of Food Additives in Addition to Calory Intake in the Pathogenesis of Obesity

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Changes of lifestyle including physical inactivity and overnutrition have led to a rise of severe obesity. The daily intake of high calory food and food additives has increased substantially during recent decades. Many of compounds used are antioxidants and may thus interfere with redox-sensitive cellular signalling.

Leptin and adiponectin are adipokines, which are secreted mainly by adipocytes, and have important effects on the metabolism and homeostasis of energy. The aim of the *in vitro* study was to investigate a potential influence of food additives on the leptin and adiponectin release of murine adipocytes. Leptin and adiponectin concentrations were analyzed in supernatants of murine adipocytes 3T3-L1 after co-incubation with lipopolysaccharide (LPS) and the widely used food additives sodium sulfite (E221, SS), sodium benzoate (E211, SB) and curcumin (E100) in various concentrations for 24 hours. Moreover, the kinetics of secretion of both adipokines were analyzed.

A significant and dose-dependent decrease of leptin production was observed after incubation of the cells with SB and curcumin after 12 and 24

hours. For SS we observed a decrease of leptin concentration after 24 hours only. SS and SB had no effect on the secretion of adiponectin. Curcumin decreased the adiponectin concentration significantly after 6h.

To analyze the effect of high calory intake on the secretion of leptin and adiponectin, we performed a within-subject crossover study, where we administered a standardized high-fat meal to 24 healthy subjects. Plasma concentrations of leptin and adiponectin were measured in the morning after an overnight fast and during postprandial lipemia, i.e. 2, 4 and 6 hours after meal ingestion (postprandial experiment). To exclude potential confounding factors affecting the adipocytokine plasma concentrations, a control experiment without meal ingestion was performed over the same time period (postabsorptive control experiment). Comparing plasma concentrations of leptin and adiponectin between the postprandial and the postabsorptive control experiments, we found no significant differences.

Our experiments show that food additives SS, SB and curcumin affect the leptin release from adipocytes in a dose- and time-dependent fashion, in an *in vitro* study. Decreased leptin release during consumption of nutrition-derived food additives could decrease the amount of circulating leptin to which the central nervous system is exposed and could therefore contribute to an adipogenic effect.

The effect of postprandial lipemia after consumption of a high fat meal has no significant effect on the plasma concentrations of leptin and adiponectin in relation to their postabsorptive plasma profiles in healthy subjects. Therefore, we conclude that prolonged states of obesity are required to affect plasma concentrations of these adipocytokines.

Serum Phenylalanine to Tyrosine Ratio in Patients with Lung Cancer and Chronic Obstructive Pulmonary Disease (COPD)

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Abnormal metabolism of aromatic amino acids tryptophan (trp) and phenylalanine (phe) accompanies conditions which go along with chronic inflammation and immune responses like cancer, infection, autoimmune syndromes, cardiovascular disorders, neurodegeneration, etc. Such diseases are often accompanied by neuropsychiatric symptoms like cognitive impairment, mood changes and depression. Part of them can be referred to subnormal trp and thus serotonin (5-hydroxytryptamine, 5HT) availability, but some patients do not respond well to treatment with selective serotonin reuptake inhibitors (SSRI). Alternatively, dopaminergic dysfunction could be involved.

Earlier it was observed that patients with hepatocellular carcinoma (1), with burns (2) or with HIV-1 infection (3) may present with increased phe concentrations. More recent data obtained from patients after trauma (4), with ovarian cancer (5) or HIV-1 infection (6) demonstrate significant associations between neopterin production and decreased activity of phenylalanine-4-hydroxylase (PAH) as is indicated by an increased phe to tyrosine (tyr) ratio (phe/tyr), and again phe/tyr correlated with neopterin concentrations.

In this study, 236 patients (48 females, 149 males, aged 62.4 ± 9.3 y) with lung cancer and 39 patients (18 females, 21 males, aged 63.3 ± 7.8 y) with chronic obstructive pulmonary disease (COPD) were investigated. Serum concentrations of phe and tyr were measured by HPLC monitoring natural fluorescence of compounds. Results were compared to neopterin concentrations measured with ELISA (BRAHMS, Hennigsdorf, Germany), to the degradation of tryptophan (trp) as reflected by the kynurenine (kyn) to trp ratio (kyn/trp) and to nitrite + nitrate production applying the Griess reaction.

Mean neopterin concentrations (lung cancer: 9.5 nmol/L vs. COPD: 7.7 nmol/L; not significant) and kyn/trp (lung cancer: 40.7 μ mol/mmol vs. COPD: 34.4 μ mol/mmol; $p = 0.02$) were found to be higher in lung cancer than in patients

with COPD, the latter presenting widely with normal neopterin concentrations, only 16% presented with neopterin concentrations >10 nmol/L). In a similar way tryptophan metabolic parameters behaved with abnormal levels preferentially in cancer patients. Eleven percent of patients with cancer compared to 8 % of patient with COPD presented with phe/tyr >1.25 indicating impaired conversion of phe. In the patients with cancer, significant correlations existed of neopterin levels with kyn/trp ($r_s = -0.587$, $p < 0.0001$) but also with tyr concentrations ($r_s = -0.266$, $p < 0.001$) as well as phe/tyr ($r_s = -0.317$, $p < 0.0001$). No such associations existed in patients with COPD in whom only the correlation between neopterin and kyn/trp remained significant ($r_s = 0.476$, $p < 0.002$).

Impaired PAH activity is indicated in a relevant percentage of patients with lung cancer but not in COPD. The impaired PAH activity parallels the increased formation of neopterin and the accelerated degradation of trp which is most probably due to enhanced activity of indoleamine 2,3-dioxygenase (IDO). The lower neopterin concentrations and kyn/trp in COPD indicates that Th1-type immunity is less strongly activated in these patients as compared with the patients with lung cancer. The correlation between phe/tyr and neopterin concentrations as well as kyn/trp in lung cancer patients points to a possible role of chronic immune activation for the abnormal activity of PAH. A role of ROS in diminishing availability of tetrahydrobiopterin (H4bip), the necessary cofactor of PAH appears likely. However, any potential relevance for the neuropsychiatric abnormalities in patients still needs to be shown. The concurrent determination of phe/tyr and kyn/trp may provide a basis for improved treatment decision whether SSRI or other neuromodulators are more appropriate for a single patient.

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Tumor Invasion Pattern and Related Serum Tryptophan and Neopterin Concentrations in Colorectal Carcinomas

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Lymphatic invasion and the depth of submucosal invasion of tumour cells are risk factors for lymph node metastasis, which is the strongest prognostic determinant for colorectal cancer patients. It was suggested that indoleamine (2,3)-dioxygenase (IDO) high-expressed colorectal tumour cells enable cancer subsets to avoid immune attack. Aim of this prospective clinical study was to investigate the impact of cellular immune response and tryptophan metabolism on peritumoral lymphocytic infiltration, lymphatic invasion score and lymph node metastasis in colorectal cancer patients. Forty-three patients without signs of malign or metabolic diseases and 44 patients with new diagnosed colorectal carcinoma were included in this study. Serum tryptophan, kynurenine and urine biopterin concentrations were determined by high performance liquid chromatography. Serum nitrite and neopterin levels were measured by ELISA. The ratio of

kynurenine to tryptophan was calculated to estimate the serum IDO activity. Histopathological evaluation of tumour grade, lymph node metastasis, peritumoral lymphocytic infiltrate, lymphatic invasion were made by H-E staining. Total tumour tissue IDO-immunostaining score was calculated with the sum of the proportion and the intensity staining scores of peritumoral infiltrating cells. In carcinoma group a significant increase in serum kynurenine/tryptophan ratio was observed ($p < 0.05$), while their serum nitrite, neopterin and urinary biopterin levels were not significantly altered ($p > 0.05$ for all). IDO high-expression in tumour tissue was significantly correlated with the lymphatic invasion and lymph node metastasis. No correlation was found between IDO expression patterns, tumour stage and serum kynurenine/tryptophan ratio ($p > 0.05$). IDO expression levels in tumour tissue significantly correlated with the presence of lymph node metastasis and lymphatic invasion. These results suggested that total IDO immunostaining score has been included in histopathological evaluation of colorectal cancer cases.

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Effects of Rheumatoid Arthritis and Coronary Artery Disease on the Expression of Chemokines and Neopterin

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In chronic inflammatory processes chemokines are abundantly present at the site of inflammation (1). Also the pteridine neopterin is elevated in various diseases with inflammatory background like Rheumatoid Arthritis (RA) and Coronary Artery Disease (CAD). Several reports have indicated a correlation of neopterin with inflammatory activity and intensification of systemic symptoms of RA (2) and CAD activity score (3). Neopterin has already been used as a marker of

inflammation during clinical management in a number of diseases (4). Monocytes/macrophages, which play an important role as infiltrating cells at the site of inflammation, produce neopterin as well as chemokines upon immune activation. Chemokines play an important role in the immune response and directing lymphocyte movement. CCL2/MCP-1 is a molecule with powerful monocyte chemotactic activity expressed by monocytes. CX3CL1/fractalkine is a mediator of inflammation and angiogenesis in RA. The B-cell attracting chemokine CXCL13/BCA-1 is expressed by fibroblasts and endothelial cells within the inflamed synovium of RA patients. The aim of the present study was to demonstrate if RA patients with enhanced neopterin levels show activation of the proinflammatory chemokines CXCL13, CX3CL1 and CCL2.

113 patients with RA (ACR criteria) including 24.8% suffering from CAD underwent a laboratory testing for neopterin, CXCL13, CX3CL1, CCL2 and CRP which were all found in high concentrations (neopterin: 11.10 ± 5.78 nmol/l). Neopterin significantly correlated with all chemokines: CXCL13 ($r = 0.368$; $p < 0.0001$), CX3CL1 ($r = 0.241$; $p < 0.02$), CCL2 ($r = 0.231$; $p < 0.02$). Further on strong correlations among all three chemokines were observed. Patients with RA plus CAD in comparison to RA patients without cardio-vascular problems showed significantly higher values of neopterin and CXCL13 ($p < 0.03$).

Increased neopterin and chemokines seem to interact in the network of cell-mediated immune response. High neopterin levels in RA patients have strong association to the chemokine induced chemotaxis of B-cells (CXCL13). Our results indicate too that the high incidence of CAD in Rheumatoid Arthritis could be associated with enhanced mononuclear- (neopterin, CCL2) and B-cell activation (CXCL13), and as a consequence increased immune activity.

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Determination of Volatile Organic Compounds in Exhaled Breath of Patients with Lung Cancer and Healthy Volunteers Using TD-GC-MS and PTR-MS

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At present, diagnosis of lung cancer very often happens late in the course of the disease since inexpensive, non-invasive, sufficiently sensitive and specific screening methods are not available. Such an early non-invasive diagnosis of lung cancer which would improve prognosis and enlarge treatment options can be analysis of exhaled breath.

Since many of the compounds observed in the breath have never been described in the biochemical literature, it is of utmost importance for further biochemical investigations to confirm the identification of detected compounds with certainty. Since identification based on spectral library match only may be misleading, additional chromatographic data e.g. retention time or retention index is necessary. Only such a validated identification allows investigating the biochemical background of the observed compound.

Therefore, the primary aim of the present work was to identify the compounds observed in exhaled breath of lung cancer patients (of all disease stages) in a careful manner. For GC-MS

analyses we have built up a database of over 230 compounds based on calibration mixtures of pure reference materials. Correct identification and quantitation is particularly difficult in case of PTR-MS analyses where the fragmentation of protonated compounds take place. Since no separation of analytes takes place before ionization, different protonated compounds may contribute to the same ion detected in the PTR-MS spectrum. Thus, considering M^{+1} ion may be misleading and for larger alcohols ($>C_2$) it gives false results.

Exhaled breath and inhaled room air samples were analyzed using proton transfer reaction mass spectrometry (PTR-MS) and adsorption on solid sorbents with subsequent gas chromatography mass spectrometry measurements (SPE-GC-MS). For the PTR-MS measurements, 220 lung cancer patients and 441 healthy volunteers were recruited. For the GC-MS measurements, we collected samples from 37 lung cancer patients and 53 healthy volunteers. Lung cancer patients were in different disease stages and under treatment with different regimes. Alveolar breath was collected with the real-time CO_2 control into Tedlar bags and transported to laboratory before PTR-MS and SPE-GC-MS analyses.

Approximately, one third of the compounds detected were hydrocarbons. We found aromatic hydrocarbons, alcohols, aldehydes, ketones, esters, ethers, sulphur compounds, nitrogen-containing compounds and halogenated compounds. Acetonitrile, groups of furans and especially dienes were found to be correlated with smoking behaviour.

Respective reaction constants and calibration correction factors elucidated from calibrations were used for determination of benzene, isoprene, acetone and methanol by PTR-MS analyses. Benzene clearly is a compound related to smoking behaviour. The respective concentrations of isoprene, acetone and methanol are lower in lung cancer patients as compared to healthy volunteers. GC-MS results shows several compounds found at elevated level in breath of lung cancer patients in comparison to healthy volunteers, whereas tetramethylurea was found to be significant, regardless of sample collection method (with or without CO_2 control).

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Evolution of Regulation, Structure and Function in Phenylalanine Hydroxylase

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Phenylalanine hydroxylase (PAH) is a tetrameric enzyme which in mammals is mainly present in the liver where it converts phenylalanine to tyrosine. In mammals this is the first step in the catabolic degradation of L-Phe. The enzyme is dependent on tetrahydrobiopterin as electron donor in the catalyzed reaction. The biopterin cofactor has an inhibitory effect on PAH activity and at the same time protects the enzyme from degradation. Strict regulation of the mammalian enzyme is important to maintain appropriate level of phenylalanine to (i) avoid the disease phenylketonuria (PKU) characterised by mental retardation and (ii) provide a continuous supply of tyrosine for protein synthesis. One important regulatory mechanism is the cooperative response to phenylalanine binding in the active sites of the four subunits.

PAHs from bacteria and lower eukaryotes, which have been associated to the synthesis of L-Tyr derivatives such as melanine-like compounds, do not show allosteric regulation. We have analyzed the sequence and structure of PAH from an evolutionary perspective in an effort to explain the structural determinants for the incorporation of regulatory properties in mammals. Sites with a high probability of being involved in a functional or regulatory change were identified and structural models of PAH from human, and *Caenorhabditis elegans* have been prepared and used to visualize the changed sites. Important areas with high probability of being involved in

allosteric regulation of mammalian PAH are discussed. Molecular dynamics simulations have been applied to these models to get insight into the conformational changes related to regulation by both the substrate and biopterin cofactor at an atomic level.

This work has identified specific amino acid residues important for regulation of enzymatic activity. It has also provided insights into the basis for the evolution of sophisticated regulatory mechanisms in an important metabolic enzyme.

Serum Neopterin Concentrations and Tryptophan Degradation in Intensive Care Unit Patients

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Increase in neopterin concentrations and tryptophan degradation are common events following the activation of cellular immune system. In neurological, cardiovascular and autoimmune disorders, different types of malignancies and infections, high neopterin levels and tryptophan degradation have been reported to be well correlated with the severity of the diseases. The present study was undertaken to evaluate serum neopterin levels and tryptophan degradation in intensive care unit patients.

Serum neopterin, tryptophan and kynurenine concentrations were determined in patients with systemic inflammatory response syndrome (n = 9), sepsis (n = 8), septic shock (n = 10), and multiple organ dysfunction syndrome (n = 7). Neopterin concentrations and tryptophan degradation were tested if they differed among the patient groups. Furthermore, the relation between neopterin concentrations, tryptophan degradation and APACHE II (Acute Physiology and Chronic

Health Evaluation) scores were investigated.

Kynurenine concentrations were significantly increased in multiple organ dysfunction syndrome and septic shock groups compared to the sepsis group. Although the survivor group had lower neopterin concentrations and tryptophan degradation, the difference compared to non-survivors was not statistically significant. Neopterin concentrations and kynurenine/tryptophan ratio as an indicator of tryptophan degradation were both correlated with APACHE II score.

Previous studies from us and others (1-4) suggested that urinary neopterin concentrations are promising in terms of determining disease progression and the resultant prognoses of the intensive care unit patients. Results of the present study confirm and extend the previous data: serum neopterin levels and tryptophan degradation were well correlated with the severity of the disease in intensive care unit patients.

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Oxygen Radical Absorbance Capacity (ORAC) of Compounds Compared to the Suppression of Stimulated Peripheral Blood Mononuclear Cells

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Conditions of oxidative stress which may contribute to pathophysiological processes arise when pro-oxidant molecules are produced by the body at a rate that exceeds its ability to neutralize them. Beside antioxidative active enzymes and molecules produced naturally, also dietary intakes and pharmaceuticals are likely to contribute to the antioxidative defence of the body. Therefore, there is an increasing demand for methods that characterize the total antioxidant capacity of substances. The cell-free Oxygen Radical Absorbance Capacity Assay (ORAC) has been widely accepted as a standard tool to measure the antioxidative activity in the nutraceutical, pharmaceutical and food industries (1). The ORAC assay principle is based on the measurement of peroxy radical damage to a photostable fluorescent probe in a time and intensity dependent manner. Antioxidants are supposed to prevent the decrease of fluorescence by inactivating the peroxy radicals. Final values are normalized to trolox, a water soluble derivate of vitamin E, providing a measure of potent antioxidative activity. Evaluation of five different substances revealed, that the food colorant curcumin (E100) showed the highest antioxidative effect ($5.16 \pm 0.17 \mu\text{mol}$ trolox equivalents (TE)). Both, the cannabinoid Δ^9 -tetrahydrocannabinol (THC) and the non-psychoactive cannabidiol (CBD) demonstrated also significant antioxidative properties (0.49 ± 0.05 and $0.61 \pm 0.06 \mu\text{mol}$ TE, respectively), while the food preservatives sodium benzoate (E211) and propionic acid (E280) did not show any considerable activity (2, 3). Furthermore the *in vitro* influence of these substances on neopterin production and tryptophan degradation in mitogen stimulated and untreated human peripheral blood mononuclear cells (PBMC) was analysed in order to monitor potential interactions with Th1-type immune response mechanisms, where pro-oxidant species are produced upon stimulation of mononuclear cells. Dose-dependent suppression of tryptophan degradation and neopterin formation in resting and in mitogen stimulated PBMC could be

observed for all compounds within the analysed concentration range (4, 5). Thus, aside differences in intensity, all tested compounds showed a capacity to suppress Th1-type immune activation pathways, which indicate anti-inflammatory properties of these substances in this *in vitro* system. In summary, substances with high ORAC values seem to mediate suppression of neopterin and tryptophan degradation *in vitro*. However this is not true for the inverse conclusion, as suppressive effects of compounds on PBMCs do not consistently correlate with high antioxidant activity in the ORAC assay. The measurement of ORAC values of food, food additives or pharmaceuticals is certainly of interest, but these values do not necessarily reflect the final effects on antioxidative levels or the radical-quenching ability after food intake. Furthermore, only peroxy radical quenching capacities of compounds have been determined by this method, without consideration of other relevant radicals or contributors to oxidative stress formed *in vivo*. The PBMC model involves a broader variety of reactive oxygen species (ROS) and provides a link to biological relevant activities, although suppressive effects on neopterin formation and tryptophan degradation might be indirect and must not be mediated by blocking ROS activity mandatory.

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Carbonyl Proteins as a Clinical Marker in Alzheimer Disease and its Relation to Tryptophan Degradation and Immune Activation

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Question arises if oxidative stress is connected with systemic immune activation in Alzheimer's disease (AD) and mildly cognitive impairment (MCI). During immune response interferon- γ stimulates the production of reactive oxygen species, of neopterin and also tryptophan-degrading enzyme indoleamine 2,3-dioxygenase (IDO) enzyme which initiates the degradation of essential amino acid L-tryptophan (Trp) along the kynurenine (Kyn) pathway. Earlier we have described increased neopterin formation and significant degradation of Trp in a subgroup of patients with AD and MCI (1, 2). In this study, we estimated in patients with AD and MCI (AD/MCI) and an aged matched healthy control group the amount of Kyn, Trp and the Kyn to Trp ratio (Kyn/Trp), but also the content of carbonyl proteins (CP) as oxidative stress parameter and homocysteine, neopterin, folate and vitamin B12. Additionally we related the oxidative stress parameter CP with the degradation of Trp creating a new quotient CP/Trp. We calculated with discriminant analysis for CP, Trp, CP/Trp and Kyn/Trp its sensitivity, specificity and cut-off value (3).

CP was significantly higher in AD/MCI (930 ± 265 pmol/mg; $p < 0.001$) patients compared to

controls (301 ± 120 pmol/mg), Trp was significantly lower in the AD/MCI group (48.9 ± 9.0 $\mu\text{mol/L}$; $p < 0.001$) than the control group (65.2 ± 10.7 $\mu\text{mol/L}$) whereas Kyn showed no significant difference between AD/MCI group (1.72 ± 0.56 $\mu\text{mol/L}$) and control group (1.53 ± 0.29 $\mu\text{mol/L}$). Kyn/Trp of AD/MCI group was significantly higher in AD/MCI (35.2 ± 8.8 ; $p < 0.001$) patients than in the control group (23.7 ± 4.2). The new quotient CP/Trp showed a more than 4 fold increase in the AD/MCI group (19.8 ± 7.76 ; $p < 0.001$) compared to control (4.79 ± 2.26). Homocysteine, folate, vitamin B12 and neopterin showed no significant difference. Discriminant analysis provided CP alone as the best clinical marker with highest sensitivity (93.8%) and highest specificity (100%) for AD/MCI followed by the ratio of CP/Trp. These results support the hypothesis that oxidative damage to proteins is directly connected with Trp degradation and the Kyn pathway in the systemic immune activation.

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Urinary Neopterin and Serum Vitamins in Patients with Head and Neck Carcinoma

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Head and neck carcinomas are tumors of predominantly squamous cell histology. In general, the prognosis of patients with head and neck carcinomas is poor. Comorbid conditions are frequent in this population, and radical therapies are associated with considerable risk of morbidity and mortality. Therefore, identification of prognostic and predictive parameters is of great importance in patients with carcinomas of the head and neck. Increased urinary concentrations of neopterin are associated with poor prognosis in tumors of different primary locations, but the data on prognostic significance of neopterin in patients with head and neck carcinomas is more limited. Retinol and alpha-tocopherol are important serum antioxidants. Neopterin, retinol and alpha-tocopherol were determined by high-performance liquid chromatography. The survival was analyzed using the Kaplan-Meier method, with survival differences being analyzed by the log-rank test. The survival of patients with urinary neopterin concentrations equal or above 214 $\mu\text{mol/mol}$ creatinine was significantly inferior ($p < 0.05$) compared to patients presenting with lower neopterin levels. Serum retinol below 1 mol/l was also associated with statistically significant inferior survival, while serum alpha-tocopherol below 19 mol/L was of border-line prognostic significance ($p = 0.07$). In conclusion, urinary neopterin and retinol are prognostic factors in patients with head and neck carcinomas.

Immune System Modulation by anti-HIV Drugs

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Several studies have indicated an increased risk of myocardial infarction (MI) in HIV populations. Triant et al. showed, that MI rates were higher in HIV-infected than -uninfected patients in all age groups, with very high rates in older patients (1). Uncontrolled HIV infection is associated with elevated markers of inflammation, including C-reactive protein (CRP) and also neopterin (2). Levels of these markers decline with treatment, but not to normal levels, and thus pro-inflammatory signals persist, which may contribute to increased risk of cardiovascular disease (CVD). The question, if there is an increased risk of MI in HIV-infected patients under treatment, has been controversially discussed. In a study of Worm et al., the investigators reported unexpected increased risk of MI with the use of nucleoside reverse transcriptase inhibitors (NRTIs) abacavir and didanosine, which created much controversy, because other groups could not confirm their findings (3).

To elucidate a possible contribution of NRTIs to increase risk of CVD, with respect to their effects on inflammation associated pathways, we investigated several NRTIs on their capacity to modulate immune activation cascades *in vitro*. For this purpose, we used the nucleoside analogues Abacavir, Tenofovir, Emtricitabine and Lamivudine to determine their influence on tryptophan degradation and neopterin formation in unstimulated and phytohemagglutinin (PHA) stimulated peripheral blood mononuclear cells (PBMC). Additionally, we evaluated the capacity of these NRTIs to modulate Toll-like receptor (TLR)-induced nuclear factor (NF- κ B) activation in THP-1-blue monocytes, which stably express a reporter plasmid, expressing a secreted embryonic alkaline phosphatase gene under the control of a promoter inducible by NF- κ B.

In unstimulated PBMC, significant effects of these NRTIs on tryptophan degradation could be observed at doses of 100 and 200 μM . While Emtricitabine showed a stimulatory activity, Abacavir and Tenofovir suppressed spontaneous IDO activity within this concentration range. Neopterin levels in the same supernatants were not affected by the treatment with these NRTIs. In

mitogen stimulated PBMC, Tenofovir showed a trend to enhance mitogen stimulated tryptophan degradation at doses of 1 μM and 10 μM , which however was not significant. Significant effects could only be observed with Tenofovir and Abacavir, but again not until doses of 100 μM , exerting a suppressive effect on mitogen induced tryptophan degradation. Neopterin levels in mitogen stimulated PBMC increased significantly only upon co-treatment with Lamivudine, already at doses of 1 μM , while treatment with Abacavir at 100 μM and Tenofovir at 200 μM , showed a capacity to diminish mitogen induced neopterin formation.

Treatment of unstimulated THP-1-blue cells with NRTIs did not affect NF- κB activity, while LPS stimulated NF- κB activity was moderately but significantly enhanced by Lamivudine at doses of 10 nM, by Abacavir at doses of 100 nM and by Emtricitabine at doses of 1 μM . Tenofovir augmented LPS induce NF- κB activity significantly not until doses of 10 μM . Due to the reported data on obtainable concentrations of NRTIs in the plasma of patients under treatment, ranging from 10 nM to a maximum of 1 μM , a relevant effect may be considered for Lamivudine, Abacavir and Emtricitabine to enhance NF- κB activity and consequently the production of associated pro-inflammatory factors such as tumor necrosis factor- α , nitric oxide, interferon- γ and several interleukins.

In summary, this study revealed, that THP-1-blue monocytes seem to be more sensitive to the action of NRTIs than PBMCs, since LPS induced NF- κB activity was significantly enhanced by Abacavir and Lamivudine at doses in the nanomolar concentration range. In contrast, PBMCs showed significant effects of NRTIs not until doses of 100 μM , except Lamivudine, which enhanced mitogen stimulated neopterin formation in PBMC at doses of 1 μM . In conclusion, results of this *in vitro* study provide a rationale that Abacavir and surprisingly also Lamivudine and Emtricitabine may enhance inflammatory pathways induced by NF- κB , and thereby potentially may contribute to increased risk of cardiovascular disease and accordingly also MI in HIV-infected patients.

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Urinary Neopterin and Prognosis of Patients with Breast Carcinoma

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Increased serum or urinary concentrations of neopterin have been described in patients with tumors of different primary locations, but reports on neopterin in patients with breast carcinoma are relatively innumerable. We have evaluated urinary neopterin in 456 patients with breast carcinoma. Urinary neopterin was determined using high-performance liquid chromatography. Neopterin was increased above the threshold of 205 $\mu\text{mol/mol}$ creatinine that was defined as upper limits of normal in an earlier study, in 113 patients (25%). Urinary neopterin was significantly higher in 55 patients with metastatic or recurrent breast carcinoma compared to the other patients (mean \pm standard deviation 224 \pm 203 vs. 176 \pm

177 $\mu\text{mol/mol}$ creatinine, $p < 0.05$). Increased urinary neopterin was associated with significantly inferior overall survival in patients with tumor expressing hormone receptors, human epidermal growth factor receptor-2, but not in patients with triple negative tumors. In conclusion, serum neopterin is increased only in a minority of patients with breast carcinoma, but high neopterin concentrations herald poor prognosis.

Neopterin and Cardiovascular Disease

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Current evidence supports a key role for inflammation in all phases of atherosclerosis, from endothelial damage through to disease progression and, ultimately, the cardiovascular complications of atherosclerosis. The growing understanding of the role of inflammation in atherogenesis has focused attention on whether circulating levels of inflammatory biomarkers may help to identify patients at risk of future cardiovascular events. Of all available biomarkers, the most widely studied has been C-reactive protein (CRP), a nonspecific acute-phase protein, which has been shown to be an independent predictor of coronary risk in patients with angina and in apparently healthy subjects.

In recent years, neopterin, an aromatic pteridine with low molecular mass (253 Da) has been suggested to be a useful tool in the assessment of cardiovascular risk. Neopterin is mainly produced by activated macrophages and thought to represent a marker of immune activation and macrophage activity. (1) Several studies have shown that macrophages play a key role in atheromatous plaque progression and disruption. Indeed, plaque vulnerability has been shown to be a function of the increased numbers of macrophages and lymphocytes. Activated lymphocytes produce interferon- γ , which reduces collagen synthesis within atheromatous plaques and activates macrophages. Activated macrophages synthesize and release matrix metalloproteinases

that contribute to the degradation of collagen in the fibrous cap. Macrophages activated by interferon- γ synthesize neopterin (1) which, in turn, can interfere with intracellular signalling pathways.

In 1991, Melichar et al. found increased urinary neopterin concentrations in patients with acute myocardial infarction (AMI). (2) Several studies have shown that circulating neopterin concentrations are higher in patients with acute coronary syndromes (ACS) than in patients with chronic stable angina pectoris (CSA) and healthy persons (3, 4). Schumacher et al. reported elevated neopterin levels in patients with chronic CAD and more pronounced increased levels in patients with AMI (3) and Gupta et al. showed significantly higher neopterin levels in patients with ACS than in patients with a previous AMI (5). Increased neopterin is associated with the presence of vulnerable or disrupted atheromatous plaques (6, 7) and represents a marker of increased risk of further events in patients with ACS. Recent studies from our group have shown that increased serum neopterin predicts rapid CAD progression (8) and the development of major adverse coronary events in patients with CSA (9, 10) and in hypertensive persons without obstructive CAD (11).

In patients with CSA, García-Moll et al. found that women who developed cardiac events during follow-up had higher baseline neopterin concentrations than those without events (10). Studies from Avanzas et al. (9, 11) demonstrated that elevated neopterin levels were an independent predictor of major adverse coronary events during a 1-year follow-up in hypertensive patients with typical angina and without significant angiographic atherosclerotic disease and also in patients with CSA, irrespective of CAD severity.

In ACS Auer et al. (12) showed that neopterin levels, measured 72 hours after the onset of the index ACS, were significantly higher in patients with a clinical history of major cardiovascular events. In a recent large study, Ray et al. demonstrated a relationship between plasma neopterin levels and acute coronary events (nonfatal MI or unstable angina) in patients with ACS enrolled in the PRavastatin Or atorVastatin Evaluation Infection Therapy-Thrombolysis In Myocardial

Infarction (PROVE IT-TIMI 22) trial (13).

The Systemic Inflammation Evaluation in Patients with non-ST segment elevation Acute coronary syndromes (SIESTA) study (14) assessed the relationship between plasma neopterin levels and adverse clinical outcomes in Mediterranean patients with non-ST segment elevation (NSTE) ACS. In this study, raised neopterin levels (> 9.56 nmol/L) were a predictor of increased risk at 180-day follow up. Moreover, the addition of neopterin to TIMI risk score increased its prognostic ability. These data taken together indicate that neopterin is an independent marker of cardiovascular risk.

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Metabolism of Fluorescence Labelled-alkyl-glycerols and Fatty Aldehydes by Cultured Cells

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In cells ether lipids have important roles in maintaining proper brain function, spermatogenesis, mediation of inflammation, anchoring proteins to membranes and lens organisation. The tetrahydrobiopterin-dependent enzyme alkylglycerol monooxygenase (glycerylether monooxygenase, E.C. 1.14.16.5) is so far the only known enzyme that is able to cleave the ether bond of these lipids producing a free fatty aldehyde and a glycerol derivative. The aldehydes thus formed are converted to the corresponding acids by fatty aldehyde dehydrogenase (FALDH; E.C. 1.2.1.48). If this enzyme is deficient, the toxic aldehydes and related metabolites accumulate and cause the autosomal recessive disorder Sjögren Larsson Syndrome (SLS) which is characterised

by ichthyosis, mental retardation and spasticity.

To follow the fate of the fluorescent labelled substrates 1-O-pyrenedecyl glycerol, pyrenedecanal and pyrenedecanol in different types of cells, we have developed sensitive methods for the quantification of various metabolites of the applied substrate in the supernatant medium of cultured adherent cells.

We found significant differences in metabolism of normal human fibroblasts (HFB) and fibroblasts of an SLS patient. After incubation with 10 μ M aldehyde substrate for 24h, in normal human fibroblast primarily the fatty acid was formed, while in SLS cells the fatty alcohol was accumulated. In both cell lines the toxic aldehyde was removed almost entirely.

Incubation with 10 μ M alcohol substrate revealed that HFB cells are able to metabolise the alcohol into the corresponding acid while this was not the case for SLS cells, in which only marginal amounts of acid was formed. Experiments with purified, recombinant rat FALDH demonstrated that the enzyme alone is not able to catalyse this reaction. This suggests that FALDH is essential but not solely responsible for the degradation of fatty alcohols.

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Dose-dependent effects of Tibetan remedies on peripheral blood mononuclear cells *in vitro*

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The Tibetan herbal remedy Padma 28 is used for the treatment of disturbances of blood circulation, varicosis, arteriosclerosis, rheumatism and chronic inflammation disorders. Padma 28 is composed of twenty different powdered plants

including natural camphor and gypsum. Padma Basic, a modification of Padma 28, contains the same active ingredients with the exception of aconiti tuber. Ethanolic extracts of Padma 28 and Padma Basic were shown to exert significant suppressive effects on neopterin production and tryptophan degradation in mitogen-stimulated peripheral blood mononuclear cells (PBMC) *in vitro*. In this study, earlier obtained data on the effects of ethanolic extracts of Padma 28 on tryptophan degradation in phytohaemagglutinin A (PHA)-stimulated and unstimulated PBMC were confirmed and also observed with ethanolic extracts of Padma Basic and water extracts of both formulas. In contrast to the ethanolic extracts, Padma 28 and Padma Basic extracted in water, enhanced tryptophan degradation in unstimulated cells. Additionally, the antioxidative capacity of all extracts were investigated using the cell-free oxygen radical absorbance capacity (ORAC)-Assay and a cellular fluorescence based antioxidant activity assay, which measures formation of reactive oxygen species (ROS) upon stimulation with peroxy radical generating 2,2'-azobis-2-methylpropanimidamide (AAPH). Furthermore, the effects of both preparations were tested on their capacity to modulate nuclear factor (NF)- κ B activity and expression of heme oxygenase-1 (HO-1). Modulation of NF- κ B activity was monitored in lipopolysaccharide (LPS)-stimulated and unstimulated THP-1-blue monocytes, stably expressing a reporter plasmid, containing secreted embryonic alkaline phosphatase gene under the control of a promoter inducible by the transcription factor NF- κ B. Expression of HO-1 was determined in PHA-stimulated PBMC, in unstimulated THP-1-blue cells and hepatocarcinoma Hep-G2 cells. Compared to the effects of Padma 28 and Padma Basic extracted in water, the ethanolic extracts of these formulas showed a stronger antioxidant capacity in the ORAC-assay and also a more pronounced inhibition of AAPH-induced intracellular formation of ROS in Hep-G2 cells. Treatment of LPS-stimulated THP-1-blue cells with both, the ethanolic and water extracts of both formulas, diminished NF- κ B activity after six hours of treatment to about 70%, at highest concentrations tested (100 μ g/ml). In contrast, twenty-four hours after treatment, LPS induced NF- κ B activity was

enhanced to about 117% and 110% by the addition of Padma 28 and Padma Basic extracted in ethanol. Both formulas extracted in water also enhanced the LPS-induced NF- κ B activity to about 127% and 173% respectively. Expression of HO-1 was induced in a dose-dependent manner by the ethanolic extracts of both formulas in PBMC, THP-1-blue cells and Hep-G2 cells. In summary, Padma 28 and Padma Basic extracted in ethanol, generally showed stronger activity compared to the formulas extracted in water, although both formulas exhibit comparable antioxidative properties and a potential to modulate inflammation induced pathways.

Changes in LNAA in Patients with Depressive Disorders

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Large neutral amino acids (valine, isoleucine, leucine, tyrosine, phenylalanine; LNAA) compete for transport across the blood-brain barrier via the L-type amino acid carrier. The molar ratio of total plasma tryptophan to the sum of other LNAA is thought to reflect brain serotonin formation. *In vitro* and *in vivo* studies suggest a link between immune activation and changes of aromatic amino acids tryptophan and phenylalanine (1-3) which are of great importance for the proper biosynthesis of neurotransmitters. In patients suffering from cancer and HIV-1 infection as well as in animal model systems a role of tryptophan-degrading enzyme indoleamine 2,3-dioxygenase (IDO) in the precipitation of depressive symptoms has been reported (4-6). In order to find a possible relevance of these immunobiochemical alterations in the development of psychoneuroimmunological alterations in patients with major depression, we compared plasma tryptophan, kynurenine, phenylalanine and tyrosine concentrations in 27 patients and in 31 healthy controls.

In parallel, concentrations of immune activation marker neopterin and of cytokines tumor necrosis factor- α and interleukin-6 (IL-6) and soluble IL-2 receptor- α and various T-cell subpopulations were analyzed.

Patients presented with significant decline of tryptophan concentrations (56.7 vs. 64.2 μ mol/L; $p < 0.01$) which was more expressed in patients with a greater Hamilton depression (HAM-D) score ($r_s = -0.48$, $p < 0.05$). Kynurenine concentrations and the kynurenine to tryptophan ratio (kyn/trp) were lower in patients, but only the difference of kynurenine levels reached the level of statistical significance (1.82 vs. 1.45 μ mol/L; $p < 0.01$). Moreover, kynurenine concentrations correlated with weight loss in patients.

Out of the immunological parameters, only neopterin differed significantly and was higher in patients (mean: 6.3 nmol/L) than in controls (mean: 5.2 nmol/L; $p = 0.01$) whereas the investigated cytokines and T-cell subsets were not different between patients and controls. Phenylalanine was significantly lower in patients and also tyrosine levels showed a tendency of lower levels in patients. Results of the General Sleep Questionnaire demonstrated significant sleep disturbances in patients with major depression. Thereby sleep duration correlated inversely with kyn/trp and difficulty falling asleep was associated with higher neopterin concentrations.

We conclude that there are significant disturbances of tryptophan and neopterin metabolism in patients with major depression, patients presenting with higher neopterin and lower tryptophan. However, also kynurenine concentrations were decreased and thus kyn/trp was not altered. Thus, data do not support a role of IDO in tryptophan metabolic changes in major depression. Pathways involved in the tryptophan metabolic changes observed might be distinct from those involved in the pathogenesis of mood disturbances in patients suffering from chronic inflammatory disorders.

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Iron Metabolic Changes and Immune Activation in Patients with HIV 1 Infection before and after Antiretroviral Therapy

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Anaemia is a frequent complication in HIV-infected patients, several studies have shown that anaemia is a major prognostic marker for an increased mortality and morbidity of patients. Cytokine-mediated suppression of haematopoiesis and disturbed iron metabolism appear to play a key role in the development of HIV-related anaemia.

In this study we examined markers of immune activation and iron metabolism in patients with HIV infection undergoing antiretroviral therapy (ART).

105 patients with HIV-infection were recruited from the department of Dermatology and Venerology (Innsbruck) and were treated with ART for 12 months. 25 patients received B-vita-

mins before ART, 30 patients were B-vitamin supplemented during ART, while 40 did not take vitamins. Neopterin, iron, transferrin, ferritin and GDF-15 were measured by ELISA, tryptophan and kynurenine were determined by HPLC. Viral load was quantified by real time PCR.

Thirty-two HIV-infected patients suffered from anaemia before ART (25 with anaemia of chronic disease), 12 patients were anaemic after ART. Significant correlations existed between markers of immune activation (neopterin, tryptophan degradation) and parameters of iron metabolism (ferritin, GDF-15) before ART. ART was very effective to decrease viral load, slow down immune activation and modulate iron metabolism: Neopterin, kynurenine and ferritin concentrations decreased significantly, while tryptophan and GDF-15 concentrations raised. Concentrations of vitamin B12 increased under ART, while folic acid levels decreased. Differences were encountered between patients who received vitamin supplementation and those who did not: Only patients, who received vitamin supplementation during ART, showed a significant increase of haemoglobin levels.

Disturbed iron metabolism and enhanced tryptophan degradation coincide with immune activation in patients with HIV-infection. B vitamin supplementation during ART appears to be beneficial for patients with anaemia.

A Murine Model of Human BH₄-responsive Phenylketonuria

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Sapropterin dihydrochloride, the synthetic form of 6(R)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH₄) was recently approved for the treatment of phenylketonuria. It corrects phenylalanine hydroxylase (PAH) deficiency and hyperphenylalaninemia by a mechanism different from

its cofactor action in a substantial share of patients. *In vitro* studies using purified PAH pointed to a stabilization of the misfolded protein against accelerated proteolytic degradation. In BH₄ deficient and wild-type mice pharmacological doses of BH₄ increased the activity and concentration of wild-type PAH protein. However, an animal model of BH₄-responsive PAH deficiency was not available so far.

The Pahenu1 mouse model was generated by germline mutagenesis earlier. It harbors the V106A mutation in the regulatory domain of PAH. Although the specific activity of purified V106A PAH was unimpaired *in vitro*, Pahenu1 mice showed hyperphenylalaninemia (median, 189 µmol/L; wild-type 76 µmol/L) and decreased phenylalanine oxidation *in vivo* (33% of wild-type) in accordance with low PAH activity in liver lysates. The decrease of PAH amount in Pahenu1 liver lysates and eukaryotic expression systems was reversible upon protease inhibitors. This pointed to a misfolding-induced instability of V106A, which was confirmed by further *in-vitro* studies. BH₄ treatment *in vivo* significantly lowered blood phenylalanine concentrations (median: 396 to 139 µmol/L), normalized the phenylalanine oxidation to wild type levels and increased the amount of PAH in liver lysates by conformational stabilization.

In conclusion, PAHenu1 is a model for human BH₄ responsive PAH deficiency, that allows to study misfolding-induced loss-of-function pathology *in vivo*. It will facilitate the further elucidation of disease specific pharmacological effects of BH₄. Moreover Pahenu1 and the methods established in this project will enable *in vivo* evaluation of novel therapeutic molecules for the treatment of BH₄-responsive PKU.

Circadian Neopterin Profiles During a Single 24-hour Shift in Anaesthetists

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Regular night shift work has been shown to evoke various adverse effects on employee's health, mostly being associated with the disruption of circadian rhythm. Unlike other professions, medical personnel are scheduled to work on 24-hour shifts several times a month. Melatonin and neopterin concentrations essentially show a 24 h-periodicity, both indicating an acrophase in the early morning hours. It was the aim of the present study to investigate whether the physiological diurnal rhythm was altered as a result of a single 24-hour shift in anaesthetists. Therefore, urinary neopterin and melatonin levels that might possibly be altered due to desynchronization were measured.

Over three consecutive days, including a 24-hour shift, urine samples were collected daily at five different time points. Neopterin and aMT6-s (6-sulfateoxymelatonin) courses were assayed using high pressure liquid chromatography and a commercially available competitive immunoassay kit, respectively. Neopterin/creatinine and melatonin/creatinine ratios were calculated.

Ten anaesthetists aged between 29 and 35 and ten medical students aged between 25 and 31 were included in the study. Urinary neopterin values ranged between morning values of (mean and 95% CI) 95.0 (76.3; 113.8) and 91.3 (73.7; 108.8) nmol/mg, late evening values of 77.4 (62.6; 92.1) and 77.1 (63.4; 90.8) nmol/mg for anaesthetists and students, respectively. Neither the factor "time" nor of the factor "group" was found to have a significant effect on neopterin courses. aMT6-s fluctuated between values of 21.6 (17.0; 26.2) and 17.6 (15.1; 20.1) pg/mg in the morning and 7.3 (3.5; 11.1) and 4.7 (2.3; 7.1) pg/mg at late night for anaesthetists and students, respectively. A marked circadian rhythm of aMT6-s courses was observed in both groups, but no effect of the factor "group" could be found calculating analyses of variance.

Similar to previous investigations of our group nocturnal urinary neopterin/creatinine ratio did not show diurnal variations over study duration. We suspect that due to those differing results compared to other studies, our results could give

evidence for an impact of a single 24-h shift on circadian disruption. On the contrary, melatonin courses did not indicate circadian disturbance. Total duration of shift work experience did not seem to have an effect with showing similar neopterin and aMT6-s courses in anaesthetists and students.

Nifedipine Affects the Course of Salmonella Infection by Pharmacological Modification of Iron Homeostasis

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Iron overload aggravates the clinical course of infections by negatively affecting cell mediated immune mechanisms of macrophages and T helper cell type 1 effector pathways. Recently, we have shown that the calcium antagonist nifedipine enhances DMT1 mediated iron transport *in vitro* and causes subsequent depletion from liver and the circulation in iron overloaded mice. Herein, we thus investigated whether nifedipine may impact on the clinical course of an invasive infection via modulation of iron homeostasis.

RAW 264.7 cells were infected with *Salmonella typhimurium* and stimulated with varying concentrations of nifedipine. Mice were fed with a control diet or iron enriched diet for three weeks, infected intraperitoneally with *Salmonella typhimurium* and treated with solvent control or nifedipine for three consecutive days. 24 hours later mice were sacrificed, the bacterial loads were quantified in the liver and the spleen, and the expression of iron regulating genes was determined by means of RT-PCR and Western blot analysis.

The cell culture model revealed a significant decrease of the bacterial load upon nifedipine treatment. In RT-PCR analysis an increase of the iron exporter ferroportin1 expression was observed following treatment with nifedipine leading to iron restriction within macrophages.

Nifedipine treated mice, independently of dietary iron overload, showed an improved survival and presented with reduced numbers of Salmonella in the liver and the spleen as compared to solvent injected animals. Even though these effects were more pronounced in the iron diet fed group. Serum iron levels were decreased as a reflection of the iron depleting effects of the drug. This was paralleled by decreased ferritin levels in the liver and spleen and increased ferroportin 1 expression as determined by means of western blot analysis. Interestingly, we did not observe differences in the expression of a panel of pro- and anti-inflammatory cytokines according to nifedipine treatment.

Our data provide evidence that nifedipine may be a promising adjunctive therapy for the treatment of infections with intracellular pathogens. Mechanistically the iron depleting effects may be due to limitation of the availability of the essential nutrient iron for intracellular pathogen as a consequence of nifedipine mediated induction of cellular iron export via ferroportin.

Dynamic Relations between Physical Activity and Cellular Immunity in Three Patients with SLE: a Time Series Analysis Approach

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with various clinical manifestations. Until today, few studies have investigated the influence of physical activity on the immune system in patients with SLE. It is suggested that these studies produced inconsistent findings because of not considering the temporal qualities of the interaction between physical activity and immune activity under real-life conditions.

Three females (46, 52, and 34 years-old) with SLE in remission participated in this study. Patients collected their entire urine and assessed their daily physical activity via a visual analogue

scale (VAS) ranging from 0 to 10 cm over a period of 56 consecutive days in 12-hour intervals (total of 112 consecutive measurements). After the investigation period, urinary neopterin and creatinine were measured applying HPLC. Before cross-correlational analyses, time series were controlled for serial dependencies applying ARIMA-modeling. In all three patients physical activity positively correlated twice with urinary neopterin concentrations, meaning that a certain time after exercise two elevations of urinary neopterin followed. In Case 1, increases of physical activity were followed by urinary neopterin increases after 12 and 144 hours, in Case 2 after 72 and 144 hours, and in Case 3 after 36 and 132 hours. As elevations of neopterin levels after physical engagement have hitherto only been seen in athletes after highly intense training (1), this study suggests that patients with SLE may react more sensitively to physical activity than healthy individuals. Although a multiphased immune response after physical engagement has already been described in literature (2), a rise in neopterin-levels after 144 hours in response to physical activity is a novum. Further integrative single-case studies combining subjective and objective measurement of physical activity have to follow in order to strengthen the findings of this study.

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Are Pterins Able to Modulate Oxidative Stress?

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Pterins (2-amino-4(3H)-oxo-pteridine derivatives) are common animal colorants which constitute heterocyclic compounds and have the highest nitrogen content of any pigment analyzed from animals. It has been reported that pterins modulate oxidative stress as these molecules are able to scavenge free radicals. Previous reports suggest three possible mechanisms that are responsible for scavenging free radicals; these are electron transfer (ET) reaction, hydrogen atom transfer (HAT) and radical addition. In this paper, the capacity to scavenge free radicals (antiradical capacity) of pterins is analyzed, using Density Functional Approximation calculations and considering two possible mechanisms: ET and HAT. For the electron transfer process, the Full Electron Donator Acceptor Map (FEDAM) of pterins indicates that the antiradical capacity of those pterins is lower than the antiradical capacity of any carotenoids. In terms of the HAT mechanism, there is a correlation between the dissociation energy involved in the removal of one hydrogen atom from pterins and its antiradical capacity. Pterins are depicted as poorer antiradicals than carotenoids in terms of the HAT mechanism, with biopterin, sepiapterin and 7,8-dihydrobiopterin representing the only exceptions. Further studies focusing on the third mechanism (radical addition) are necessary in order to fully understand the antiradical capacity of these substances. For this reason, work continues in order to clarify these aspects.

Pharmacological Chaperones for the Aromatic Amino Acid Hydroxylases

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The aromatic amino acid hydroxylase (AAAH) enzyme family includes phenylalanine hydroxylase (PAH), tyrosine hydroxylase (TH), and the tryptophan hydroxylases (TPH-1 and 2). These enzymes are structurally and functionally related and in mammals they are mainly distributed in liver and kidney (PAH) and neuronal and neuroendocrine tissues (TH and TPH-1 and 2). The AAAHs share a number of ligands, such as the cofactor tetrahydrobiopterin (BH₄) and inhibitors. In fact the AAAHs do not show strict substrate selectivity, and can utilize all three aromatic amino acids as substrates to some extent. It is thus important to investigate binding selectivity for compounds used in therapeutic interventions aiming the modulation of each AAAH, such as BH₄, stabilizers (pharmacological chaperones) and inhibitors. Through high-throughput screening we recently discovered four compounds (I-IV) that functioned as pharmacological chaperones for PAH (Pey et al. (2008) J Clin Invest 118, 2858-2867). These compounds stabilized the functional tetrameric conformation of recombinant wild-type PAH and PKU mutants, and increased steady-state PAH protein levels in eukaryote cells and liver of treated mice. We now investigated the effect of these compounds on TH and TPH2 *in vitro*, comparative to PAH, by differential scanning fluorimetry. For the most effective compounds (II-IV) we also measured their *in vivo* effect. Only compound III (3-amino-2-benzyl-7-nitro-4-(2-quinoly)-1,2-dihydroisoquinolin-1-one) bound and stabilized all three purified enzymes (PAH, TH (isoform 1) and TPH2), and increased TH activity in treated mice. For the other compounds, different effects were measured for their action *in vitro* and *in vivo* for each AAAH. Our results will be discussed in the context of the potential of these compounds as pharmacological chaperones for the treatment of disorders associated with AAAH misfolding.

Prognostic Significance of Urinary Neopterin in Colorectal Cancer Patients Treated with Cetuximab

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An increase of urinary neopterin has been described after administration of different anti-cancer agents. Cetuximab is a chimeric antibody used in the therapy of advanced colorectal carcinoma that may act partly through activation of host immune response. We have studied urinary neopterin in 44 pre-treated patients with metastatic colorectal carcinoma during therapy with cetuximab, in the majority of cases in combination with irinotecan, 5-fluorouracil and leucovorin. Urinary neopterin was determined by high performance liquid chromatography. A significant correlation was observed between urinary neopterin and peripheral blood leukocyte count ($r_s = 0.38$; $p < 0.05$), hemoglobin ($r_s = -0.34$; $p < 0.05$) and CEA ($r_s = 0.33$; $p < 0.05$) concentration. Seventeen patients had urinary neopterin equal or above 214 $\mu\text{mol/mol}$ creatinine. Survival of patients with urinary neopterin concentration of 214 $\mu\text{mol/mol}$ creatinine or above was significantly inferior compared to patients with initial urinary neopterin below 214 $\mu\text{mol/mol}$ creatinine (median 10.1 vs. 17.5 months, $p < 0.05$). Urinary neopterin concentrations increased during the therapy only in patients with normal pre-treatment neopterin concentrations. In conclusion, an increase of urinary neopterin in patients treated indicates the activation of systemic immune response by the therapy that could be responsible for anti-tumor activity of cetuximab. Urinary

neopterin is a prognostic factor in patients with metastatic colorectal carcinoma treated with cetuximab.

Serum Homocysteine, C-reactive Protein, Retinol and Alpha-tocopherol and Urinary Neopterin in Patients with Breast Carcinoma Treated with Bevacizumab

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Increased serum or urinary concentrations of neopterin, an indicator of systemic immune response, have been described in patients with tumors of different primary locations, and further increase has been observed during anticancer therapy. An increase of urinary neopterin has been described after administration of cytokines, cytotoxic chemotherapy or external beam radiation, but much less is currently known about the effects of targeted agents on systemic immune response. We have studied serum homocysteine, C-reactive protein, alpha-tocopherol and retinol, and urinary neopterin in patients with metastatic breast cancer treated with bevacizumab, taxane and carboplatin. Homocysteine and C-reactive protein were determined immunochemically. Alpha-tocopherol, retinol and urinary neopterin were determined by high performance liquid chromatography. Homocysteine, CRP and urinary neopterin decreased, while retinol and alpha-tocopherol increased during the therapy. In conclusion, a decrease of urinary neopterin and C-reactive protein during bevacizumab therapy was associated with decreased serum homocysteine and

increased retinol and alpha-tocopherol concentrations.

Native and Adaptive Immune Response: Serum Neopterin Concentrations in Healthy Blood Donors are not Influenced by MBL Polymorphism

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Mannose-binding lectin (MBL) is an important component of the immune defence able to bind to repeating mannose based structural patterns typical of microbial surface. Importantly, it also activates the complement system through a distinctive third pathway, independent of both antibody and the C1 complex. The serum MBL level is variable in healthy population. The mean serum MBL level in an individual is genetically determined by three single nucleotide substitutions in exon-1 of the human MBL2 gene also referred as D, B, and C variants whereas the wild type allele is referred as A. These mutations result in amino acid substitution in the collagen-like domain, thereby reducing the amount of functional serum MBL level. In addition, three pairs of allelic dimorphisms can occur in the downstream promoter. The combination of structural gene and promoter polymorphisms results in a dramatic variation in MBL concentration in apparently healthy individuals of up to 1000 fold (Caucasian <20-10,000ng/ml). The variant alleles are very frequent in normal, healthy populations, where they are present in 20 to 50% of the individuals. Epidemiological studies have suggested that genetically determined variations in MBL serum concentrations influence the susceptibility to and the course of different types of infectious, autoimmune, neoplastic, metabolic and cardiovascular diseases.

In this study we investigated the influence of MBL polymorphisms on serum neopterin concentrations.

Blood samples were obtained from 67 unrelated healthy platelet apheresis donors. Genomic DNA was isolated from mononuclear cells using Nucleon BACC2 kit from Amersham Biosciences and screened for the relevant MBL variants with PCR-SSPs based on the same techniques, as applied for routine typing of HLA. Additional MBL serum levels were measured using MBL Ologomer Elisa Kit from BioPorto. For comparison serum neopterin concentrations from the last 10 donations of every donor were used.

According to the literature donors were grouped into low, intermediate and high MBL levels.

Based on our results 22.4%, 11.9% and 65.7% of the donors belonged to the low, intermediate and high group, respectively, confirming the finding of other European studies. There was no difference in serum neopterin concentrations between the 3 groups, median ranges from 3.6 to 6.8 nmol/L in the low, 3.6 to 6.9 nmol/L in the intermediate, and 3.8 to 6.2 nmol/L in the high group. There were 2 donations from 2 different donors with neopterin levels >10 nmol/L both belonging to the high group.

Inflammatory Markers in Post-partum Period

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The most commonly used marker to detect infectious and inflammatory responses in the clinics today is C-reactive protein (CRP), e.g., clinicians in gynecology or surgery use CRP, sometimes procalcitonin (PCT) is applied. Other markers as macrophage product neopterin and some interleukins seem to be only introduced as scientific parameters. Also gravidity can be associated with inflammatory complications which very often take place in the puerperal period. The aim of this study

was to compare some inflammatory markers in

the early puerperal period in normal gravidity without infection, with normal gestation age and healthy newborns.

Blood specimens were obtained from 46 mothers (23 with spontaneous delivery/23 Cesarean section) together with cord blood aged median 31/30, range 22-37/24-46 years. Delivery was at week 40 ± 1 , the Apgar scores of newborns ranged within 8-10, all were without puerperal infection. In the plasma (EDTA) samples we analysed concentrations of neopterin (ELISA, BRAHMS, Hennigsdorf, Germany), reference range: 5.3 ± 2.7 nmol/L, PCT (TRACE - Kryptor, BRAHMS Germany) reference range: <0.05 ug/L and ultrasensitive CRP (CRPus; TRACE - Kryptor, BRAHMS Germany) reference range: 0.07 - 8.2 mg/L. In total 5 specimens of each mother/child pair were examined, 4 specimens obtained from the mother (days 1, 3, 4, 5) and cord blood. Blood cell counts were measured and residual plasma was divided into 2-3 aliquots and frozen at -35 °C. In the early puerperium mean neopterin concentrations were 9.3 (S.D.: ± 2.7 , median: 8.2; d1), 10.1 (± 2.9 , 10.0; d3), 8.5 (± 2.4 , 7.7; d4) and 8.4 (± 2.3 , 9.0; d5) nmol/L in samples obtained after normal delivery and slightly but not significantly higher in samples obtained after Cesarean section was performed 9.35 (± 2.4 , 10.0; d1), 10.2 (± 3.4 , 9.5; d3), 11.0 (± 4.0 , 9.8; d4) and 9.8 (± 3.2 , 8.4; d5). Neopterin concentrations were about twice as high in cord blood as compared with concentrations obtained in plasma specimens from mothers, again there was no significant difference between samples obtained after normal delivery (17.4 ± 4.9 , 17.9; cord blood) as compared with Cesarean section (20.4 ± 5.7 , 20.7; cord blood). PCT concentrations showed a similar pattern as neopterin in mothers, but also cord blood specimens contained a comparable PCT concentration as compared with blood specimens of mothers. CRP levels were significantly higher in days 3-5 as compared with day 1, CRP was almost undetectable in cord blood specimens. CRP levels after normal vaginal delivery how similar dynamic compare to Cesarean section, with higher levels after Cesarean section most probably due to damage of tissue. In both groups is a wide variation scale, which complicates the use of this marker alone in the diagnostic of early

puerperal infection.

In sum, there are significant changes of neopterin, CRP and PCT in females after delivery. However, to dissect their possible clinical relevance further studies on the comparison between healthy normals and newborns with complications are still required.

N-Terminal Sequencing of Pterin Deaminase from *Micrococcus luteus* BI252 using MALDI-TOF

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The most unexplored and possibly a potent anticancer drug is the enzyme pterin deaminase belonging to class of hydrolase, which deaminates pterin to products lumazine and ammonia. The enzyme pterin deaminase has been patented for the antitumour property against leukaemia. In spite of the patent, very few works has been carried out in exploring the structural properties of the enzyme.

In the present study, an attempt has been made to purify the enzyme pterin deaminase to homogeneity from *Micrococcus luteus* BI252 and sequence the N-terminal using MALDI-TOF. The enzyme was purified to homogeneity in a sequential manner using alcohol precipitation, Gel filtration and Ion exchange column chromatography. The purified enzyme was run on Non-denaturing gel electrophoresis and the obtained band was digested using trypsin. The tryptic digest was subjected to MALDI-TOF analysis and the spectrum was predicted using MASCOT software. The N-terminal sequence was then explored in the protein database for the identification of protein using BLAST software and found to be novel with less than 50% homogeneity.

Thus the present study would throw light for the structural characterization of the enzyme

pterin deaminase which could be explored or manipulated for the production of a potent anti-cancer drug.

Antioxidant Property Exhibited by Novel Enzyme Pterin Deaminase

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With the advent of the enzymes as drugs, cancer therapy has highlighted one to focus and venture in search of an efficient enzyme as anti-tumour drug. The most unexplored and possibly a potent drug is the enzyme pterin deaminase, which deaminates folic acid to products 2-deamino-2-hydroxy folic acid and ammonia.

In spite of the patent and exploration of the preliminary antitumour property of enzyme pterin deaminase in prokaryotes and eukaryotes, very few works has been carried out in elucidating the various roles or properties of the enzyme. The reasons behind the lack of work are unclear currently and this tempted us to speculate and investigate the properties of pterin deaminase from rat liver.

In the present study, we have isolated and characterized the enzyme pterin deaminase from rat liver. The enzyme was purified to homogeneity in a sequence by precipitation and ion-exchange chromatography. The purified enzyme was checked for its antioxidant properties. The enzyme pterin deaminase exhibited antioxidant property by scavenging the hydrogen peroxide free radicals compared to the free radical induced by DPPH.

Thus the present work has thrown a lime light on the antioxidant property of the enzyme pterin deaminase apart from the anti-tumour property.

Phenylalanine to Tyrosine Ratio (phe/tyr ratio) in Children with Neuropediatric Abnormalities

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Phenylalanine (4)-hydroxylase (PAH) converts phenylalanine (phe) to tyrosine (tyr). An altered phe/tyr ratio is indicative for a disturbed hydroxylation reaction which can be detected by parallel measurements of phe and tyr (1). 5,6,7,8-Tetrahydrobiopterin (BH₄), the cofactor of PAH, is easily oxidized and in situations with oxidative stress this will lead to a decreased hydroxylation of phe. Consequently, phe/tyr ratio is elevated.

In this retrospective study, we calculated phe/tyr in cerebrospinal fluid (CSF) and plasma of children with neurologic diseases, who underwent simultaneous lumbar and venous puncture. Phe and tyr concentrations were determined using ion exchange chromatography with ninhydrine detection (2). From 70 punctures (68 patients with neurologic diseases of unknown origin, see reference (2) for inclusion and exclusion criteria for this group) 38 were excluded because of therapy with antiepileptic drugs, 3 because of elevated neopterin concentrations and 3 because of meanwhile known diagnosis.

The 'reference' values for phe/tyr were calculated in 26 patients (15 girls, 11 boys; mean age 5.1 years, median 4.2 years, range 0.5-17.2 years). In CSF phe/tyr was 0.88 ± 0.29 (mean \pm SD), in plasma phe/tyr was 0.95 ± 0.17 . Two patients of the 'reference' group had elevated CSF phe/tyr of 1.60 and 1.62. The corresponding plasma phe/tyr was only slightly elevated 1.28 and 1.22.

In additional 155 patients with neuropediatric disorders and inherited disorders of metabolism we calculated phe/tyr in CSF and plasma. With a cut-off value of 1.15 for both compartments, 17 patients had elevated ratios in plasma and 20 patients had elevated ratios in CSF. The highest

ratios were measured in a patient with dihydropteridine reductase (DHPR)-deficiency (BH₄-deficiency, CSF 2.82 and plasma 2.28). Additionally, the ratios were elevated in some patients with immunologic or infectious disorders like cerebellitis.

We conclude that the parallel monitoring of phe and tyr and calculating phe/tyr in plasma and CSF may not only support diagnosis of phenylketonurias but also of mild disturbances of phe and tyr metabolism in children and juveniles with inflammatory disorders and/or neuropsychiatric abnormalities.

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Serum Neopterin Production and Tryptophan Degradation in Patients with Atopic Dermatitis

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Atopic dermatitis is an eczematous, highly pruritic chronic and recurrent inflammatory skin disease starting at young age. Although much research has already been done, its causes and immunological mechanisms are only partly understood. It is generally accepted that besides genetic predisposition, a epidermal barrier dysfunction and heightened susceptibility to viral, fungal and bacterial infection, increasing inflammation, the increased prevalence of atopic disease is mainly due to a disturbed balance of type-1 and type-2 T-helper cells (Th1 and Th2-type) immunity, and as a consequence, pathways triggered by Th1-type cytokines might be altered. During Th1-type immune response large amounts of neopterin

are released by human monocytes/macrophages especially upon stimulation by the Th1-type cytokine interferon- γ (IFN- γ). Therefore, determination of neopterin concentrations represents an indirect measurement of IFN- γ levels and allows to monitor Th1-type immune response (1). In parallel, IFN- γ induces indoleamine 2,3-dioxygenase (IDO) which degrades tryptophan to kynurenine. IDO enzyme activity can be estimated by calculating the ratio of serum/plasma concentrations of kynurenine to tryptophan (kyn/trp).

In this study, we evaluated whether neopterin concentrations or tryptophan degradation in atopic dermatitis patients could be markers to reflect disease activity and severity in actually determined serum neopterin, tryptophan and kynurenine levels concentrations in 15 patients (9 women, 6 men) with severe atopic dermatitis. Results were compared with concentrations in 38 serum specimens collected from healthy blood donors. Serum neopterin and tryptophan concentrations did not differ between the two groups, but kynurenine levels and kyn/trp -although still rather in the low range- were significantly higher in atopic dermatitis patients ($2.13 \pm 0.16 \mu\text{M}$; $36.3 \pm 3.38 \mu\text{mol/mmol}$) than in blood donors ($1.4 \pm 0.06 \mu\text{M}$; $24.5 \pm 1.02 \mu\text{mol/mmol}$) (both $p < 0.001$), respectively. In patients we found a positive correlation between neopterin levels and tryptophan degradation ($r_s = 0.58$, $p < 0.05$). All neopterin concentrations measured in patients and healthy controls were $< 8.7 \text{ nM}$, the upper limit of the normal (= 95th percentile).

Although in our study serum neopterin levels did not differ between healthy controls and atopic dermatitis patients, whereas tryptophan degradation reflected by kynurenine concentrations and kyn/trp ratio was slightly increased, we may conclude that IDO is involved in the tryptophan metabolic changes. The significant correlation found between neopterin concentrations and kyn/trp underlines this conclusion. However, such it is in contrast to what was found in pollinosis patients earlier, when a subgroup of patients was identified with highly increased serum tryptophan concentrations which predicted unresponsiveness to hyposensitisation therapy (2). In these patients no association was observed between neopterin and kyn/trp. However, the number of

patients studied is still small and any conclusion has to be substantiated in further studies with higher patient numbers.

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Immunomodulatory Effects of Vitamin K-antagonist Acenocoumarol (Sintrom)

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Vitamin K-antagonists belong to the group of anticoagulants and are commonly used for the treatment and prevention of deep venous thrombosis, pulmonary embolism, myocardial infarction and stroke. Their antithrombotic effects and the mechanisms involved in blood coagulation are well documented, but little is known about their interaction with the immune system, though inflammation and immune activation are crucially involved in the pathogenesis of atherosclerosis and cardiovascular disease. Within cell-mediated (= Th1-type) immune response pro-inflammatory cytokines especially interferon- γ activate GTP-cyclohydrolase I to form neopterin in human macrophages and dendritic cells. In parallel, indoleamine 2,3-dioxygenase (IDO) is induced and degrades tryptophan (trp) to kynurenine (kyn), IDO enzyme activity can be estimated by calculating the ratio of kyn to trp concentrations (kyn/trp).

This *in vitro* study investigated the effects of vitamin K-antagonist acenocoumarol (Sintrom) on freshly isolated peripheral blood mononuclear cells (PBMC) from healthy blood donors. PBMC were incubated with accelerating doses of aceno-

coumarol and were either left unstimulated or stimulated with T-cell mitogen phytohaemagglutinin (PHA) after 30 minutes. Concentrations of neopterin, trp and kyn were measured in supernatants after 48 hours of stimulation.

IDO-activity and neopterin formation were significantly higher in PHA-stimulated PBMC than in unstimulated cells. Acenocoumarol dose-dependently inhibited trp degradation and neopterin production in parallel, and at concentrations as low as 10 µg/ml a significant effect was demonstrated in PHA-stimulated cells, whereas in unstimulated cells no such effect was observed.

Though plasma levels of acenocoumarol in treated patients are below the concentrations of our *in vitro* experiments, our data suggests that the immunomodulatory capacity of acenocoumarol could be part of its positive effects in antithrombotic treatment and prophylaxis.

Neutrophil Gelatinase-associated Lipocalin (NGAL) Promotes Granulocytes Emigration

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Neutrophil gelatinase-associated lipocalin (NGAL, 24p3 or lipocalin-2, Lcn2) is a 21 kDa protein of the lipocalin superfamily. NGAL has been shown to affect cellular iron homeostasis transport, cellular apoptosis, and the course of infections with gram negative bacteria via binding of bacterial siderophores. Since lipocalin is produced and released in large quantities by granulocytes, we questioned whether this peptide may affect the immunological function of these cells.

Human or murine neutrophil granulocytes were purified and attracted *in vitro* with varying concentrations of human interleukin-8 (hIL-8), mL-8 (KC), mRLcn2, hLcn2 and/or an anti-mLcn2-Ab. Neutrophil migration assays were performed using a modified 48-well Boyden microchemotaxis chamber.

To investigate the adhesion of neutrophils we coated 12-well plates with mouse serum and allowed cells to adhere for 40min. To study neutrophil function upon microbial challenge a single intradermale injection of *Salmonella typhimurium* (300 CFU) was given to wild-type and Lcn2 ^{-/-} C57BL6 mice. After 24h, the skin at each injection site was excised and fixed in formalin for histological analysis.

We found that the migration of human and murine granulocytes was significantly increased upon the addition of Lcn2, which was not linked to increased formation of IL-8/KC. Mechanistically, this could be traced back to Lcn2 mediated changes of ERK1/2 signalling in these cells. Accordingly, we found significantly reduced neutrophil migration into intradermale bacterial lesions of Lcn2 ^{-/-} as compared to their wild type littermates. In addition to a reduced chemotactic activity, neutrophils from Lcn2^{-/-} mice also presented with an impaired adherence to surfaces.

We herein describe a novel role of Lcn2 as an important paracrine chemo-attractant and an indispensable factor for neutrophil function in bacterial infections.

The Relationship between Alcohol Intake and Cellular Immune Activity in Systemic Lupus Erythematosus May Change from Inhibitory to Stimulatory within Two Months of Study

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In recent years, interest has been growing regarding the role of alcohol intake on systemic lupus erythematosus (SLE) disease activity. This integrative single-case study (1) investigated the dynamic interdependencies between alcohol consumption, various emotional states and cellular immune activity in SLE using time series analysis (i.e., ARIMA modeling, cross-correlational analysis). We determined neopterin concentrations (HPLC) in a 52-year-old woman suffering from SLE, who collected her entire urine on a twice-

daily basis for a period of 56 days (total: 112 time-points). Aside from the central interest of our studies, i.e., the impact of everyday incidents on cellular immune system dynamics, the patient also provided daily information on intake of alcoholic beverages (type and quantity) and various emotional state variables (mental activity, irritation, mood). Cross-correlational analyses revealed that moderate alcohol consumption had different effects on urinary neopterin levels depending on whether time series data were analyzed before or after the occurrence of an inflammatory event (acute paranasal sinusitis) that had taken place halfway through the study period (time-points 45 - 54). Before sinusitis (1 - 44), increases in alcohol consumption were parallelized by decreases in urinary neopterin levels (lag0: $r = -0.371$; $p < 0.05$) whereas after sinusitis (55 - 112), increases in alcohol intake preceded increases in urinary neopterin levels with a temporal delay of 12 hours (lag1: $r = +0.308$; $p < 0.05$). The emotional states under study did not interfere with the associations between alcohol intake and neopterin levels. As to the biochemical mechanisms of the alcohol-immune effects seen in this study, ethanol as well as antioxidative compounds such as resveratrol or humulones contained in wine or beer may have been responsible for suppression of inflammation (2, 3). However, the change in the direction of effect between alcohol consumption and neopterin levels before and after sinusitis cannot be explained solely on a biochemical basis. Rather, the clear influence of psychological factors on stress system activity might suggest that the patient's health perception during sinusitis modified the meaning alcohol had for her and, consequently, how drinking alcohol affected her immune system. Though the underlying mechanisms of the current findings remain far from clear, this study shows that even within the same patient with SLE the relationship between alcohol intake and neopterin levels might not always be stable suggesting a possible reason for inconsistent evidence from conventional group studies.

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***Staphylococcus aureus* Lipoproteins Affect Immune Response and Body Iron Homeostasis**

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Toll-like receptor 2 (TLR2) is a receptor for lipoproteins (lpp) and signals by the adaptor MyD88. Interactions of TLRs with lpps are known to be crucial for eliciting an adequate host response against bacterial infections. As the impact of lpp on TLR2-MyD88 activation for *S. aureus* in systemic infection is unknown, we studied the effects of lpp maturation in two *S. aureus* strains (SA113 and Newman) deficient in the enzyme prolipoprotein diacylglycerol transferase (Δ lgt).

C57BL/6 mice were injected either 1×10^6 to 1×10^8 cfu of *S. aureus* 113/Newman wt or *S. aureus* lgt (dissolved in 200 μ L of 0.9% NaCl) or 0.9% NaCl into the lateral tail vein. The animals were sacrificed after 24 hours or 7 days after initiation of the infection. We extracted RNA and protein from spleen and liver and studied the expression of critical genes in iron homeostasis and innate immunity by means of QRT-PCR and Western blots. Iron content of organs was quantified by a colorimetric assay.

The injection of *Staphylococcus aureus* resulted in a severe sepsis with stimulation of pro-

inflammatory and anti-inflammatory cytokine formation. Δ lgt staphylococcal infection resulted in impaired immune response, reflected by a decreased expression of the pro-inflammatory cytokine TNF- α as compared to wt staphylococcal infection, along with a modulation in the expression of iron homeostasis genes in the liver and most pronounced in the spleen which was most evident when investigating the expression of divalent metal transporter-1 and the iron export protein ferroportin. We further observed significant differences of iron content in organs between SA113/Newman wt infected mice and animals exposed to SA113/Newman Δ lgt. In addition, we found an impaired bacterial growth of Δ lgt staphylococci under iron restricted conditions as compared to wt staphylococci. Finally, we could demonstrate that excessive iron supply nullified the growth deficits of Δ lgt *S. aureus*.

In conclusion, these results indicate that lpp induction of inflammatory cytokines has an impact on iron transport mechanisms. Changes in iron metabolism are known to influence host immune effector functions as well as bacterial growth rates and survival and may therefore contribute to the disease severity in *S. aureus* sepsis.

TDS-GCMS Analysis of Volatile Metabolites of Human Lung Cancer and Non-carcinogenic Cells *in vitro*

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Analysis of volatile organic compounds (VOCs) in the exhaled breath for the detection of human diseases is a non-invasive diagnostic method. Breath can be sampled as often as it is desirable, even during operations or at an inten-

sive care unit. Since the field of breath analysis is relatively new, and the advances in analytical technology occur so fast, many compounds in exhaled breath have been detected whose biochemical origin has not yet been studied. Even less is known about the relationship of volatile compounds to cellular processes like malignant transformation.

A particular focus of breath research is on patients suffering from lung cancer. Investigations of exhaled breath from cancer patients showed that concentrations of specific compounds may be increased or decreased in comparison with healthy age-matched controls. So far, little is known about the underlying metabolic mechanisms and different sources leading to the production of volatile organic compounds (VOCs). Since tumours are complex and heterogeneous systems it is possible that tumorous and also non-tumorous components like immune cells and even microorganisms contribute to VOCs. The objective of this work was to investigate VOCs released or consumed by various lung cells of carcinogenic origin as well as healthy control cells from lung and dermis *in vitro*.

We investigated four lung cancer cell lines (A549, NCI-H2087, NCI-H1666 and CALU-1), primary lung epithelial cells (HBEpC) and immortalized fibroblasts originating from human dermis (hFB, human fibroblast) for the release or consumption of VOCs by GC-MS after thermal desorption. Cells were trypsinized and up to 100×10^6 cells were incubated in a sealed fermenter for 18 hours. Prior to gas chromatography mass spectrometry (GC-MS) analyses samples from the headspace of the culture vessel were collected with simultaneous preconcentration by adsorption on solid sorbents and then VOCs were thermodesorbed for analysis by GC-MS. For comparison we analyzed the VOCs present in the headspace of the medium control.

Using this approach, we identified VOCs that were similar in a particular transformed and in non-transformed cells independently if the latter had been derived from lung or skin. For instance, concentrations of 2-pentanone and 2,4-dimethyl-1-heptene were found to be increased in HBEpCs and hFB cells and the A549 lung cancer cell line. A similar observation was made for 2,3,3-

trimethylpentane which appeared in increased concentrations in control cells and CALU-1 lung cancer cells. In addition, the ethers methyl *tert*-butyl ether and ethyl *tert*-butyl ether could be detected at elevated levels in A549 cells and one of the untransformed cell lines. However, especially branched hydrocarbons and alcohols were seen increased more frequently in untransformed than transformed cells. Interestingly, a big variety of predominantly aldehydes and the ester *n*-butyl acetate were present at decreased concentrations in the headspace of all cell lines tested compared with medium controls. Again greater variety was shown by hFB and HBEpC cells compared to carcinogenic cell lines. For example 2-butenal was metabolized only by these two types of control cells, but none of the cancer cell lines.

In summary, our findings confirm that several VOCs like unsaturated hydrocarbons and oxygen-containing compounds are released, while different aldehydes and the ester *n*-butyl acetate are consumed or metabolized by the cell lines and primary cells studied here. The healthy primary cells display more compounds with increased or decreased concentration (compared to medium) than the tumorigenic cell lines.

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Urinary Neopterin in Metastatic Renal Cell Carcinoma Patients Treated with Bevacizumab-Based Therapy

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The introduction of targeted agents has profoundly affected the treatment of metastatic renal cell carcinoma (RCC). Increased urinary concentrations of neopterin are associated with poor prognosis in tumors of different primary locations. However, the data on prognostic significance of neopterin in patients with RCC is more limited. Neopterin was determined by high-performance liquid chromatography. The survival was analyzed using the Kaplan-Meier method, with survival differences being analyzed by the log-rank test. Fifty-five patients with metastatic RCC were studied. No differences were observed between previously treated and systemic treatment-naïve patients. Patients ineligible for inclusion into clinical trial tended to have higher urinary neopterin. The survival of patients with increased urinary neopterin concentrations using different cutoff points was significantly inferior ($p < 0.05$) in the whole group as well as in patients with no prior systemic therapy. In conclusion, urinary neopterin is a prognostic factor in metastatic RCC.

Clinical Status in Long Term Successfully Transplanted Patients

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Allogeneic kidney transplantation is a well established treatment for endstage renal disease; early postoperative organ function and survival rates are estimated over 90%, less is known about the clinical status of patients with graft function of more than 10 years.

Our clinical retrospective study in 29 kidney transplant recipients (22 males, 7 females, mean age 56 ± 10 years), mean graft survival of 18 ± 6 ,

range 10 -30 years) investigated the actual physical status, renal function, blood pressure (bp), incidence of malignancies and intake of immunosuppressive medication.

By clinical examination, 27/29 patients were in a good physical condition (mean BMI 24.9), all non-smokers, 8 were active in sports, one patient was a well treated type I diabetic, two suffered from a metabolic syndrome, one patient presented donor specific alloantibodies (35% panel-reactive antibodies). Mean creatinine values were 1.27 ± 0.4 mg/dl; glomerular filtration rate (GFR) was 63 ± 20 ml/min/1.73 m²/MDRD (MDRD = Modification of Diet in Renal Disease). Patients treated with calcineurin inhibitors (CNI+), showed no different graft function compared to those with calcineurin free (CNI-) medication. Mild proteinuria was found in 11/29 (38%) patients.

Before transplantation, blood pressure was hypertensive in 26/29 patients, after engraftment, two patients presented mild hypertension, two were normotensive without and 25 were normotensive under medication. Mean blood pressure values were 125:78 mm Hg and not statistically different in CNI+ or CNI- treated patients.

Immunosuppressive therapy consisted in CNI-monotherapy in 17, CNI-combination in 4 and CNI-free medication in 7 patients respectively. One patient refused any immunosuppressive medication since 12 years. Malignancies were observed in 3 patients (10.3%) (2 skin cancers, 1 post-transplant lymphoproliferative disorder = PTLN); all patients were treated successfully without impairment of the graft function.

In summary, longtime graft survival was observed in a cohort of 29 patients with excellent compliance, careful antihypertensive and immunosuppressive treatment. Further investigations on operative tolerance are in progress.

Distribution of Alkylglycerol Monooxygenase and Fatty Aldehyde Dehydrogenase Activities in Tissues and Subcellular Compartments

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The degradation of alkylglycerols in mammalia occurs by the consecutive action of two membrane bound enzymes. The first step is catalysed by alkylglycerol monooxygenase (glycerylether monooxygenase, EC 1.14.16.5), a tetrahydrobiopterin-dependent enzyme, and consists in the cleavage of the ether bond. The resulting aldehyde is then oxidized and thereby detoxified by the NAD-dependent enzyme fatty aldehyde dehydrogenase (EC 1.2.1.48). By alternative splicing, fatty aldehyde dehydrogenase can have two distinct C-termini, one localising the enzyme to the endoplasmic reticulum and the other targeting it to peroxisomes. As these two enzymes are acting in such a metabolic proximity, we wanted to investigate the distribution of alkylglycerol monooxygenase in comparison to fatty aldehyde dehydrogenase in mouse tissues and subcellular compartments. For this aim we have measured the activities of both enzymes using our established HPLC methods based on fluorescent substrates in a variety of mouse tissues and in fractions of subcellular density gradients.

Activity could be detected in almost all tissues with peak levels in liver, fat tissues and in the gastrointestinal tract. Interestingly, even though having a similar distribution pattern, fatty aldehyde dehydrogenase activity always exceeded alkylglycerol monooxygenase activity by at least ten fold ensuring clearing of the toxic intermediate product. Also the subcellular distribution was similar for the two enzymatic activities with peak levels found in the endoplasmic reticulum but revealing a potential peroxisomal contribution also for alkylglycerol monooxygenase. In further experiments we also tested for the influence of streptozotocin-induced diabetes in mice, fenofibrate feeding and absence of the substrate in

etherlipid deficient mice on the activities of alkyl-glycerol monooxygenase and fatty aldehyde dehydrogenase.

In conclusion, both enzymes responsible for degradation of saturated etherlipids are similarly regulated and expressed in a wide range of mouse tissues and subcellular compartments.

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Serum Neopterin and Tryptophan Concentrations in Obese Children

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Neopterin is synthesized from guanosine triphosphate by enzyme GTP cyclohydrolase I. It is released preferentially from human monocyte-derived macrophages upon stimulation with the Th1-type cytokine interferon- γ that is the probably most important mediator of antimicrobial and antitumor defence *in vivo*. Thus, neopterin concentrations in body fluids usually correlate with immune activation and are significant predictors of chronic infections, various types of cancer and cardiovascular disease (1, 2). In adults, an association between increased body mass index and neopterin concentrations was observed (3), but although there is evidence for a proinflammatory state accompanied by impaired vascular endothelial function scarce in childhood and adolescence, such data are still scarce (4). Moreover, an earlier study indicated that neopterin concentrations are decreased rather than increased in juvenile obesity (5). In a cross-sectional approach we investigated serum neopterin concentrations as well as

tryptophan metabolism in a cohort of 356 overweight and obese (aged 11.3 ± 2.97 years; mean \pm S.D.), otherwise healthy children and 32 non-obese controls which were recruited at the University Hospital of Salzburg, Department of Pediatrics. Distribution of female to male was equal in both groups. Neopterin concentrations were measured using ELISA (BRAHMS, Hennigsdorf, Germany), and tryptophan and kynurenine concentrations were determined by HPLC.

Body mass index (BMI) differed significantly between obese and non obese probands (28 ± 5.64 vs. 18 ± 2.19 SD kg/m² U = 9.24, p <0.0001, Mann Whitney U-test). Obese children had significantly higher serum triglyceride 109 ± 63.9 vs. 51 ± 30.5 (U = 6.67 p <0.0001) lower HDL-cholesterol (50 ± 12.3 vs. 63 ± 13.1 mg/dl; U = 4.9 p <0.0001), higher fasting glucose levels (88 ± 9.4 vs. 75 ± 7.6 mg/dl; U = 7.3 p <0.0001) and were significantly more Insulin resistant (HOMA-IR 1.0 vs. 3.5; p <0.0001). Serum neopterin concentrations were low in both groups with an average of 5.13 ± 1.59 in normal weight controls vs. 5.85 ± 2.86 nmol/L in obese juveniles (not significant). By contrast, there was no difference in kynurenine concentrations and the kynurenine to tryptophan ratio (kyn/trp), although tryptophan concentrations were significantly lower in the obese (80.7 ± 12.7 μ mol/L) vs. non-obese subjects (74.7 ± 18.8 μ mol/L; U = 2.384, p = 0.017). Tryptophan concentrations correlated with size and weight in both groups, but neither tryptophan nor kynurenine concentrations nor kyn/trp correlated with neopterin concentrations.

This study shows that obesity in juveniles is not associated with increased neopterin concentrations. Results suggest that obesity at least in the early course of the disease does not lead directly to Th1-type immune activation and associated cardiovascular disease at this stage. Only in the later course a switch to Th-1 type immune activation and associated cardiovascular diseases may take place. Chronic infections or other cofactors might be important to trigger cytokine production and elevated neopterin concentrations at the later stage.

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Mechanisms of Tetrahydrobiopterin Stimulation of Enzymes - an Overview

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Currently five enzymatic reactions are agreed to require tetrahydrobiopterin a cofactor: Three are the aromatic amino acid hydroxylases phenylalanine 4-monooxygenase (E.C. 1.14.16.1), tyrosine 3-monooxygenase (E.C. 1.14.16.2) and tryptophan 5-monooxygenase (E.C. 1.14.16.4), which are encoded by single genes (PAH and TH), and by two genes (TPH1 and TPH2), respectively. The fourth enzymatic reaction is the group of nitric oxide synthases (E.C. 1.14.13.39), which occur in three isoforms encoded by three genes in mammals, NOS1, NOS2 and NOS3. The fifth reaction requiring tetrahydrobiopterin is alkylglycerol monooxygenase (glyceryl ether monooxygenase, E.C.1.14.16.5), the sequence of

which has not been characterized yet.

The aromatic amino acid hydroxylase proteins show significant sequence homology among each other, and share a common reaction mechanism characterized by the use of a non-heme iron, stoichiometric oxidation of the tetrahydrobiopterin cofactor, use of tetrahydrobiopterin in oxygen activation and the need for external recycling of the tetrahydrobiopterin cofactor which leaves the reaction as 4a carbinolamine derivative.

The nitric oxide synthases show significant sequence homology among each other, but share no homology with aromatic amino acid hydroxylases. The mechanism of tetrahydrobiopterin stimulation of nitric oxide synthases is fundamentally different from that of aromatic amino acid hydroxylases: The tetrahydrobiopterin cofactor is not involved in oxygen activation, is not consumed stoichiometrically and does not need to be recycled by external enzymes. In contrast, tetrahydrobiopterin donates a single electron and a proton, thus forming a radical intermediate which is recycled to the active tetrahydrobiopterin by nitric oxide synthase itself. Another difference to the aromatic amino acid hydroxylases is that nitric oxide synthases contain a catalytically active heme iron.

Alkylglycerol monooxygenase, a membrane bound enzyme, has many features similar to the aromatic amino acid hydroxylases in terms of tetrahydrobiopterin cofactor handling. Tetrahydrobiopterin is required stoichiometrically, and is recycled by external enzymes. In addition, alkylglycerol monooxygenase can be inhibited by the iron chelator 1,10-phenanthroline, suggesting that it does also contain a non-heme iron. Since no sequence homologous to aromatic amino acid hydroxylases and nitric oxide synthases is found in databases, we assume that alkylglycerol monooxygenase may form a third, distinct class of tetrahydrobiopterin dependent enzyme.

Increased Plasma Phenylalanine to Tyrosine Ratio in HIV-1 Infection and Correction Following Effective Antiretroviral Therapy

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Abnormal metabolism of aromatic amino acids tryptophan, phenylalanine and tyrosine accompanies conditions which go along with chronic inflammation and immune responses, e.g., cancer, infection, coronary artery disease, neurodegeneration and normal ageing (1, 2). Several years ago, higher serum and plasma concentrations of the essential amino acid phenylalanine have been documented in patients with HIV-1 infection (3). They may relate to a diminished conversion of phe to tyrosine (tyr) by the enzyme phenylalanine-hydroxylase (PAH). PAH is rate-limiting in the biosynthesis of dopamine, and impaired PAH activity is reflected by an increased phe to tyr ratio (phe/tyr). In this study, plasma phe/tyr was measured in 107 patients with HIV-1 infection before and after 12 months of effective antiretroviral therapy (ART) (4). Results were compared with CD4+ cell counts, HIV-1 RNA levels and concentrations of immune activation marker neopterin.

Before ART, phe/tyr was mean \pm S.D.: $0.99 \pm 0.57 \mu\text{mol}/\mu\text{mol}$ and correlated significantly with plasma and urine neopterin concentrations (both $p < 0.001$) and less strongly with HIV-RNA levels and CD4+ counts (both $p < 0.05$). After ART, phe/tyr dropped ($p = 0.01$) which was due to a decline of phe concentrations from $73.1 \pm 34.0 \mu\text{mol/L}$ at baseline to $62.9 \pm 17.8 \mu\text{mol/L}$ after ART ($p = 0.01$) and a concomitant increase of tyr concentrations ($p = 0.01$). In parallel, significant reductions of plasma and urine neopterin concentrations were observed during ART.

Increased phe/tyr is frequent in patients with HIV-1 infection and is related to immune activation. ART was found to decrease phe/tyr and this change could indicate and influence on PAH

activity.

Increase of phenylalanine is probably due to diminished activity of PAH which is probably due to a role of ROS in diminishing BH₄ appears (5). Any relevance for the neuropsychiatric abnormalities in patients still needs to be shown. Future studies might be able to show whether the decline of phe/tyr under ART may concur with the often improved neuropsychiatric status in treated patients.

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Urinary Neopterin in Patients with Gastrointestinal Stromal Tumors Treated with Imatinib

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Increased serum or urinary concentrations of neopterin have been described in patients with tumors of different primary locations, but so far there were no reports on neopterin in patients with gastrointestinal stromal tumors (GIST). We have studied urinary neopterin in 26 patients with GIST. Urinary neopterin was determined by high performance liquid chromatography. Compared to controls (n = 16), urinary neopterin was increased in patients with advanced/metastatic GIST (324 ± 306 , range 78 - 1425 vs. 124 ± 54 , range 34 - 229

$\mu\text{mol/mol}$ creatinine, $p = 0.001$). No statistically significant changes of urinary neopterin were observed during the therapy with imatinib, but a marked decrease was observed occasionally during the first days of imatinib treatment, in association with tumor control. The survival of patients with increased urinary neopterin was shorter, but the difference in survival was not statistically significant. In conclusion, urinary neopterin is increased in patients with advanced/metastatic GIST. No significant changes were observed in association with imatinib therapy, but a decreasing trend of urinary neopterin concentrations was evident in individual patients.