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Abstracts

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Correlation of neopterin levels in sepsis premature infant to sign and symptoms of neonatal sepsis using Tollner score

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Neonatal sepsis in premature infants is difficult to be diagnosed and markers that exist still vary in its value while the death rate dues to infection are very high. Neopterin is derivatives of pyrazino-pyrimidine produced by macrophages when stimulated by gamma interferon (IFN). This study aimed to measure and compare the value of serum neopterin between groups with and without sepsis and its correlation to signs and symptoms of neonatal sepsis using Tollner score. Analytic observational study with a cross-sectional design was implemented to subjects of 46 premature infants consisted of 23 infants each, with and without neonatal sepsis. Data analysis was done using Mann-Whitney test to compare the levels of neopterin in premature infants with and without neonatal sepsis and Spearman rank correlation test to see levels of neopterin correlation to Tollner score. Neopterin levels in the sepsis group was higher than non-sepsis group ie 116.65 ng/mL to 41.13 ng/mL with $p<0.001$. This study also showed a positive correlation between levels of neopterin in premature infants with neonatal sepsis to Tollner scores, with correlation coefficients of 0.776 which was significant at the 0.01 level (2-tailed) with $p<0.001$. Neopterin levels are higher in premature infants with neonatal sepsis than without neonatal sepsis. Neopterin levels are positively correlated with the signs and symptoms of neonatal sepsis that is indicated by Tollner score which means the higher the level of neopterin, the higher the score Tollner.

Intracellular signaling pathways controlling redox stress

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Reactive oxygen species (ROS) constitute important signaling molecules but when excessively produced efficiently contribute to cell damage and organ malfunction. Reducing intracellular ROS offers therapeutic options in the treatment of pathologies ranging from neurodegenerative diseases to ischemia/reperfusion injury (IRI), while further enhancing cellular ROS levels increases cell death and thus may inhibit tumor growth. ROS are derived from mitochondrial and non-mitochondrial sources and both are important in pathological settings. Intracellular ROS levels are tightly controlled by production and detoxification. However, achieving redox balance through the use of antioxidants had little effect on the prevention of oxidative damage in the clinical setting. Very recently the concept that intracellular signaling pathways may control cellular ROS levels has obtained experimental support. Regulation may be on the expression of antioxidant systems, but also on the production of ROS, e.g. through phosphorylation of components of the mitochondrial electron transport chain. In our own work we analyzed the link between intracellular signaling and cellular ROS levels in two settings: In the study of the prosurvival activity of the human RAF oncogenes we could demonstrate that RAF prevented the accumulation of excessive ROS levels and cell death as efficiently as antioxidants, which otherwise through the increase of mitochondrial Ca^{2+} triggered cell death. Similar effects we could demonstrate for the survival proteins Bcl-2 and AKT. More recently we also were able to show that oncogenic RAF signaling had no effects on the total antioxidant capacity of RAF-protected cells suggesting that the production of ROS could be targeted by RAF signaling. The second approach involved *in vitro* and *in vivo* models for the development of ischemia/reperfusion (IR)-associated tissue injury (IRI), a major contributor to organ dysfunction or failure. ROS are extensively produced during early reperfusion and contribute to damage through effects on biomolecules and also through their role as signaling molecules leading to e.g. enhanced production of inflammatory cytokines. Preventing IRI with antioxidants failed in the clinic, most likely due to the difficulty to timely and efficiently target them to the site of ROS production and action. Early reperfusion is also characterized by changes in the activity of intracellular signaling molecules. For one of them, the stress kinase p38MAPK, we obtained evidence that it is activated during reperfusion in various models of solid organ transplantation but also *in vitro* using reooxygenation following hypoxia to mimic the conditions of ischemia and early reperfusion. We obtained evidence that increased p38MAPK activity lead to elevated mitochondrial ROS levels *in vitro*, suggesting a role for p38MAPK upstream of ROS. To explore the therapeutic potential of inhibiting p38MAPK a stringent kidney clamping model in the Lewis rat was used. p38MAPK activity increased upon reperfusion and p38MAPK inhibition by the small molecular weight inhibitor BIRB796 almost completely prevented severe functional impairment caused by IR. Histological and molecular analyses suggested that protection resulted from decreased redox stress and suppressed apoptotic cell death. Moreover, a significant protection was also achieved in a rat kidney transplant model, establishing p38MAPK as a critical regulator of early damage associated with IR.

The role of Jak2 in hepcidin-dependent ferroportin internalisation in macrophages

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Macrophages play a key role in iron homeostasis. Especially with regard to their iron recycling function. Macrophages in the spleen and liver phagocytize damaged or senescent erythrocytes and by degrading heme, recycle iron. Iron can be stored intracellulary or released into the circulation by Ferroportin1 (Fpn1), a transmembrane protein and the only known iron exporter. Posttranslational regulation of Fpn1 is mediated by Hepcidin, the central iron regulator. Binding of hepcidin to Fpn1 results in Fpn1 internalisation, degradation and iron retention in macrophages. High levels of hepcidin are present in the anemia of chronic disease (ACD), a major form of anemia, developing

in patients with chronic inflammations (cancer, HIV, rheumatoid arthritis), resulting in a much worse outcome. The exact mechanisms of Hepcidin-dependent Fpn1 internalisation are incompletely understood and highly debated, especially with regard to the role of Jak2. There is conflicting evidence about the requirement of Jak2 mediated phosphorylation of Fpn1, induced by Hepcidin binding to Fpn1, leading to its internalization and degradation. Therefore, we established a mouse model of macrophage-specific Jak2 deficiency, by using a LysMCre-LoxP System, to study the role of Jak2 in hepcidin-dependent Fpn1 internalisation in macrophages, especially in states of inflammation and iron homeostatis disturbances. Bone marrow derived macrophages (BMDM) of Jak2 cKO (fl/fl; lc/lc) and Jak2 WT (fl/fl; wt/wt) were cultivated and analysed by qRT-PCR and immunoblotting. Our preliminary results show, that hepcidin is still able to induce the Fpn1 internalisation in Jak2 cKO BMDMs, lacking functional Jak2. Therefore it is likely, that Jak2 is dispensable for hepcidin-mediated Fpn1 internalisation. But there seems to be a role of Jak2 in the intracellular iron metabolism, by influencing Transferrin receptor 1 protein expression/stability, which needs further investigations.

In vitro effects of thymol

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Thymol, a naturally occurring phenolic monoterpene, has been known for its antioxidant, antimicrobial and antispasmodic activity. Furthermore it has been reported that thymol mediates cytoprotection against radiation induced damage and protects from DNA damage [1]. Most of the effects are suggested to be mediated through radical scavenging properties. Antioxidants can act in a direct manner by neutralizing reactive oxidative species (ROS) or indirectly, by influencing on cellular antioxidant mechanisms, such as the transcription of detoxifying or cytoprotective enzymes. The aim of this *in vitro* study was to further investigate the immunomodulatory properties of thymol by using peripheral mononuclear cells (PBMC) [2] and human myelomonocytic leukemia THP-1 cells and its descendant reporter cell line THP-1-Blue [3]. In this study we determined the impact of thymol on cell viability, tryptophan breakdown, as well as activation of the redox-sensitive transcription factor nuclear factor-κB (NF-κB). NF-κB is a key element in the regulation of immune activation and stress response and was measured by using the stably transfected reporter cell line THP-1-Blue [3]. During Th1-type immune response interferon-γ (IFN-γ), which is primarily produced by activated T-cells, stimulates the enzyme indoleamine 2,3-dioxygenase (IDO) that converts the essential amino acid tryptophan into kynurenine [5]. Antioxidants are known to suppress this type of immune response by counteracting redox-sensitive signal transduction cascades like NF-κB and IFN-γ [4].

Cytotoxic effects of thymol in normal human PBMC and THP-1 cells appeared at concentrations exceeding 250 μM after 48h of treatment. Non-toxic concentrations (125-250 μM) of thymol were able to suppress IDO activity in mitogen-stimulated PBMC and THP-1 cells stimulated with lipopolysaccharides (LPS). However, thymol treatment could not reduce NF-κB/AP-1 dependent reporter gene transcription in LPS stimulated THP-1-Blue cells up to a concentration of 850 μM, where the compound already exerts cytotoxic effects. Data indicates that thymol might have stronger influences on immunological mechanism by interfering with IDO activity than by influencing on NF-κB in isolated monocytic cells.

[1] Archana PR, et al, Mutat Res 2011;726:136-45.
[2] Jenny M, et al, Inflamm Res 2011;60:127-35.
[3] Schroecksnadel S, et al, Biochem Biophys Res Comm 2010;399:642-6.
[4] Schroecksnadel K, et al, Drug Metab Lett 2007;1:166-71.
[5] Schröcksnadel K, et al, Clin Chim Acta 2006;364:82-90.

Effects of Ag and Al_2O_3 nanoparticles on immunoregulatory circuits *in vitro*

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Nanomaterials are increasingly produced and used throughout recent years. Consequently the probability of exposure to nanoparticles has drastically risen. Because of their small 1-100 nm size, the physicochemical properties of nanomaterials may differ from standard bulk materials and may pose a threat to human health. Only little is known about the effects of nanoparticles on the human immune system [1]. In this study, we investigated the effects of Ag and Al_2O_3 nanoparticles (Sigma, Vienna, Austria) employing the *in vitro* model of human peripheral blood mononuclear cells (PBMC) [2], cytokine-induced neopterin formation and tryptophan breakdown were monitored, all experiments in duplicates and repeated twice (total n = 3). Both biochemical processes are closely related to the course of diseases like infections, atherogenesis and neurodegeneration.

Treatment with the nanoparticles increased neopterin production in unstimulated and stimulated PBMC cells significantly (all $p<0.05$). No effect on tryptophan breakdown was detected in unstimulated cells, whereas in stimulated cells, tryptophan breakdown and IFN-γ production were suppressed by the nanoparticles ($p<0.05$). Treatment with Ag showed a significant and dose-dependent inhibition of tryptophan breakdown and IFN-γ production in stimulated cells ($p<0.05$), whereas, Al_2O_3 nanoparticle treatment did not significantly influence IFN-γ production.

When results were compared to that obtained with TiO_2 nanoparticles that were investigated earlier using the same cellular system [3], the effects of Ag and Al_2O_3 nanoparticles were similar to OCTi60 TiO_2, while P25 TiO_2 (25 nm diameter) nanoparticles had comparably little influence. It seems that the T cell-dependent responses such as tryptophan breakdown and IFN-γ and neopterin production in PBMC are suppressed by Ag and Al_2O_3 nanoparticles. The higher neopterin concentrations observed may result from high output production of the compound by macrophages which are presumably directly activated by nanoparticles. Because nanoparticles stimulated neopterin production but suppressed tryptophan breakdown by anti-inflammatory indoleamine 2,3-dioxygenase (IDO) in parallel, data suggests that the net effect of particles would be even stronger pro-inflammatory.

[1] Thomas T, et al. Toxicol Sci 2006;91:14-19.
[2] Jenna M, et al. Inflamm Res 2011;60:127-35.

[3] Becker K, et al. Food Chem Toxicol 2013;65C:63-69.
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Tryptophan catabolism during HIV infection: incidental occurrence or pathogenic mechanism

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The human immunodeficiency virus type 1 (HIV-1) is the causative agent of the acquired immunodeficiency syndrome (AIDS) in the current global pandemic. HIV-1 causes a progressive impairment of immune responses, which eventually leads to increased susceptibility to otherwise innocuous opportunistic infections. HIV-1 infection is associated with chronic and uncontrolled pan-immune activation, which contributes to functional immune exhaustion. Since the early 1990's, increased tryptophan (Trp) catabolism via the Kynurenine (Kyn) pathway has been described in HIV-1-infected patients [1]. Indoleamine 2,3-dioxygenase (IDO) catabolizes the first and rate limiting step of Trp degradation via the Kyn pathway. We demonstrated that HIV-1 is a potent inducer of IDO in plasmacytoid dendritic cells (pDC), a cell subset specialized in the production of high amounts of type I interferon during innate antiviral immune responses [2]. We found increased IDO expression and activity in blood and lymphoid tissues of HIV-1 patients and simian immunodeficiency virus (SIV)-infected rhesus macaques [3]. IDO expression in lymphoid tissues correlated with accumulation of regulatory T cells and high HIV/SIV replication [3]. IDO blockade using the competitive inhibitor 1-methyl-D-tryptophan (D-1mT) enhanced the ability of T lymphocytes from HIV-1 patients to proliferate in response to *in vitro* stimulation, and D-1mT administration to SIV-infected macaques improved the responsiveness to antiretroviral therapy [2,4]. More recently, we showed that the ability of HIV-1 to induce IDO in pDC is due to a functional substructure on the viral envelope, the virion-associated lipid raft [5]. Finally, our most recent data show that HIV-2, a naturally occurring attenuated form of HIV which is immunologically controlled *in vivo*, is a less potent inducer of pDC activation and IDO activity compared to the more pathogenic HIV-1. IDO may represent a critical mechanism linking HIV-1-induced innate immune response and immune exhaustion.

[1] Fuchs D, et al. Immunol Lett 1991;28:207-11.
[2] Boasso A, et al. Blood 2007;109:3351-9.
[3] Nilsson J, et al. Blood 2006;108:3808-17.
[4] Boasso A, et al. J Immunol 2009;182:4313-20.
[5] Boasso A, et al. Blood 2011;118:5152-62.

HFE deficiency critically affects cholesterol homeostasis in mice

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Iron metabolism plays a crucial role in diseases characterized by chronic inflammation including atherosclerosis. Specifically, iron overload has been linked to an increased risk for atherosclerosis development, and with its sequel stroke and myocardial infarction. However, the underlying mechanism has not been elucidated thus far. To study this we used apoE-/- mice as a model of dyslipemia which develop spontaneous atherosclerosis over time and crossed these mice HFE-/-, a model of genetic iron overload. When studying lipid homeostasis in double KO mice (HFE-/- apoE-/-) we observed markedly higher plasma cholesterol levels than wild-type littermates and apoE -/- or Hfe-/- single knockout mice.

Furthermore, keeping mice on a low-iron diet we observed an even more pronounced increase in plasma cholesterol, characterized by an atherogenic lipoprotein profile with markedly increased low-density lipoprotein (LDL) cholesterol levels. Accordingly, qRT-PCR analysis of hepatic genes involved in cholesterol metabolism revealed a significant down-regulation of the LDL receptor in double KOs fed a low-iron diet. In a subsequent long-term experiment of 20 weeks, double KOs on a low-iron diet displayed a significant 2.5-fold increase in atherosclerotic lesion area of the thoracic aorta. In summary, we show for the first time that the Hfe mutation affects cholesterol metabolism and atherogenesis, presumably through regulation of hepatic LDL receptor and that this effect is even aggravated by a low iron diet.

The arachidonic acid metabolome as conserved regulator of cholesterol metabolism

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Cholesterol metabolism is closely interrelated with cardiovascular disease in humans. Dietary supplementation with omega-6 polyunsaturated fatty acids including arachidonic acid (AA) was shown to affect plasma LDL-C and HDL-C in a favorable way. However, the underlying mechanisms are poorly understood. By combining data from a GWAS screening in >100,000 individuals of European Ancestry, mediator lipidomics, and functional validation studies in mice, we identify arachidonate 5-lipoxygenase (Alox5) as an important modulator of LDL-C and HDL-C levels, capable of impacting one key function of HDL, namely reverse cholesterol transport (RCT). Importantly, we show that aspirin promotes RCT by stimulating bile acid excretion via induction of the bile salt export pump (Abcb11) through Alox5-dependent generation of leukotrienes and lipoxins. Moreover, we identify lipoxin mimetics as therapeutics to lower plasma LDL-C. Our results define the AA metabolome as conserved regulator of cholesterol metabolism, and identify AA-based compounds as promising therapeutics to counteract cardiovascular disease in humans.

IFNg +847T/A polymorphism: improvement of genotyping and genetic analysis of the STYJOBS/ EDECTA cohort

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Interferon-gamma (IFN-γ) is a potent inflammatory mediator that activates indoleamine 2,3-dioxygenase (IDO). IDO facilitates the conversion of tryptophan (Trp) to kynurenine (Kyn), which forms an alternative pathway to serotonin formation from Trp [1]. A polymorphism (+874A/T; rs2430561) in the first intron of IFN-γ is located within a canonical binding site of the activating transcription factor NF-κB and its functional importance was shown as the T-allele gives rise to increased levels of IFN-γ. Genotyping IFN-γ +847T/A is a cumbersome task as the A/T transversion is located in the las dinucleotide of a CA(n) repeat [2]. For each DNA to be determined, two polymerase chain reaction (PCR) need to be performed and analyzed on agarose gels. We improved this method and designed a new primer for the A-allele, which is more specific than the primer widely used due to additional mutations within the binding sequence, and that is approximately 30 nucleotides longer than the T-allele specific primer. PCR is carried out with the two allele specific primers and a common antisense primer. On an agarose gel, the A- and T- allele can easily be distinguished by length. This improvement not only decreases hands-on time by half, it also is more specific. We detected approximately 2% false genotype calls using the traditional 2-reaction PCR. We genotyped the IFN-γ +874 A/T polymorphism in 508 patients (60% females) of the StyJobs/EDECTA cohort, which has been set up to investigate the preclinical phase of obesity by a well defined cohort of young and middle aged overweight/obese and normal weight subjects [3]. As in an earlier investigation [4], serum Trp concentrations were significantly higher in male than in female individuals. While in females Trp levels decreased over age, no effect of age was detected in males. Body Mass index (BMI) was positively correlated with Trp serum levels in females, while in males there was no such correlation. Genetic associations were detected for AST/GOT ($p = 0.02$) and nitrite ($p < 0.05$) in the general population. Stratification for sex showed an effect of IFN-γ +874 on AST/GOT ($p = 0.03$), ALT/GPT ($p = 0.02$) and family history of stroke ($p = 0.02$) in females, but not in males. Thus, we confirmed earlier data [4] showing higher Trp levels in males compared to females. On the other hand, we detected no association between the IFN-γ +874 T/A polymorphism and Trp levels as shown before by Hurme's group [5]. This might be due to different structures and age of the two study populations.

[1] Xu H, et al. Immunol Lett 2008;121:1-6.
[2]Pravica A, et al. Hum Imunol 2000;61: 863-6.
[3] Mangge H, et al. Obesity (Silver Spring) 2013;21:E71-7.
[4] Widner B, et al. Clin Chem 1997;43:2424-5.
[5] Raitala A, et al. Scand J Immunol 2005;61:387-90.
*STYJOBS/EDECTA (*http://clinicaltrials.gov/ct2/show/NCT00482924*)*

Cytotoxic and interferon-gamma independent effects of glucose and insulin *in vitro*

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Glucose is a critical component in the pro-inflammatory response of macrophages. Since macrophages contribute to diabetes and obesity-induced inflammation, it is important to understand how substrate metabolism may alter its inflammatory function. Hyperglycemia is a chronic abnormality in diabetes. It is known that exposure of proteins to high glucose leads to the formation of advanced glycation end-products. This oxidative stress induces structural and functional damage of protein residues. However, it remains unclear how exposure to high glucose changes cellular responses to inflammatory stimuli. We hypothesized that exposure to high glucose might render the macrophages more sensitive to oxidative stress and immune activation. Therefore, we have tested whether the high concentration of glucose induces DNA damage, neopterin expression and tryptophan degradation by U937 macrophages without IFN-gamma stimulation. The human U937 monocytic cells were exposed to glucose alone and glucose plus 10, 40, 60 uU/ml insulin in seven different concentrations at three different time-periods. To determine toxicity levels of glucose and glucose plus insulin, cell viability was estimated by MTT test. Average value of each point was calculated by measuring eight samples. In order to indicate the DNA damage, Comet assay was used. Neopterin levels were measured by ELISA, kynurenine and tryptophan concentrations were measured by high performance liquid chromatography. U937 cells in the presence of high glucose (>200 mg/dl) had a significant DNA damage which confirmed genotoxicity. While with the increasing glucose concentrations with or without insulin U937 cells had higher cell viability at the end of twenty-four-period, at seventy-two- hours MTT tests indicated a glucose concentration-dependent decrease in metabolic activity. In glucose-only groups, a negative correlation was calculated between the MTT and neopterin/per cell. However a positive correlation was obtained between MTT/per cell and neopterin/per cell. Regardless of the presence of insulin, a negative correlation was evident between tryptophan and kynurenine. But we could not find any relationship between neopterin and kynurenine/tryptophan ratio. Decreases in neopterin per cell, tryptophan and kynurenine were tightly associated with genotoxicity. These findings suggest that the human U937 monocytic cells were most vulnerable to high glucose- induced oxidative stress. This study also suggests that glycotoxicity is in genotoxic nature. On the basis of these data, neopterin and kynurenine biosynthesis of U937 cells does not necessarily relate to IFN-gamma stimulation. However the mechanisms of glucotoxicity related tryptophan depletion and cytokine release by U937 cells are needed to be clarified.

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Role of the aryl hydrocarbon receptor (AhR) and tryptophan catabolism in endotoxin tolerance

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The study of disease tolerance pathways could provide new approaches for treating infections and other inflammatory diseases. Candidate pathways include sensors of pathogen presence, host metabolism, and intrinsic danger- or damage-associated molecules. Typically, an initial exposure to bacterial Lipopolysaccharide (LPS) induces a state of protective tolerance to further LPS challenge ("endotoxin tolerance"). The complex events underlying this phenomenon remain poorly understood. Here we demonstrate that endotoxin tolerance reprograms Toll-like receptor 4 signaling *via* the combined effects of tryptophan metabolites activating the ligand-operated transcription factor aryl hydrocarbon receptor, and the cytokine transforming growth factor β. The protective, LPS-triggered tolerant state is not restricted to LPS-or gram-negative bacteria-induced immunopathology, in that it also specifically targets specific signaling pathways in a *Streptococcus*-induced multifocal septic arthritis model. Thus interfering with the trade-offs that the defense systems in both pathogens and their hosts impose on host fitness could ultimately help to selectively enhance pathways responsible for strong and rapid inflammatory responses that lead to fast pathogen clearance, yet do not involve immunopathology.

The structure and function of phenylalanine hydroxylase from *Legionella pneumophila*, a thermostable enzyme with a major functional role in pyomelanin synthesis

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Legionella pneumophila is a bacterium that can cause Legionnaires' disease and other non-pneumonic infections in humans. Phenylalanine hydroxylase from *L. pneumophila* (lpPAH) has a major functional role in the synthesis of the pigment pyomelanin, which is a potential virulence factor with ferric reductase activity. We have investigated the role of lpPAH, the product of the phhA gene and found that lpPAH has a major functional role in pigment synthesis and promotes growth in low-tyrosine media. We also cloned and characterized lpPAH, which showed many characteristics of other PAHs studied so far, including Fe(II) requirement for activity. However, it also showed many particular properties such as dimerization, a high conformational thermal stability, with a midpoint denaturation temperature (T_m) = 79 ± 0.5°C, a high specific activity at 37°C (10.2 ± 0.3 μmol L-Tyr/mg/min) and low affinity for the substrate (K_m (L-Phe) = 735 ± 50 μM [1]). The crystal structure of lpPAH (PDB 4BPT [2]) reveals a unique dimerization interface, which together with a number of aromatic clusters appears to contribute to the high thermal stability of lpPAH. The crystal structure also explains the increased aggregation of the enzyme in the presence of salt and the low affinity for the substrate L-Phe. This is the first structure of a dimeric bacterial PAH and provides a framework for interpreting the molecular and kinetic properties of lpPAH and for further investigating the regulation of the enzyme. The high thermal stability of lpPAH might reflect the adaptation of the enzyme to withstand relatively high survival temperatures. These results will be presented and discussed.

[1] Flydal MI, et al. PLoS One 2012;7:e46209.
[2] Leiros HK, et al. FEBS Open Bio 2013;3:370-8.

Dynamic regulation of phenylalanine hydroxylase

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Phenylalanine hydroxylase (PAH) is the key enzyme in phenylalanine metabolism catalyzing its oxidative degradation to tyrosine at a non-heme iron center using tetrahydrobiopterin and molecular oxygen as co-factors. Due to the key role in the well-studied genetic disease phenylketonuria (PKU), regulation of PAH has been extensively examined using several experimental and computational techniques. Thereby, several regulatory mechanisms at different levels have been discovered over the last decades. Tetrahydrobiopterin acts as a pharmacological chaperone, directed to the active site of the protein and is thus used in the treatment of phenylketonuria [1]. Fluorescence experiments highlighted major conformational changes in the protein upon activation although it is unclear whether the activating Phe binds to the active site itself or to a remote site [2]. Additionally, several allosteric mechanisms of enzyme regulation have been described and were recently reviewed by Fitzpatrick [3]. A loop near the active site (Tyr138-loop) was described to change its position dependent on the enzyme activation state [4]. Additionally, phosphorylation at Ser-16 [5] and changes in the tetrameric assembly of the protein [6] are known to regulate enzyme turnover. Based on structural data and in combination with experimental assays, molecular dynamics simulations are a powerful computational technique to rationalize changes in protein structure and dynamics. Using extensive molecular dynamics simulation we could come up with a structural explanation for the experimentally observed down-regulation of PAH in situations of oxidative stress [7]. Furthermore, we could show how binding of phenylalanine distal from the active site influences enzyme dynamics and thus regulates substrate binding [8]. With the increase of structural data on PAH, more and more regulatory mechanisms can be understood at atomistic level. Protein dynamics add another layer of complexity to biomolecular recognition. Therefore, we hope that molecular dynamics simulations will allow to decipher further mechanisms of regulation in PAH in the near future.

[1] Blau N, et al. Lancet 2010;376:1417-27.
[2] Flydal MI & Martinez A. IUBMB Life 2013;65:341-9.
[3] Fitzpatrick PF. Arch Biochem Biophys 2012;519:194-201.
[4] Andersen OA, et al. J Mol Biol 2003;333:747-57.
[5] Li J & Fitzpatrick PF. Arch Biochem Biophys 2013;535:115-9.
[6] Jaffe EK, et al. Arch Biochem Biophys 2013;530:73-82.
[7] Fuchs JE, et al. PLOS One 2012;7:e53005.
[8] Ronau JA, et al. Eur Biophys J 2013;42:691-708.

Serum tryptophan, kynurenine, phenylalanine, tyrosine, nitrite and neopterin concentrations in 100 healthy blood donors

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Inflammation processes are considered to play a relevant role in the pathogenesis of a variety of diseases like cancer, infections and autoimmune disorders. Thereby elevated concentrations of several biomarkers and the effect of cytokines on the monoamine metabolism were found to closely correlate with depression, cognitive alternations and mood disorders. Recently it was observed that tryptophan breakdown and phenylalanine metabolism may provide a biochemical link between immune activation, inflammation and neuropsychiatric sequelae [1-3]. Tryptophan, a precursor of the neurotransmitter serotonin, can be metabolized to kynurenine which further leads to neuroactive substances like quinolinic acid and kynurenic acid. Phenylalanine and tyrosine are precursors for dopamine, L-DOPA, adrenalin and noradrenalin which play a crucial role in the regulation of mood and cognitive processes. Neopterin as an unspecific marker of the immune system correlates strongly with tryptophan and phenylalanine metabolism [1,2]. As a consequence of the new considerations the analytics for measurements of all these metabolites came into focus. The goal of this investigation was to establish reference values for the markers in a healthy population, and therefore one hundred successive individuals were included in the study who donated blood at the University Clinics of Innsbruck, Austria and who had passed anamnestic examination at entry and are therefore considered as healthy. The mean age of participants was 49 (SD = 11.4) years, 18 were older than 60 years. Neopterin concentrations were measured by ELISA (BRAHMS, Hennigsdorf, Germany), all other analytes were determined by HPLC applying previously published methods [4,5]. Kynurenine and tryptophan concentrations were found as 1.78 ± 0.42 µmol/L and 67.4 ± 10.19 µmol/L, and Kyn/Trp was 26.7 ± 6.20 µmol/mmol). The phenylalanine and tyrosine concentrations were 65.2 ± 11.05 µmol/L and 90.6 ± 22.9 µmol/L, and Phe/Tyr was 0.75 ± 0.14 µmol/µmol. Neopterin concentrations were 5.9 ± 1.58 nmol/L, 98 donors presented with neopterin concentrations below 10 nmol/L. Males presented with significantly higher tryptophan and tyrosine concentrations than females (both $p < 0.05$) and males were taller and heavier (both $p < 0.01$), but body mass index (BMI) did not differ significantly by gender. There existed several significant correlations between neopterin and the other biomarker concentrations: kynurenine rs = 0.368, kynurenine to tryptophan ratio (Kyn/Trp, an estimate for the activity of tryptophan-degrading enzyme indoleamine 2,3-dioxygenase, IDO [1]) rs = 0.453 and tyrosine -0.267 (all $p < 0.01$) and the phenylalanine to tyrosine ratio (Phe/Tyr, an estimate for the activity of phenylalanine-converting enzyme phenylalanine hydroxylase, PAH [2]) rs = -0.236 ($p < 0.05$). Thus, data demonstrate that also in a population of healthy individuals an association exists between "low grade" immune activation and biochemical alterations which can be of relevance for neurotransmitter biology.

[1] Widner B, et al. Brain Behav Immunity 2002;16:590-5.
[2] Neurauter G, et al. Curr Drug Metabol 2008;9:622-7.
[3] Haroon E, et al. Neuropsychopharmacology 2012;37:137-62.
[4] Laich A, et al. Clin Chem 2002;48:579-81.
[5] Neurauter et al. Clin Biochem 2013;46:1848-51.

Influence of antibiotic gentamicin on mitogen-induced tryptophan breakdown *in vitro*, effects similar to standard food preservatives

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The exposure to antimicrobial agents has increased in the Western World and ranges from antibiotics, antiseptics, disinfectants to preservatives. According to the "hygiene hypothesis", the decrease of infections contributes to the high incidence of allergies and asthma. Changes of the human microbiome play a major role in the modulation of immune responses [1]. Another possibility to predispose for T helper (Th)-type 2 immunity is the suppression of Th 1- type immune responses, as Th1 and Th2 reactions cross-regulate each other. Several food additives and colorants, as well as a wide range of antioxidant substances, were shown to suppress Th1-type immune reactions *in vitro* [2].

Tryptophan breakdown via interferon γ (IFNγ)-inducible indoleamine 2,3-dioxygenase (IDO) is a central biochemical pathway within Th1 type immunity. Tryptophan deprivation serves as an antiproliferative strategy for invading pathogens and is a negative feedback regulator for Th1-type responses. IFNγ induces also the formation of the cellular immune activation marker neopterin. Beside other biological functions, neopterin enhances the bactericidal effects of several reactive oxygen species (ROS) and peroxynitrite [3].

The aim of our study was to analyze if the aminoglycoside complex gentamicin, a broad spectrum antibiotic, which inhibits growth of many Gram-positive and Gram-negative bacteria, interferes with Th1-type immune reactions *in vitro*. We used mitogen-stimulated human peripheral blood mononuclear cells (PBMC) as a model system [4]. IDO activity, estimated by the kynurenine to tryptophan ratio in culture supernatants, and neopterin formation was used as a readout. Gentamicin could significantly suppress IDO activity and neopterin formation in a dose-dependent manner. Also, the metabolic activity of stimulated-cells was negatively affected.

Our data suggest that gentamicin is able to suppress Th1-type reactions by similar mechanism as was shown for standard food preservatives. These effects may polarize immunity towards Th2-type reactions [3,5]. Such property of antibiotics like gentamicin may relate to the allergy-promoting potential of the widely used therapeutics. Earlier studies reporting the potential of gentamicin and other antibiotics to inhibit the production of IFNγ in stimulated mouse lymphocytes [6] and activation of rabbit peritoneal macrophages [7] are in line with our hypothesis. Furthermore, antibiotic exposure in early life or in utero was found to be associated with an increased risk of childhood asthma [7].

[1] Okada H1, et al. Clin Exp Immunol 2010;160:1-9.
[2] Zaknun D, et al. Int Arch Allergy Immunol 2012;157:113-24.
[3]Murr C, et al. Curr Drug Metab 2002;3:175-87.
[4] Jenny M, et al. Inflamm Res 2011;60:127-35.

[5] GostnerJ, et al. Curr Pharm Des 2013; PMID: 23701561.
[6] Sacha PT, et al. Med Dosw Mikrobiol 1999;51:413-9.
[7] Sacha PT, et al. Med Dosw Mikrobiol 1999;51:399-412.
[8] Murk W, et al. Pediatrics 2011;127:1125-38.

Effects of kaempferol on *in vitro* models of inflammation

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The flavonoid campherol is nearly ubiquitously distributed in edible and medicinal plants and was shown to exert a broad range of pharmacological activities. Daily campherol uptake from a plant-rich diet may reach values in the lower milligram range [1]. In this study, we investigated the interference of campherol with T helper (Th) type 1 immune response signaling in human peripheral blood mononuclear cells (PBMC) and in myelomonocytic THP-1Blue cells. Activated T cells are a major source of interferon gamma (IFNγ), a central regulator of the immune response that activates biochemical pathways such as tryptophan breakdown via indoleamine 2,3-dioxygenase (IDO1) and formation of the oxidative stress marker neopterin via GTP-cyclohydrolase I. Activation of redox-sensitive transcription factors e.g. transcription factor nuclear factor-κB (NF-κB) and signal transducer and activator of transcription 1 (STAT1) leads to upregulation of differential gene sets, including chemokines and cytokines [2].

Treatment of mitogen-stimulated PBMC with campherol at non-lethal concentrations decreased IDO-mediated tryptophan breakdown significantly in a dose-dependent manner. In parallel, neopterin levels were reduced. No effect of campherol treatment was observed in unstimulated PBMC. Parallel interference of campherol with tryptophan breakdown and neopterin formation suggests the targeting of a shared upstream signal, however also direct enzymatic inhibition may play a role. *In silico* modeling analysis by docking of campherol to the crystal structure of human IDO (pdb: 2D0T, [3]) predicts a single favorable binding mode to the catalytic center.

Treatment of the lipopolysaccaride (LPS)-stimulated NF-κB reporter cell line THP-1Blue with campherol at non-lethal concentrations superinduced NF-κB activity. Analysing the expression of the cytokines tumor necrosis factor alpha (TNF), interleukins 1B (IL1B) and 6 (IL6) as well as of transcription factors NFKB1 and STAT1 with qPCR in cells treated with LPS and campherol (50 μM) resulted in increased expression of TNF, IL1B and NF-κB, while IL6 expression was decreased. Beside NF-κB expression, these effects could not be observed with 25 μM treatment. Of note, neither IDO nor expression of the cytoprotective enzyme heme oxygenase was affected upon LPS treatment under these experimental conditions.

All together these *in vitro* results suggest a different impact of campherol on isolated macrophage-like cells such as THP-1Blue than on PBMC, which can be used as model of lymphocyte/macrophage interplay.

[1] Calderón-Montaño JM, et al. Mini Rev Med Chem 2011;11:298-344.
[2] Saha B, et al. Cytokine 2010;50:1-14.
[3] Sugimoto H, et al. Proc Natl Acad Sci USA 2006;103:2611-6.

Comparison of effects of tranilast, cinnamic acid and anthranilic acid on tryptophan breakdown in human PBMC

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Breakdown of the essential amino acid tryptophan by indoleamine 2,3-dioxygenase (IDO) is a metabolic checkpoint for immune activation [1]. Tryptophan catabolites of the kynurenine pathway are considered to be involved in the regulation of various immunological and biological processes [2,3]. Tranilast (*N*-(3,4-dimethoxycinnamoyl) anthranilic acid) was approved in the 80s for the management of allergic disorders due to its capacity to inhibit histamine release [4]. Recent studies discuss anti-inflammatory properties of tranilast based on its structural and functional homology to the tryptophan catabolite 3-hydroxyanthranilic acid (3-HAA). However, also the hydroxycinnamic acid moiety may play a role [5]. In this study, we aimed to investigate the effect of 3-HAA, anthranilic acid (AA), cinnamic acid (CA) and tranilast in human peripheral blood mononuclear cells (PBMC). Mitogen-stimulated PBMC are a cellular model system for T helper (Th)-type 1 immune response, were IDO activity, measured by the kynurenine to tryptophan ratio, and formation of the cellular activation marker neopterin can be used as readout [6]. While AA treatment did not show an effect in the tested concentration range [1 to 100 μM], 3-HAA reduced mitogen-induced tryptophan degradation at the highest concentration. Cell viability was reduced with both treatments. CA slightly influenced neopterin formation and a strong and dose-dependent inhibition of the central Th1-type cytokine interferon γ (IFNγ) was found, indicating an anti-inflammatory activity of CA. No significant effects on cell viability were observed.

Treatment of mitogen-stimulated cells with tranilast solved in DMSO decreased tryptophan breakdown at higher concentrations. Production of IFNγ was strongly decreased. However, also DMSO alone could significantly reduce IDO activity and IFNγ concentrations. Neopterin formation was reduced at higher tranilast concentrations. This effect was not observed for DMSO alone. Both DMSO and tranilast-DMSO treatments negatively affected cell viability. Although a decrease of tryptophan and IFNγ was observed for tranilast-DMSO, it will be difficult to distinguish between effects of tranilast and solvent. The impact on neopterin formation appears to be less solvent-dependent. It might be possible that some of the reported *in vitro* effects of tranilast on immune cells relate to the activity of the tranilast-DMSO combination. Furthermore, results with CA indicate, that the CA part of the structure is important for an anti-inflammatory activity. In general, effects on IFNγ were observed at lower treatment concentrations than changes in tryptophan breakdown and neopterin formation.

[1] Munn DH, et al. Trends Immunol 2013;34:137-43.
[2] Stone TW, et al. Nat Rev Drug Discov 2002;1:609-20.
[3] Krause D, et al. Am J Pathol 2011;179:1360-72.
[4] Azuma H, et al. Br J Pharmacol 1976;58:483-8.
[5] Sova M. Mini Rev Med Chem 2012;12:749-67.
[6] Jenny M, et al. Inflamm Res 2011;60:127-35.

Simultaneous determination of tetrahydropbiopterin, dihydrobiopterin and dihydroneopterin in cerebrospinal fluid

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Tetrahydrobiopterin (BH_4) is an essential cofactor for several amino acid hydroxylases as well as for three nitric oxide synthases, and glyceryl-ether mono-oxygenase. Defects in the metabolism or regeneration of BH_4 leading to BH_4 deficiency are associated with neurological deterioration including progressive mental and physical retardation, central hypotonia and peripheral spasticity, seizures and microcephaly. BH_4 synthesis or regeneration can be affected by five inborn errors of metabolism leading to deficiency of five enzymes: GTPC-I, PTPS, SR, DHPR and pterin-4α-carbinolamine dehydratase [1]. The diagnosis of all these conditions mainly relies on the determination of pterins [neopterin (N), BH_4 and its oxidized form dihydrobiopterin (BH_2) in the cerebrospinal fluid (CSF). Classical methods of pterin determination in CSF involve HPLC coupled with fluorescence detection. As BH_4 and BH_2 are not natively fluorescent, pre column chemical oxidation of BH_4 and BH_2 into biopterin (B), which is fluorescent, must be performed [2]. Thus, these methods do not allow the simultaneous determination of BH_4 and BH_2. Here we describe a new method to simultaneously measure BH_4, BH_2, B, NH_2 and N in CSF samples by HPLC coupled to fluorescence detection after post-column coulometric oxidation. This new assay has been successfully applied to measure these pterins in more than one hundred CSF samples including samples from patients with known BH_4 deficiency.

[1] Blau N, Thöny B. In: Blau N, Duran M, Gibson KM. Laboratory guide to the methods in biochemical genetics. Berlin Heidelberg, Germany 2008.
[2] Fukushima T, Nixon JC. Anal Biochem 1980;102:176-88.

Comparison of ELISA and HPLC assays to determine blood kynurenine concentrations in men and mice

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Th1-type immune response represents an important aspect of adaptive immunity during which activated T-cells release several characteristic cytokines like interferon γ (IFNγ) and interleukin-2. Among other functions, IFN-γ is primarily responsible for the expression of specific enzymes indoleamine 2,3-dioxygenase (IDO) and GTP-cyclohydrolase I in various target cells. As part of an antiproliferative strategy, IDO degrades the essential amino acid tryptophan (Trp) to kynurenine (Kyn) and thereby limits tryptophan availability for protein biosynthesis. In parallel GTP-cyclohydrolase I produces neopterin in human monocyte-derived macrophages and dendritic cells, other cells and cells from other species form enzyme cofactor tetrahydrobiopterin (BH4) instead, BH4 representing the necessary cofactor of nitric oxide (NO·) formation and other mono-oxygenases. Therefore the increase formation of Kyn and NO by IFN-γ occurs usually in parallel in a variety of cells of human and non-human origin, and only in human monocytic cells neopterin is produced at the expense of BH4 but high output NO· production is lacking. To estimate Th1-type immune response, measurement of neopterin production and Trp breakdown as indicated by the Kyn to Trp ratio (Kyn/Trp) is useful [1].

Trp and Kyn can be measured simultaneously by using HPLC with fluorescence detection at extinction 286 nm and 366 nm emission wavelengths and UV detection at 360 nm [2,3]. For HPLC, samples are deproteinized with 2M trichloroacetic acid (TCA). Thereby nitrite which mainly derives from NO· under acidic conditions can react with Kyn to form N-nitroso intermediates that may rapidly decompose, and thus Kyn is lost during acidification pretreatment of specimens [4]. This is of greater relevance in specimens collected from mice or rats which especially during immune activation conditions may contain large amounts of nitrite derived from NO· that is formed and released by the cytokine-inducible NO· synthase (iNOS) [5]. As a consequence, acidic treatment should be avoided for Kyn measurement when nitrite is present in specimens. This effect of endogenous nitrite is in addition to the inhibition by NO· of IDO expression which functionally links oxidative arginine and tryptophan metabolism [5].

We measured Kyn in 83 specimens of mixed origin namely culture supernatant and plasma/serum from human and mouse, using HPLC [3] and a competitive enzyme linked immunoassay (L-Kynurenin ELISA Kit, ImmunDiagnostik, Bensheim, Germany). When measurements in all samples were compared, there existed a significant positive correlation ($r_s = 0.787$, $p < 0.001$). The same was true when only the mouse cell culture supernatants were calculated ($r_s = 0.803$, $p < 0.001$; $n = 53$) and in human plasma specimens ($r_s = 0.451$, $p = 0.027$; $n = 24$). No significant correlation was found in mouse plasma ($r_s = 0.232$, $p = 0.658$), but the number of specimens measured was only 6. We conclude that deproteinization of samples by acidic pretreatment of samples should be avoided for the Kyn assay when NO· is anticipated to be produced in a high rate. This does not seem to represent a major problem in murine (and presumably also in human) cell culture specimens, and also in human plasma/serum specimens there still existed a significant correlation albeit rather weak between results of both assays. The measurement of Kyn in mice specimens appears to be more critical, but the number of specimens measured thus far is certainly too small for a final conclusion.

[1] Schroecksnadel K, et al. Clin Chim Acta 2006;364:82-90.
[2] Widner B, et al. Clin Chem 1997;43:2424-6.
[3] Laich A, et al. Clin Chem 2002;48:579-81.
[4] Hara T, et al. J Immunol Methods 2008;332:162-9.
[5] Thomas SR, et al. J Biol Chem 1994;269:14457-64.

Iron homeostasis and immune function in macrophages infected with different intracellular pathogens

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Following an infection with intracellular bacteria a struggle for the essential nutrient iron between host phagocytes and the invading pathogens ensues. Consequently, competitive interactions between macrophage iron transporters and microbial iron acquisition systems form a central battlefield that determines the course of disease. While *Salmonella typhimurium* is mainly contained in the phagolysosome, *Listeria monocytogenes* is able to gain access to the host cell cytosol. Therefore it is possible that different defense strategies of macrophages on the one side and iron acquisition abilities of the pathogens on the other side are present. We used the murine macrophage cell line RAW264.7 either stably transfected with a functional allele of a cation transporter present on the late phagolysosome, Nramp1 (RAW-37), or a non-functional control of the same transporter (RAW-21).

We found varying intramacrophage survival of *Listeria* and *Salmonella* when stimulated with different iron sources, i.e. iron salts versus iron isomaltoside. This effect was reproducible in the RAW-21 and RAW-37 cell line with significant lower bacterial load in the latter, indicating an important function of Nramp1 in host defense against *Salmonella*, but interestingly also for *Listeria*, which is able to escape the phagolysosome. In contrast to Salmonella infection this effect in Listeria was not due to iron limitation through Nramp1.

The ability of bacteria to use external iron sources depends on their uptake, compartmentalization and utilization by macrophages. Nramp1 exerts anti-bacterial activity through restriction of iron availability and promoting macrophage immune effector functions.

The role of homocysteine and B-vitamins in degenerative bone disease

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Hyperhomocysteinemia (HHCY) has been suggested as a new risk factor for osteoporosis. Recent epidemiological, clinical and experimental studies provide a growing body of data, which is reviewed in this article. Epidemiological and (randomized) clinical trials suggest that HHCY increases fracture risk, but has minor effects on bone mineral density. Measurement of biochemical bone turnover markers indicates a shift of bone metabolism towards bone resorption. Animal studies confirm these observations showing a reduced bone quality and stimulation of bone resorption in hyperhomocysteinemic animals. Homocysteine (HCY) has been found to accumulate in bone by collagen binding. Cell culture studies demonstrate that high HCY levels stimulate osteoclasts but not osteoblasts, indicating again a shift of bone metabolism towards bone resorption. Regarding B-vitamins, only a few in vivo studies with equivocal results have been published. However, two large cell culture studies confirm the results obtained with exogenous HCY administration. In addition, HHCY seems to have adverse affects on extracellular bone matrix by disturbing collagen crosslinking. In conclusion, existing data suggest that HHCY (and possibly B-vitamin deficiencies) adversely affects bone quality by a stimulation of bone resorption and disturbance of collagen crosslinking.

Effect of occupational zinc exposure on erythrocyte folate levels

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Zinc-coating process named hot-dip galvanization is used for protecting metal items from corrosion. The greatest risk in galvanization process is exposure of workers to zinc fume rising from the metal bath surface. Physiologically, zinc is an essential element which has many important functions such as maintenance of immunity, cell integrity, signalling, cellular respiration, neuromodulation, etc. As well as zinc, folate also plays a crucial role in various biochemical pathways. Exposure to some xenobiotics may lead to folate deficiency. In this study, it was aimed to evaluate the effect of zinc nanoaerosols on erythrocyte folates which are present in virtually every known organism and cell type. In the present study, erythrocyte folate levels and blood zinc concentrations were evaluated in 63 galvanization workers and 23 office personnel as a control group. Folate levels were found to be decreased while zinc levels were increased in workers compared to the controls (both, $p<0.01$). Additionally, in galvanization workers a significant but rather weak negative correlation was observed between blood folate and zinc concentrations ($p<0.05$). It was estimated that zinc exposure leads to a decrease in erythrocyte folate levels. Besides, it may be speculated that galvanization procedure can cause an elevation in zinc concentration of workers and the chronically occupational zinc exposure may trigger changes in erythrocyte folate status which reflects tissue folates.

[1] Sarac ES, et al. Biol Trace Elem Res 2013;151:330-4.
[2] El Safty A, et al. Ann N Y Acad Sci 2008;1140:256-62.
[3] Sipahi H, et al. Pteridines 2007;18:106-13.
[4] Wagner C, et al. Clin Res Reg Affairs 2001;18:161-80.

Increased stool calprotectin in patients with Alzheimer's disease is associated with low serum tryptophan and phenylalanine

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Activated immune response and inflammation are detectable in the majority of patients suffering from various forms of dementias and other neurodegenerative disorders. Thereby increased serum neopterin and kynurenine to tryptophan concentrations indicate that the activated immune system is not confined to the brain rather also systemic immune abnormalities are common [1]. Increased neopterin production and tryptophan breakdown may result from an activated Th1-type immune response in patients, and e.g. in patients with Alzheimer's disease (AD), the increased neopterin levels were found

to correlate with cytomegalovirus (CMV) antibody titers [2]. Thus, an increased pathogen load might underlie the process of immune activation. Earlier in patients with rectal carcinoma, Melichar et al. described a significant association between intestinal permeability and urine and serum neopterin concentrations [3]. Data posed the question whether bacterial translocation due to hampered gut barrier function could also play a role in the chronic immune activation process which is detectable in AD patients. To address this question in a first step we examined stool concentrations of calprotectin and α1-antitrypsin in 23 patients with AD using ELISA test kits (PhicalTest, Calpro AS, Oslo, Norway) and compared results with concentrations of neopterin, tryptophan, phenylalanine and tyrosine (all measured by ELISA or HPLC) in serum collected on the same day. Calprotectin and antitrypsin concentrations were not associated with serum neopterin and C-reactive protein concentrations but correlated inversely with serum levels of tryptophan (rs = -0.414), tyrosine (rs = -0.427; both $p < 0.05$) and phenylalanine (rs = -0.587, $p < 0.01$), antitrypsin levels correlated with tyrosine (rs = -0.381; $p < 0.05$). No other significant relationships were apparent. Because increased concentrations of stool calprotectin and α1-antityrpsin levels are indicative for a disturbed intestinal barrier function (IBF), our data indicate that a disturbed IBF could play a role in the lowering of essential aromatic amino acids concentrations in the blood. Recent studies indicate that such disturbances of IBF can be positively influenced by probiotics, available data also imply that proper dietary supplements might be able to improve not only IBF but also the disturbed resorption of aromatic amino acids like tryptophan, phenylalanine and tyrosine that are of utmost relevance, e.g., for neurotransmitter biosynthesis and thus for the neuropsychiatric status of patients. Further studies are under way in which it is tested whether administration of probiotics (e.g. Omnibiotic, Allergosan, Graz, Austria) may help to improve blood concentrations of these particular amino acids.

[1] Widner et al. J Neural Transm 2000;107:343-53.
[2] Blasko I, et al. J Psychiatr Res 2007;41:694-701.
[3] Dvorák J, et al. Med Oncol 2010;27:690-6.
[4] Kuitunen M, et al. J Pediatr Gastroenterol Nutr 2009;49:626-30.
[5] van Hemert, et al. Adv Microbiol 2013,3:212-21.

Role of hepatic IDO expression in HCV infection

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Hepatitis C virus is a member of the *Flaviviridae* family and highly successful at establishing persistent infection. An increased serum kynurenine/tryptophan ratio, which is an index for IDO activity, has been previously reported in patients with chronic HCV infection when compared to patients with resolved HCV infection and healthy individuals [1-4]. The molecular mechanism of IDO induction in HCV infection and its role in antiviral immune response remain unknown. Using primary human hepatocytes, we show that HCV infection stimulates IDO expression. IDO gene induction was transient and coincided with the expression of type I and type III interferons (IFNs) and IFN-stimulated genes (ISGs) in HCV-infected hepatocytes. Hepatic IDO protein was enzymatically active as evidenced by conversion of tryptophan to kynurenine. Using the *in vitro* HCV replication system based on HCV genotype 2a (JFH1) we found that an ongoing HCV replication significantly upregulated IFN-γ induced hepatic IDO expression. Gene overexpression and silencing studies demonstrated that interferon-regulatory factor 1 (IRF1) regulated hepatic IDO expression. Overexpression of hepatic IDO prior to HCV infection markedly impaired HCV replication in hepatocytes suggesting that IDO limits the spread of HCV within the liver. However, hepatic IDO expression had a significant inhibitory effect on CD4+ T cell proliferation. Together, our data suggest that hepatic IDO plays a dual and opposing role during HCV infection by retarding viral replication and also regulating host immune responses. Further studies are warranted to determine the dominant effect of IDO – anti-HCV activity or immune regulation – in HCV infection.

[1] Cozzi A, et al. J Viral Hepat 2006;13:402-8.
[2] Larrea E, et al. J Virol 2007;81:3662-6.
[3] Zignego AL, et al. Dig Liver Dis 2007;39 Suppl 1:S107-11.
[4] Saito K, et al. J Gastroenterol 2013;48:660-70.

Neopterin rapid test-indicator of infection in ICU, our first experience

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Neopterin is a small molecular weight molecule produced and released by the antigen presenting cells in the activation of the cellular immune response. Cells that mediate cellular immunity, during which IFN gamma is produced, that binds to macrophages and dendritic cells triggering a cascade of intracellular enzymes that including the release of neopterin from GTP [1]. Accordingly, the neopterin concentration can be a good indicator of the diagnosis of a disease mediated cellular immune response, such as infection, malignancy and autoimmune disease [2,3]. Goal of the work is to assess the diagnostic value of determining the presence of neopterin in serum of patients treated in intensive care units KBC Zemun.

The study included two groups of patients: Totally we tested 30 patients. In first group were 15 patients with malignancies and the second 15 patients with acute coronary syndrome. Neopterin levels were determined by commercial test <Infect Check unloaded (qualitative)> (Concile, Freiburg, Germany) based on the principle of a competitive immunoassay chromatography and by Neopterin ELISA (IBL International, Hamburg, Germany). All procedures followed were in accordance with the ethical standards of the Ethical Committee of CHC Zemun Belgrade. Data of both groups were analyzed with standard statistical methods. In patients with malignant diseases neopterin was positive in 60% of cases, whereas in patients with ACS neopterin was positive in 40% of patients. Comparing results both of, neopterin rapid test and ELISA testing we found matching in 58% of cases. According to the data, it can be said that the determination of neopterin has a legitimate role in demonstrating the activation of the immune response. Generally the value of 10 nmol/L is estimated as a standard reference concentration of neopterin in serum.

Detection of markers of cell oxidative stress is a significant advancement in the diagnosis of chronic infectious, malignant, and of acute pathological states [4]. Neopterin as a stable and safe indicator of activation of cellular immune responses, may reasonably

find a place in screening healthy population and used in the early diagnosis of the disease before its clinical manifestation. Neopterin rapid test can be used as preliminary test. Also, due to the wider range of emerging infectious diseases transmitted through blood, testing voluntary blood donors in this unique marker will contribute to improved safety and quality of donated blood units, which is the imperative of modern transfusion services [5].

[1] Murr C, et al. Curr Drug Metab 2002;3:175-87.
[2] Plata-Nazar K, et al. Pteridines 2011;22:77-89.
[3] Mitaka C. Clin Chim Acta 2005;351:17-29.
[4] Zahorec R, et al. Bratisl Lek Listy 2000;101:552-7.
[5] Schennach H, et al. J Infect Dis 2002;186:1494-7.

The immune-inflammatory metabolic dysbalance-Medusa's head in obesity and atherosclerosis

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Atherosclerosis (AS) leading to myocardial infarction (MI) or stroke is associated with a systemic chronic low grade immune-mediated inflammation (scLGI) coccurring preferentially in the biologic surrounding of the so called metabolic syndrome (MetS). The innate and the adaptive immune response, as well as adipokines, chemokines, cytokines, and their receptors are involved in the initiation and perpetuation of scLGI, and play an important role in the scenario of atherosclerotic vascular plaque lesions [1-4]. In particular, the proinflammatory Th1-type cytokine interferon-γ (IFN-γ) is a key mediator [4]. IFN-γ activates the enzyme indoleamine 2,3-dioxygenase (IDO) in monocyte-derived macrophages, dendritic, and other cells, which, in turn, decreases serum levels of the essential amino acid tryptophan (TRP). Hence, people with cardiovascular disease (CVD) show an increased serum kynurenine to tryptophan ratio (KYN/TRP) as a result of an increased TRP breakdown [1]. Importantly, increased KYN/TRP indicates a higher likelihood of fatal cardiovascular events. The proinflammatory adipokine leptin represents another central link between obesity, AS, and cardiovascular disease. It has also been shown by own observations to play an important role for the initiation of scLGI in young obese [5]. Tryptophan is not only an important source for protein production but also for the generation of the basic neurotransmitter, 5-hydroxytryptamine (serotonin). In prolonged states of scLGI, availability of free serum TRP is strongly diminished, affecting serotonin synthesis, particularly in the brain [1]. Notably, accumulation of neurotoxic KYN metabolites such as quinolinic acid produced by microglia, can contribute to fatal courses of scLGI in AS and other inflammatory diseases. In conclusion, the "quality" of scLGI represented by individual biomarker profiles, and the KYN/TRYP ratio are important predictors of an unfavourable outcome in patients with CVD. It will be important to investigate if these parameters can provide a basis for more successful therapeutic protocols to further reduce cardiovascular morbidity and mortality by an improved understanding of the immune-inflammatory metabolic dysbalance.

[1] Mangge H, et al. Curr Med Chem 2013;20:2330-7.
[2] Mangge H, et al. Obesity 2014;22:195-201.
[3] Mangge H, et al. Exp Clin Endocrinol Diabetes 2004;112:378-82.
[4] Mangge H, et al. Curr Med Chem 2010;17:4511-20.
[5] Mangge H, et al. Transl Res 2012 159(2):118-24.

Tryptophan breakdown in alcoholism

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Post-withdrawal alcohol-dependent patients suffer from anxiety, craving, sleep disorder, irritability, mild depressive symptoms and high stress sensitization [1]. These symptoms persist for weeks to months after the beginning of abstinence and raise the risk of alcohol relapse. The understanding of tryptophan metabolism during different stages of alcohol withdrawal is important because of its role in serotonin synthesis. Under normal conditions, tryptophan is metabolized by the combined activity of the hepatic tryptophan 2,3-dioxygenase (TDO) and the indoleamine 2,3-dioxygenase (IDO). The activity of TDO seems to be sensitive to cortisol concentrations while IDO-activation occurs mostly through cytokines [2]. We investigated the parameters of tryptophan metabolism and in particular the role of immune-mediated tryptophan breakdown in two studies of alcohol dependent patients: in an acute withdrawal study (N=23; days 1, 5 and 10 of abstinence) and during the medium-term withdrawal (N=54; weeks 4-12 of abstinence). We observed substantial signs of IDO-activation in the first 10 days of alcohol withdrawal, as well as signs of moderate IDO-activation at the week 4 of alcohol abstinence. No signs of IDO-activation at week 12 of alcohol withdrawal were observed in this study. Kynurenine concentrations and kynurenie to tryptophan ratio (Kyn/Trp) increased significantly between week 4 and 12 of withdrawal, while Kyn/Trp correlated significantly to some behavioural symptoms associated with chronic stress. We suggest that IDO plays an important role in tryptophan metabolism during the excessive alcohol consumption and during the first days of withdrawal. During the medium-term withdrawal, tryptophan is predominantly metabolized by TDO. Further studies on the relationship between the TDO-activity and cortisol levels as well as on katabolism of kynurenine in medium-term alcohol withdrawal appear to be important for understanding of the recovery processes in abstinent alcohol dependent patients.

[1] Heilig M, et al. Neurosci Biobehav Rev 2010;35:334-44.
[2] Oxenkrug GF. Isr J Psychiatry Relat Sci 2010;47:56-63.

Association between neopterin concentrations and other biomarkers in patients with breast cancer

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Early diagnosis and effective therapy result in cure in most patients affected with breast cancer. In previous studies, the systemic immune

activation was noted in breast cancer survivors. In earlier studies the administration of neoadjuvant dose-dense chemotherapy was also shown to be associated with systemic immune activation, reflected in increased neopterin concentrations, and with a decrease of hemoglobin levels. In the present study, systemic immune activation and parameters of iron metabolism are followed in patients treated with neoadjuvant chemotherapy. Marked fluctuations of iron levels were accompanied by increasing neopterin concentrations. We have also examined correlations of urinary and serum neopterin concentrations with laboratory parameters of atherosclerosis risk and peripheral blood cell count in breast cancer survivors. Serum neopterin exhibited significant positive correlation with age, triglycerides, uric acid, homocysteine, albuminuria and C-reactive protein, and a negative correlation with HDL cholesterol. Urinary neopterin showed a significant negative correlation with HDL cholesterol and a positive correlation with C-reactive protein. In addition, a negative correlation was observed between urinary neopterin and hemoglobin concentrations and between relative lymphocyte counts and serum neopterin concentrations. These observations indicate that in breast cancer survivors immune activation reflected in neopterin concentrations is associated with parameters of atherosclerosis risk and immune dysfunction.

Low tryptophan-associated with immune activation-predicts mortality in coronary artery disease

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The essential amino acid tryptophan is necessary for protein synthesis, formation of the neurotransmitter serotonin and may exert immunoregulatory functions. Serum concentrations of tryptophan, neopterin and high sensitivity C-reactive protein (hsCRP) were measured in 1196 patients derived from the LUdwigshafen RIsk and Cardiovascular Health (LURIC) study, a cohort study among patients referred for coronary angiography. Serum concentrations of tryptophan did not differ between patients with (mean ± SD: 40.1 ± 9.8 µmol/L) or without angiographic coronary artery disease (CAD) (42.3 ± 23.9 µmol/L; Welch's t test: p = n.s.) but patients with CAD had higher neopterin (9.1 ± 8.2 nmol/L) and hsCRP (9.3 ± 18.5 mg/L) concentrations compared to patients without (neopterin: 7.6 ± 4.7 nmol/L; p <0.0001; hsCRP: 5.8 ± 7.6 mg/lL; p <0.0001). There was an inverse correlation between serum tryptophan and neopterin (Spearman's rank correlation: r_s = -0.273) and hsCRP (r_s = -0.163; both p <0.0001) concentrations.

Median observation period was 10.5 years. 385 patients had died during follow-up, 244 due to cardiovascular, 132 due to non-cardiovascular causes, in 9 cases no sufficient information for classification was available. After adjustments for cardiovascular risk factors and other possible confounders, the hazard ratio (with 95% CI) in the first when compared with the fourth tryptophan quartile of the study population was 1.96 (1.57-2.45; p = 0.0002) for total mortality, 2.33 (1.77-3.07; p = 0.007) for cardiovascular and 1.66 (1.16-2.38; p = 0.0015) for non-cardiovascular mortality, respectively, thus indicating a significantly higher risk of death in patients with tryptophan concentrations of the lowest quartile (<34 µmol/L). In conclusion, low serum tryptophan in patients is associated with immune activation and may indicate reduced life expectancy.

[1] Pedersen ER, et al. Arterioscler Thromb Vasc Biol 2011;31:698-704.
[2] Sulo G, et al. Int J Cardiol 2013;168:1435-40.
[3] Grammer TB, et al. Clin Chem 2009;55:1135-46.
[4] Schroecksnadel K, et al. Clin Chim Acta 2006;364:82-90.
[5] Sucher R, et al. Pteridines 2013;24:149-64.

Immune activation and inflammation in patients with cardiovascular disease are associated with higher phenylalanine to tyrosine ratios, the Ludwigshafen Risk and Cardiovascular Health (LURIC) study

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Higher serum neopterin is associated with increased mortality in patients with coronary artery disease (CAD) [1]. Preferentially Th1-type cytokine interferon-γ stimulates neopterin production by GTP cychlohydrolase I (GCH-I) in parallel in monocyte-derived macrophages and dendritic cells. In other cells, activation of GCH-I leads to the formation of 5,6,7,8-tetrahydrobiopterin (BH_4), the necessary cofactor of amino acid hydroxylases like phenylalanine 4-hydroxylase (PAH). Higher neopterin concentrations in patients suffering from various chronic inflammatory diseases were found to coincide with moderately increased serum phenylalanine and phenylalanine to tyrosine (Phe/Tyr) concentrations [2]. In a pilot study similar associations were reported in patients with CAD [3]. In this study, serum concentrations of phenylalanine, tyrosine, neopterin and and high sensitivity C-reactive protein (hsCRP) were measured in 1196 patients derived from the LUdwigshafen RIsk and Cardiovascular Health (LURIC) study, a cohort study among patients referred for coronary angiography. Phe/Tyr served as an estimate of phenylalanine hydroxylase (PAH) enzyme activity. Serum concentrations of phenylalanine and tyrosine and of Phe/Tyr did not differ between individuals with (mean ± SD: phenylalanine: 58±12 µmol/L; tyrosine: 64 ± 16 µmol/L; Phe/Tyr: 0.9 ± 0.2) or without CAD (phenylalanine: 57 ± 12 µmol/L; tyrosine: 65 ± 16 µmol/L; Phe/Tyr: 0.9 ± 0.2; Welch's t test: p = n.s.). Higher neopterin (9.1 ± 8.2 nmol/L) and hsCRP (9.3 ± 18.5 mg/L) concentrations were observed in CAD patients compared to controls (neopterin: 7.6 ± 4.7 nmol/L; p <0.0001; hsCRP: 5.8 ± 7.6 mg/L p<0.0001) and they correlated with Phe/Tyr (Spearman's rank correlation neopterin: r_s = 0.216 and hsCRP: r_s = 0.122; both p <0.0001) concentrations. We conclude that immune activation is associated with higher Phe/Tyr in CAD patients. Data indicates subnormal PAH activity which might be involved in the precipitation of neuropsychiatric symptoms in patients.

[1] Fuchs D, et al. Curr Med Chem 2009;16:4644-53.
[2] Neurauter G, et al. Curr Drug Metabol 2008;9:622-7.

[3] Mangge H, et al. Pteridines 2013;24:51-5.
[4] Murr C, et al. J Amino Acids 2014, ID 783730.

Investigating the NAD metabolome in Ewing Sarcoma

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Ewing Sarcoma (ES) is the second most common bone cancer in children and adolescents with a high metastatic potential. Tumor development is driven by the specific t(11;22)(q24;q12) chromosomal translocation resulting in generation of the chimeric transcription factor EWS-FLI1.

Recently, ES has been reported to be exquisitely sensitive to inhibitors of poly(ADP-ribose) polymerase 1 (PARP1). This enzyme uses NAD^+ as substrate and was demonstrated to regulate EWS-FLI1 in a feed-back mechanism. Another major mammalian NAD^+ consumer is the deacetylase SIRT1 which we observed to be highly expressed in ES metastases, validated with immunohistochemistry of 250 primary tumors and 30 metastases. PARP1 and SIRT1 play pivotal roles in coupling cellular metabolism to transcriptional gene regulation as well as to stress response. Both of them regulate pro-apoptotic transcription factors that are suppressed in ES including p53, FOXO1 and 3, and NF-κB. It has been demonstrated that PARP1 activity is at least partially dependent on acetylation which is counteracted by SIRT1 activity. Severe cell stress such as DNA damage leads to massive activation of PARP1, thus resulting in depletion of the cellular NAD^+ pool and finally cell death. Usually, NAD^+ is regenerated from nicotinamide via NAMPT or from the reduction of pyruvate via LDHA (Warburg effect), but can also be synthesized *de novo* from tryptophan. Interestingly, the knockdown of EWS-FLI1 in ES cells comes along with increased TDO and suppressed KMO expression, both being critical enzymes in tryptophan metabolism and NAD^+ *de novo* synthesis. As a consequence, cellular tryptophan consumption drastically increases, which might indicate a regulatory function of EWS-FLI1 in maintaining the balance of the cellular NAD^+ metabolism. In addition, we observed a striking sensitivity of ES cells to interferon gamma treatment at very low doses (5U/ml) resulting in the induction of the tryptophan metabolizing enzyme IDO and the production of the immune-suppressive metabolite kynurenine. We are currently studying in how far these changes in tryptophan metabolism affect cellular NAD^+ pools and SIRT1 and PARP1 activities in ES. This study serves to better understand the role of EWS-FLI1 and of the microenvironment in the post-translational control of SIRT1 and PARP1 regulated gene transcription and the consequences for PARP1 and/or SIRT1 directed therapies of ES.

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Tetrahydrobiopterin saves pancreatic isografts from brain death exacerbated ischemia reperfusion injury

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Brain death (BD) has been shown to immunologically prime grafts in part by aggravating ischemia reperfusion injury (IRI). Herein we assessed the effects of BD on IRI in an experimental setting furthermore the therapeutic potential of tetrahydrobiopterin (BH4), an essential NOS-cofactor was tested. Pancreas transplantation was performed using C57BL/6-mice. Animals underwent BD induction and were followed for 3h. Experimental groups included: non-treated BD-donors, BD-donors treated with 50mg/kg BH4, ventilated non-treated donors and living donors. Following 2 hours of reperfusion, microcirculation (functional capillary density, FCD; capillary diameter, CD) and cell viability was assessed by intravital fluorescence microscopy. Parenchymal graft damage was assessed by histology, NOS were quantified by immunohistochemistry against nitrotyrosine and mRNA expression of inflammatory candidate markers was measured by real-time RT-PCR.

Compared with controls, BD exacerbated IRI reflected by significantly reduced FCD and CD values ($p < 0.05$). Moreover BD induced IL-1ß, TNF-α, IL-6 and ICAM-1 mRNA expression. In contrast treated grafts displayed significantly higher FCD and CD values ($p < 0.05$). BD had devastating impact on cell viability whereas treatment resulted in significantly higher numbers of viable cells after reperfusion ($p < 0.01$). Parenchymal damage in grafts from BD-donors was significantly more pronounced when compared to controls ($p < 0.05$). Treatment resulted in significantly better histology. Nitrotyrosine immunostaining showed significantly higher score values in grafts from BD donors when compared to BH4 treated pancreata ($p < 0.05$).

In conclusion, our data gain new insights into the impact of BD on pancreatic grafts. Donor pre-treatment with BH4 offers a novel option for preventing BD exacerbated IRI.

TAU Alzheimer's vaccine in clinical trial – first in man, first in class

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Alzheimer's disease is still being recognized today as an unmet medical need. There is no disease modifying therapy for Alzheimer's disease available [1]. Currently, most prevalent is symptomatic therapy, which is not able to stop progression of the disease. Therefore much attention is now being directed at the development of approaches that counteract the fundamental pathological processes of the disease. Tau immunotherapy represents the prospective approach in the treatment of AD sufferers. Towards this end we have developed active tau peptide vaccine addressing key region on tau protein responsible and essential for pathological tau-tau interaction. We have discovered the novel drug target for Alzheimer's disease – Alzheimer tau [2]. The pathogenicity of this molecule was validated in the transgenic rat model that fully recapitulated the tau neurodegenerative cascade. In the next step we have identified the most vulnerable area on tau protein which

is responsible for pathological tau-tau interaction. To characterize the novel tau immunotherapy approach, we have treated transgenic rats with active vaccine. We found that vaccination significantly improved neurobehavioral deficit of the animals, reduced neurofibrillary degeneration and reduced mortality of transgenic animals. Toxicology and safety pharmacology studies were done on mice, rats, rabbits and dogs. These studies established no adverse effect level testing the doses planned for human clinical trials. The first phase of human clinical trials started in July 2013, featuring a three month double blind design followed by a three month open labelled study with administration of up to six doses of AADvac1. As of today patients have received up to six doses of AADvac1 with no adverse effects.

[1] Levarska L, et al. J Alzheimer Dis 2013;37:569-77.
[2] Zilka N, et al. Biochem Soc Trans 2012;40:681-6.

Quantitative analysis of tryptophan metabolites in human plasma by liquid chromatography-high resolution mass spectrometry

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The kynurenine (KYN) pathway is the most important pathway in tryptophan (TRP) catabolism which accounts for more than 90% of TRP breakdown in mammals. Important TRP metabolites include KYN, kynurenic acid (KYNA) and quinolinic acid (QUIN), and they are known to be active in the nervous and/or immune systems. For studying the physiological roles of TRP and its metabolites, the availability of reliable, sensitive and comprehensive quantitative methods is of utmost importance. Herein, we introduce liquid chromatography (LC) coupled to high resolution mass spectrometry (HRMS) as a new technique for measuring TRP, KYN, KYNA, and QUIN in human plasma.

50 µL of plasma were prepared for LC-HRMS analysis by protein precipitation with acetonitrile. Deuterated analogues of TRP, KYNA, and QUIN served as internal standards. TRP metabolite-free plasma obtained from treatment with activated charcoal was used as surrogate matrix for preparing calibration standards and quality control samples. Analytes were separated on a reversed-phase column (Synergi Hydro-RP, 150×1.00 mm, 4 µm, 80 A, Phenomenex, Torrance, CA, USA) using a gradient of acetonitrile in aqueous ammonium formate (5 mM, pH 5), and detected on a quadrupole-quadrupole- time-of-flight instrument (TripleTOF5600+, ABSciex, Foster City, CA, USA) using electrospray ionization (ESI) in negative ion mode. The applied acquisition strategy involved the collection of full scan MS/MS spectra and post-acquisition extraction of fragment ion mass traces very specific for the analytes of interest. The developed method was validated. The parameters tested for each compound included calibration model, limit of quantification, accuracy, reproducibility, sample stability, recovery, and matrix effects. With the exception of matrix effects, the assay was well within tolerances prescribed by regulatory guidance for validation of quantitative LC/MS assays.

The homologous lipocalins dog Can f 1 and human Lcn-1 direct dendritic cells to induce divergent immune responses corresponding to their allergenicity

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The role of the indoleamine (2,3)-dioxygenase (IDO) in regulating immune responses was a matter of controversy but it is relatively clear now that the induction of the tryptophan degradation pathway reduces allergic inflammation. Enhanced systemic IDO activity may contribute to the containment of allergic Th2 responses. Why and when the immune system skews to Th2 mediated allergic immune responses is still poorly characterized. With two homologous recombinant proteins of the lipocalin family, the major respiratory dog allergen Can f 1 and the human endogenous, not allergenic Lipocalin-1, we investigated their impact on human monocyte derived dendritic cells. Effects involved in directing the type of immune responses including antigen uptake, maturation induction, tryptophan breakdown and cytokine production by human monocyte derived dendritic cells were measured. The type of the induced immune response was characterized in allogenic dendritic cell-T cell co-cultures by key cytokine secretion (IFN-γ for Th1, IL-13 for Th2) of T cells. The two homologous lipocalins had differential effects on dendritic cells according to their allergenic potential. The dog allergen Can f 1 persistently induced less of the Th1 skewing maturation marker expression, tryptophan breakdown and IL-12 production in human monocyte derived dendritic cells when compared to the endogenous non-allergenic Lipocalin-1. As a consequence, T cells stimulated by dendritic cells treated with Can f 1 produced more of the Th2 signature cytokine IL-13 and less of the Th1 signature cytokine IFN-γ than T cells stimulated by Lipocalin-1 treated dendritic cells. These data show that the crosstalk of dendritic cells with lipocalins alone has the potential to direct the type of the immune response induced. With this study we contribute to a better understanding of the induction phase of a Th2 immune response.

Latent neurotropic pathogens, low grade inflammation, and personality traits implicated in suicidal self-directed violence

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Aggression and impulsivity are known suicide endophenotypes. Recent data suggest that individuals with low dopamine transmission are more vulnerable to reactive/impulsive aggression in response to provocation, while older data in animals suggested the opposite.

Inflammation, recently found associated with suicidal behavior can impair the function of the enzyme Phenylalanine hydroxylase (PAH), which catalyzes the conversion of phenylalanine(Phe) to tyrosine (Tyr), the precursor of dopamine, leading to elevated Phe and reduced Tyr (thus dopamine) levels. Subtle alterations in personality and human behavior, including increased risk for suicidal behavior, have been associated with latent infection with *Toxoplasma gondii* (*T. gondii),* a widespread neurotropic pathogen that establishes latency under immune pressure. *T. gondii* also possesses adequate enzymatic equipment to synthetize dopamine. We thus investigated associations between *T. gondii* seropositivity, Phe/Tyr ratio and aggressive personality traits.

One thousand healthy adults free of Axis and I and Axis II disorders by SCID-DSM-IV were recruited as part of a case-control study of schizophrenia and tested on personality dimensions using the aggression (Questionnaire for Measuring Factors of Aggression [FAF]) and trait impulsivity (Sensation-Seeking Scale-V [SSS-V]). Plasma samples were tested for IgG antibodies to *T.gondii* by ELISA and non-fasting plasma phenylalanine and tyrosine were measured with HPLC. Global multivariate tests were used as initial tests of association between the Phe/Tyr and ratio and FAF-aggression scores (FAF). Adjusted linear regression models, accounting for BMI, age, education level and sex were then used to determine if the Phe/Tyr were related to specific subscales of aggression. All models were stratified by *T. gondii* and sex due to previous evidence of sex-dependent shifts in personality traits associated with *T.gondii* infection.

T.gondii-positivity was significantly associated with higher FAF-Reactive Aggression scores among women (p <0.05) and higher FAF-Self-Aggression scores among women of post-menopausal age (p <0.05) while *Toxoplasma*-positive men exhibited significantly lowered aggression and self-aggression. *Toxoplasma*-positivity was associated with higher impulsive sensation-seeking among men below the sample median age of 60 (p <0.05). Higher Phe/Tyr ratios was associated with increased aggression scores among *Toxo*-positive males (p = 0.024) but not among *Toxo*-negative males (p = 0.964). A similar pattern was found for FAF Self-Aggression among *Toxo*-positive (p = 0.035) but not *Toxo*-negative males (p = 0.578). Among FAF-Aggression subscales, effects of heightened Phe/Tyr among Toxo-positive males were strongest for Spontaneous Aggression (p = 0.007) and Reactive Aggression (p = 0.034) but not significantly elevated for Irritability (p = 0.711). Elevated Phe/Tyr ratios were not associated with FAF-Aggression scores among women regardless of Toxoplasma status.

Latent toxoplasmosis was associated with higher reactive aggression in women, higher trait self-aggression in older women, and heightened impulsivity in younger males. Additionally, in *T. gondii* positive males, higher Phe/ Tyr ratio was associated with increased aggression, suggesting that possible decreased dopamine production secondary to Tyr depletion as a consequence of immune activation, or elevated neurotoxic effects via Phe may moderate, or mediate effects of *T. gondii* infection on personality traits in males. These findings may lead to novel treatment targets for reducing vulnerabilities and triggering of aggression and suicidal behavior.

QT-AIM analysis of neutral, cationic and anionic pterin

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Quantum theory of atoms in molecules (QT-AIM) has become an important tool for the analysis of molecular wave functions obtained by quantum chemical calculations. According to QTAIM, molecular structure is revealed by the stationary points of the electron density together with the gradient paths of the electron density that originate and terminate at these "critical" points [1]. The theory, while being rigorously grounded in quantum mechanics, relates basic concepts of chemistry (chemical structure, chemical bonding, transferability of functional groups, and chemical reactivity) to the topology of the underlying electron density distribution of a molecule which itself may be obtained theoretically by quantum chemical calculations or experimentally by X-ray diffraction studies.

Pteridines are an important class of heterocyclic molecules which play important roles in many biological systems. Quantum chemical treatment of various pteridine systems has been published since many years; however, most papers on the subject have appeared at times when computer power was quite limited in comparison with today and hence, quantum chemical methods of limited accuracy were used. Using more timely density functional techniques, Soniat and Martin [2,3] recently studied the relative energies of the numerous possible tautomeric forms of neutral and anionic 6-methyl pterin (which they erroneously designated as "pterin").

The present paper reports first results of a QT-AIM analysis of neutral pterin as well as its anionic and cationic forms. Starting with structures resembling the minimum energy tautomeric forms found by Soniat and Martin for 6-methyl pterin, first a geometry optimization using density functional theory (B3LYP/6-31G(d)) is performed, followed by a vibrational analysis in order to ensure that a true minimum structure was found. Using this optimized structure, a more accurate single-point computation at the B3LYP/6-311+G(2d,p) level is done yielding the molecular wave function together with an QT-AIM analysis of the atomic partial charges and bond orders of the molecules. In addition, the electron density and the electrostatic potential (ESP) functions are obtained in cubes surrounding the molecules and employing a 192x192x192 grid. These calculations are performed for gas phase as well as for solution, using the SMD model [4]. All quantum chemical calculations are done using G09W software (Gaussian Inc., Pittsburgh, PA, USA). Molecular graphs and graphical representation of the gradient fields as well as of the nuclear basins of attraction are obtained by AIM2000 software version 2.0 (Innovative Software, F. Biegler-König, J. Schönbohm, Bielefeld, Germany) and by the free software ParaView 4.0.1 32 Bit (www.paraview.org) together with the free program DGRID (invaluable help by Drs. M Kohout and A Baranov from the Max Planck Institute for Chemical Physics of Solids, Dresden, Germany, is gratefully acknowledged). Visualization of electron density function, ESP and the Laplacian of electron density function is done with AVS Express 5.3 software (Advanced Visual Systems Inc., Waltham, MA, USA).

The results demonstrate that quantum chemical techniques in combination with QT-AIM procedures are well-suited to describe the bonding structure as well as the major atomic and bonding features of neutral pterin and its cationic and anionic forms. Specifically, adding a proton to N1 of neutral pterin results in weakening of the aromatic character of the pyrimidine moiety in comparison to neutral pterin, while at the same time the double bond characters between C2 and the amino group and between C4 and the oxygen atom are slightly strengthened. On the contrary, abstracting a proton from N3 of neutral pterin leads to stronger aromaticity of the pyrimidine moiety, while the double bond characters between C2 and the amino group and between C4 and the oxygen atom are markedly weakened.

Effects of these molecular changes on the pyrazine moiety are comparably small.

In agreement with the literature [5] the data demonstrate that the chosen level of theory (B3LYP/6-311+G(2d,p)//6-31G(d)) yields chemically reasonable results while being computationally not too expensive. Thus, the results provide a sound basis for further theoretical chemical treatment of pteridines.

[1] Bader RWF. Atoms in Molecules: A Quantum Theory. Oxford University Press: Oxford, U.K., 1990.
[2] Soniat M, Martin CB. Pteridines 2008;19:120-4.
[3] Soniat M, Martin CB. Pteridines 2009;20:124-9.
[4] Marenich AV, et al. J Phys Chem B 2009;113:78-96.
[5] Foresman JB, Aeleen Frisch. Exploring Chemistry with Electronic Structure Methods. 2nd Ed. Gaussian Inc.,Pittsburgh, PA, USA, 1996.

Serum phenylalanine and tyrosine concentrations in euthymic bipolar disorder

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Catecholamine dysfunction is amply documented in individuals with bipolar disorder (BD). Catecholamines are synthesized from essential amino acids phenylalanine (PHE) and tyrosine (TYR). Peripheral PHE and TYR and consequently the PHE/TYR ratio present an opportunity to proxy central monoaminergic regulation. Interplay between catecholamine regulation and metabolic function is instantiated as insulin resistance associates with high peripheral concentrations of PHE and TYR. Peripheral metabolic abnormalities in BD are associated with a more complex BD presentation. This provides the impetus for exploring whether euthymic adults with BD (n=104) and comorbid metabolic disorders evince a different peripheral concentration of PHE, TYR, and/or PHE/TYR. All 104 euthymic BD patients and 56 controls took part in the BIPFAT study (at the MedUniGraz, Psychiatry) that aims to characterize disparate biological markers that subserve the association between BD and metabolic morbidity. Measures include: psychiatric history (SCID I- interview), anthropometric measure, fasting blood, cognitive testing, magnet resonance tomography, EEG, stool sample and different questionnaires. A positive correlation between the number of episodes and PHE/TYR ($r = 0.278$, $p = 0.009$) was found. Depressive episodes correlated positively with PHE/TYR ($r = 0.327$, $p = 0.002$). The total number of episodes was significantly greater in individuals with BD and increased PHE/TYR ratio compared to the sample with normal PHE/TYR (mean 48 *vs*. 21 episodes; $p = 0.007$, $F = 7.67$). PHE and TYR were significantly higher in overweight females, but not in males. In females PHE, TYR and PHE/TYR were highly associated with insulin resistance (HOMA-IR). The results herein empirically support the hypothesis that metabolic comorbidity in BD is associated with proxy measures of central monoaminergic dysregulation implicated in affect regulation and cognitive function. Increases in PHE/TYR with reduced conversion to TYR might result from inflammation and immune activation. Moreover, elevations of PHE and TYR were most apparent in women and correlated with insulin resistance. This should be considered when adjudicating diabetes risk. Disturbed insulin metabolism had been associated with neurocognitive dysfunction, pathological HPA-axis activation and immune dysregulation which might play a role in cognitive deficits in BD.

The impact of everyday life barometric pressure on urinary neopterin concentrations in a woman with systemic lupus erythematosus

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This study on a 52-year old female SLE patient in remission investigated the dynamic impact of atmospheric pressure changes on cortisol and neopterin concentrations using a study approach specifically designed for the investigation of stress system activity under conditions of "life as it is lived". Her entire urine was collected by the patient in 12-h intervals over a period of 56 days. For generating the atmospheric pressure time series, consecutive 10 min. measurements of air pressure in hectopascal (hPa) provided by the Zentralanstalt für Meteorologie und Geodynamik (ZAMG) Innsbruck were averaged over 12-h intervals. Cross-correlational analyses after ARIMA modeling of urinary neopterin per creatinine [AR(2)] and urinary cortisol per creatinine [AR(2)] time series revealed a significant and delayed influence of air pressure on the patient's immune system and HPA axis. Specifically, increases in air pressure were followed by decreases in urinary neopterin concentrations after 24 h (lag2: -0.240; $p < 0.05$) and were preceded by increases in urinary neopterin levels by 36-108 h. Urinary cortisol concentrations, on the other hand, increased 36-48 and 48-60 h after increases in atmospheric pressure (lag3: +0.247; $p < 0.05$, lag4: +0.200; $p < 0.05$). As positive daily incidents in this patient were otherwise shown to be also associated with ultimate increases in urinary cortisol levels and ultimate decreases in urinary neopterin levels [1], it can be suggested that atmospheric pressure had a beneficial effect on the patient's stress system. Further studies have to be undertaken for generalization of results.

[1] Schubert C, et al. Stress Health 2006;22:215-27.

Interaction of *Carthamus tinctorius* lignin arctigenin with the binding site of tryptophan-degrading enzyme indoleamine 2,3-dioxygenase

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The herbaceous *Asteraceae* plant *Carthamus tinctorius* (Safflower) has been used in traditional Chinese medicine to promote circulation and menstruation and to treat neuropsychological disorders such as major depression. In the Mediterranean area, *C. tinctorius* extracts play a role in the treatment of cancer and are known for antihelmintic, antiseptic, diuretic and febrifugal properties. Most of the clinical conditions mentioned are associated with immune activation and inflammation which is characterized by specific biochemical alterations. Recently *C. tinctorius* lignans arctigenin and trachelogenin but not matairesinol were described to interfere with the activity of tryptophan-degrading enzyme indoleamine 2,3-dioxygenase (IDO) in peripheral blood mononuclear cells (PBMC) *in vitro* [1]. Interestingly, the three structurally closely related isolates exert differing activity profiles on IDO; arctigenin showed the highest activity (IC50 26.5 μM) followed by trachelogenin (IC50 57.4 μM), while matairesinol showed only a weak inhibitory activity on IDO activity in PBMC. We examined a potential direct influence of compounds on IDO enzyme activity applying computational calculations based on 3D geometry of the compounds [2]. The interaction pattern analysis and force field-based minimization was performed within LigandScout 3.03, the docking simulation with MOE 2011.10 using the X-ray crystal structure of IDO. The three compounds were docked into a published X-ray crystal structure of human IDO [3]. The docking results were refined by energetically minimizing the binding poses within the enzyme's active site using the MMFF94 force field. Molecular modeling calculation results showed that arctigenin and to a lesser extent also trachelogenin strongly interact with the IDO binding site, whereas matairesinol was predicted to be less stabilized within the binding pocket [2]. Especially a hydrogen bond between the ligand and Ser235 was predicted as crucial for activity. Results confirmed the possibility of an intense interaction of arctigenin and trachelogenin with the binding site of the enzyme, while matairesinol had no such effect. Any effect of arctigenin and possibly also of trachelogenin to directly inhibit IDO activity would have several therapeutic consequences for antidepressant and immunomodulatory treatment strategies. It might explain treatment effects observed with *C. tinctorius* extracts in traditional medicine. However, further studies are needed to clarify this point and the possible application of arctigenin as an IDO inhibitor with additional immunomodulatory properties needs to be further tested in appropriate animal model systems. Results confirm the possibility of an intense interaction of arctigenin and trachelogenin with the binding site of the enzyme. The inhibitory effect of compounds on IDO cannot imply a similar influence on tryptophan 2,3-dioxygenase (TDO), because structural homology between TDO and IDO is rather limited. While both the enzymes act via an activated heme, the binding pocket anatomy is very different. We assume it is unlikely that compounds like arctigenin or trachelogenin would also bind to TDO.

[1] Kuehnl et al. Phytomedicine 2013;20:1190-5.
[2] Temml et al. FEBS Open Bio 2013;3:450-2.
[3] Sugimoto et al. Proc Natl Acad Sci U S A 2006;103:2611-6.
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Development and validation of an online-SPE LC-MS/MS method for the quantification of iohexol: A milestone towards the clinical use of the iohexol clearance method for the determination of the glomerular filtration rate

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It is a well established fact that the assessment of the renal glomerular clearance capacity by measuring endogenous filtration markers as creatinine and calculating an estimated glomerular filtration rate (eGFR) has its limitations –due to the complex metabolism of the markers used, the interplay between glomerular and tubular clearance, the mathematical models applied and limitations in the precision and accuracy of analytical measurements [1,2]. Assessing the plasma and ideally also the urine levels of "ideal exogenous filtration markers" (e.g. ^{57}Cr-EDTA, inulin, iothalamate, or iohexol; all of which are freely filtered in the glomerulum but inert concerning other renal cellular compartments) is considered gold standard for the measurement of the GFR (mGFR) and is generally acknowledged as reference in modeling the eGFR [3,4]. However, due to applicative and technological limitations mGFR is still hardly used in routine patient care.

Consequently, with LC-MS/MS (considered a gold standard analytical technology in plasma and urine small molecule quantification [5]) at hands, the establishment of an mGFR measurement platform was envisioned. Iohexol, a clinically well established contrast agent and an acknowledged "ideal exogenous filtration marker" was chosen as target analyte, the online-SPE-LC-MS/MS instrumentation of the ZIMCL [6] served as analytical platform. Utilizing an iohexol stable isotope labeled internal standard in the sample preparation, relying on gradient elution techniques in the LC domain, and measuring iohexol with two selected reaction monitoring (SRM) mass transitions as MS/MS readout, a sufficient sensitive (linear range 10 mg/l – 1000 mg/l), accurate (inter day bias less than ±5.8%), and precise (inter-day coefficient of variation less than 7.2%) quantitative iohexol assay was established. After undergoing a performance evaluation study in accordance with the Austrian MPG and the IVD directive (98/79/EG) of the European Community this assay can be used for mGFR measurements in clinical settings.

[1] Myers GL, et al. Clin Chem 2006;52:5-18.
[2] Coresh J and Auguste P. Scand J Clin Lab Invest Suppl 2008;241:30-8.
[3] Stevens LA, et al. J Am Soc Nephrol 2009;20:2305-13.
[4] Levey AS, et al. Ann Intern Med 1999;130:461-70.
[5] Vogeser M and Seger C. Clin Biochem 2008;41:649-62.
[6]Seger C, et al. Nat Protoc 2009;4:526-34.

Bioactivites of two common polyphenolic compounds: verbascoside and catechin

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In recent years, the researches on biologically active plant isolates have attracted considerable attention. The aim of this study was to evaluate and to compare the cytotoxic effects of two polyphenolic compounds, verbascoside and catechin on human peripheral blood mononuclear cells (PBMC) and the myelomonocytic cell line THP-1 *in vitro*. In addition, the effects of the compounds on immune activation markers such as indoleamine 2,3-dioxygenase (IDO), neopterin and nuclear factor-κB (NF-κB) expression were investigated. Cytotoxicity of the compounds was tested on THP-1 cell line and PBMC using the Cell-Titer Blue assay. PBMC supernatants were collected and concentrations of tryptophan (Trp) and kynurenine (Kyn) were analyzed by HPLC and neopterin by ELISA (BRAHMS, Hennigsdorf, Germany). The effects of the compounds on the expression of the signal transduction element NF-κB were determined on human THP-1 Blue cells by Quanti-Blue assay. According to our results, verbascoside exhibited significant suppressive effects on the cell viability of mitogen stimulated PBMC as well as on IDO activity and the production of neopterin, but these immune-suppressive effects was not compatible with the effects on NF-κB activity. On the other hand, the other polyphenol, catechin had no important effects on the tested inflammation markers except NF-κB expression. Certain concentrations of catechin suppressed NF-κB activity stimulated with lipopolysaccharide (LPS). The *in vitro* results indicate that sub-cytotoxic concentrations of verbascoside may prove to be potent anti-inflammatory response.

Inflammation-induced disturbances of neurotransmitter precursor amino acids is relevant only in a subgroup of patients with depression

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A possible connection between chronic inflammation and the development of depression has received increasing attention during the last 10 years. Elevated biomarkers of inflammation, including pro-inflammatory cytokines and neopterin, have been found in depressed patients, and administration of inflammatory stimuli has been associated with the development of depressive symptoms [1-3]. Furthermore data have demonstrated that inflammatory cytokines can interact with pathways known to be involved in the development of depression, such as the monoamine metabolism [4]. On the other hand the question arises if this possible pathomechanism is relevant in all patients with major depression (MDD) or only in a subgroup. In order to investigate possible influencing factors we studied the tryptophan-kynurenine and the phenylalanine-tyrosine pathways in physically healthy patients with MDD, in breast cancer patients with and without MDD and in healthy individuals.

Neopterin, tryptophan and phenylalanine metabolites have been analysed in 46 MDD patients, 84 breast cancer patients of whom 35 women were also suffering from MDD and 43 healthy matched controls.

An explorative analysis between the different groups showed highest tryptophan degradation rates in patients with cancer and depression; in this group the same was found for the phenylalanine-tyrosine metabolism. To explore the influences of physical status (breast cancer) and mental status (depression) on the two pathways in greater detail a two-way-analysis of (co) variance (ANCOVA) was used. The model investigated the main effects of mental status and physical status as well as their interaction effect on the metabolites. Although the mental status "depression" showed a significantly decreasing effect on tryptophan and kynurenine values ($p<0.001$, $p=0.002$), the Kyn/Trp ratio reflecting the degradation rate was not changed due to this influence. The physical status "breast cancer" showed a significantly increasing effect on kynurenine values ($p<0.001$) as well as on the Kyn/Trp ratio ($p <0.001$). For the tryptophan-kynurenine metabolism no statistically significant interaction between the mental status "depression" and the physical status "breast cancer" was found. Neopterin was found significantly increased ($p <0.009$) due to the effect of depression. Significant interactions of mental status and physical status were found for phenylalanine, tyrosine and the Phe/Tyr ratio ($p <0.001$) i.e. the impact of "depression" on this pathway metabolites was "breast cancer" specific and *vice versa*.

Data of this study show that the inflammatory response which is often observed in patients with cancer could affect two of the major neurotransmitter pathways and thereby influence the development of depression in these patients. On the other hand these results also suggest that not all types of depressive disorders are related to disturbances of these neurotransmitter precursor amino acids.

[1] Haroon E, et al. Neuropsychopharmacology 2012;37:137-62.
[2] Dantzer R, et al. Nat Rev Neursci 2008;9:46-56.
[3] Raison CL, et al. Trends Immunol 2006;27:24-31.
[4] Sperner-Unterweger B, et al. Prog Neuropsychopharmacol Biol Psychiatry 2014;48:268-76.

Effects of a caloric restriction weight loss diet on tryptophan metabolism and inflammatory biomarkers in overweight adults

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Recent data suggest that chronic low-grade inflammation, a characteristic of obesity, is associated with altered tryptophan and tyrosine metabolism and plays a role in neuropsychiatric symptoms [1]. The present study assessed the effect of an extreme short term diet on tryptophan breakdown and inflammatory biomarkers in overweight adults. Materials/Methods: Thirty-eight overweight participants (16 women, 22 men; average body mass index: 29 kg/m^2, mean age 52.8 years) were randomized into two diet groups: a very low kcal diet group (VLCD; ∅ 600 kcal/day, n=21), and a low kcal diet group

(LCD; ∅ 1200 kcal/day, n=17). Assays included the measurement of tryptophan, kynurenine, and their ratio, neopterin, phenylalanine, tyrosine, as biological markers; leptin, plasma insulin, glucose, and HOMA-IR; and interleukin-6, tumor necrosis factor-alpha, and C-reactive-protein, as biochemical and inflammatory markers at baseline and after 2 weeks of treatment. Results: Weight loss diet lowered leptin levels in both groups by 46%, although not reaching significance. Tryptophan and kynurenine decreased significantly by 21% and 16% for VLCD and by 15% and 17% for the LCD group, respectively. A significant reduction of phenylalanine was only seen after VLCD. Inflammatory biomarkers, neopterin and tyrosine were not significantly altered during the study period. Leptin was significantly correlated with tryptophan breakdown before and after the intervention ($p<0.02$). Conclusions: Since disturbed metabolism of tryptophan affects biosynthesis of serotonin, and might be associated with increased susceptibility for mood disturbances and carbohydrate craving, strategies to supplement tryptophan while dieting could be highly useful in treating uncontrolled weight gain or in preventing neuropsychiatric symptoms.

[1] Mangge H, et al. Curr Med Chem 2013;20:2330-37.

Prognostic significance of urinary neopterin in ovarian cancer-a study of the Austrian gynecology oncology group

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Most studies in patients with malignant diseases found an association between higher neopterin concentrations with reduced survival and impaired prognosis [1]. Thereby neopterin is not a classical tumor marker since it is not produced by cancer cells but derives from activated human macrophages and dendritic cells upon stimulation with Th1-type cytokine interferon-gamma (IFN-γ). Thus, elevated concentrations in patients indicate cellular immune response. In a study of the Austrian Gynecologic Oncology Group in 114 patients with cystadenomas and 17 patients with borderline ovarian tumors as well as 223 patients with invasive ovarian cancer urinary neopterin was determined before and after primary therapy. Elevated levels (cutoff 250 µmol/mol creatinine) were found less frequently in women with benign ovarian cystadenomas (24%) and borderline tumors (29%) compared to patients with malignant disease. There existed a significant stage dependency with 16%, 25%, 65% and 91% in FIGO stage I-IV, respectively. By univariate analysis residual tumor, FIGO stage, age, histological type, and neopterin was significantly associated with overall and progression-free survival (OS and PFS). Median overall survival (OS) was 81 versus 24 months for patients with normal and elevated neopterin, median progression-free survival (PFS) was 52 and 12 months respectively ($p<0.001$ for both). In a multivariate Cox regression analysis, residual tumor, neopterin and age were independently associated with OS, while only residual tumor was predictive for PFS. Thirty patients with surgically staged FIGO I or II invasive ovarian cancer were analyzed separately. Two of three patients with elevated levels died of disease in contrast to 2/27 deaths in women with normal neopterin ($p = 0.004$, Fisher's exact test). In conclusion, in ovarian cancer the negative impact of pretreatment urinary neopterin indicates a detrimental role of immune activation for the course of the disease. The chronic immune activation and neopterin production may relate to anti-proliferative strategies of the immune system that hamper the functional immune response and also are promote regulatory T-cells via expression of tryptophan-degrading enzyme indoleamine 2,3-dioxygenase (IDO) [2].

[1] Volgger B, et al. Cancer Lett 2008;262:183-9.
[2] Sucher R, et al. Cancer Lett 2010;287:13-22.

Alkylglycerol monooxygenase in murine macrophages: Regulation in differentiation and dependence on intracellular tetrahydrobiopterin

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Alkylglycerols are important players in lens organisation, spermatogenesis and also signalling. A prominent member of this class of lipids is the pleiotropic inflammatory mediator platelet activating factor (PAF). The only enzyme that can degrade lyso-alkylglycerols including lyso-PAF is alkylglycerol monooxygenase, an integral membrane protein the sequence of which we assigned in 2010 [1]. Alkylglycerol monooxygenase belongs to the family of tetrahydrobiopterin-dependent enzymes but so far the influence of cofactor concentration on alkylglycerol turnover in the living cells has not been shown.

We therefore manipulated tetrahydrobiopterin content in the murine macrophage cell line RAW264.7 by pharmacological inhibition and lentiviral short hairpin RNA knockdown of GCH1, the key enzyme in cofactor biosynthesis. By monitoring the fate of a pyrene-labelled alkylglycerol using a novel live cell assay [2] we show that alkylglycerol metabolism is severely affected in absence of sufficient amounts of tetrahydrobiopterin indicating that alkylglycerol monooxygenase may limit PAF signalling by degrading lyso-PAF and thereby preventing its recycling to the active signalling molecule.

We also investigated expression and enzymatic activity of alkylglycerol monooxygenase in murine bone-marrow derived macrophages. These primary cells display pronounced amounts of alkylglycerol monooxygenase mRNA and activity both of which were strongly regulated by cytokines: Alkylglycerol monooxygenase levels were reduced in inflammatory conditions where PAF must exert its function and high lyso-PAF levels are needed to ensure enough PAF supply. In contrast, alkylglycerol monooxygenase was elevated in anti-inflammation, where PAF function is possibly attenuated by cleavage of its precursor.

We therefore propose a central role of alkylglycerol monooxygenase in tuning PAF function.

[1] Watschinger K, et al. Proc Natl Acad Sci 2010;107:13672-7.
[2] Keller MA, et al. J Lipid Res 2012;53:1410-6

Biosynthesis of pteridines from GTP in different species: an enzymological update based on genome sequence analysis data

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Nature forms a rich variety of pteridine cofactors from guanosine 5′ triphopshate (GTP). These include molybdopterin, which is synthesized and required by most organisms, e.g. for xanthine and sulfite oxidase. Another class of widespread cofactors, the flavins such as FAD and FMN is derived from riboflavin. Riboflavin is not synthesized by animals, but by bacteria and plants from GTP. Upon cleavage of GTP by GTP cyclohydrolase 1, 7,8-dihydroneopterin triphosphate is formed which is a key intermediate for three important classes of biologically active compounds. In animals, 7,8-dihydroneopterin triphosphate is converted to tetrahydrobiopterin. Bacteria and plants from folic acid derivatives from 7,8-dihydroneopterin triphosphate. Finally, bacteria convert 7,8-dihydroneopterin triphosphate to a modified nucleotide, queuosine. Animals and humans have to take up this modified nucleotide from bacteria because it is required as an essential constituent of four distinct transfer RNAs.

Large scale genome sequencing of organisms has provided a rich source of data for the occurrence of these pathways among living organisms. A first surprise was that several bacterial species lack a reading frame with similarity to GTP cyclohydrolase 1, the first enzyme for folate biosynthesis bacteria. These bacteria do form folate, and comparative genomics revealed a cluster of orthologous groups of proteins (*COG*) which was found to encode a protein capable of converting GTP to dihydroneopterin triphosphate. Remarkably, this protein has no significant primary amino acid sequence homology with the previously known form of GTP cyclohydrolase 1, but shares many features of its three dimensional structure.

Automated annotation of genomes lead to some confusion in the labeling of dihydroneopterin-triphosphate metabolising enzymes. Theses enzymes share sequence homology due to the use of the common substrate, 7,8-dihydroneopterin triphosphate. Although only one of these enzymes, 6-pyruvoyl tetrahydropterin synthase (ptps) initates the formation of tetrahydrobiopterin, also the homologues are labeled ptps-like, although they initiate the formation of folate or queuosine, respectively. Even when these annotation problems are solved, it turns out that several non animal species might have the capacity to synthesize tetrahydrobiopterin. The rich genomic data can provide a guide for future research into the biochemistry and physiology of this cofactor in non-animal organisms.

Radiation dose, gut toxicity and systemic immune activation in rectal cancer patients treated with neoadjuvant chemoradiation

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Gastrointestinal toxicity is the principal side effect of neoadjuvant chemoradiation. This toxicity is still assessed mostly based on patient-reported symptoms, and biomarkers that would diagnose and predict the toxicity are urgently needed. Plasma concentrations of citrulline, a biomarker of gut damage, and both urinary and serum concentrations of neopterin, a biomarker of immune activation were measured in patients with rectal cancer undergoing neoadjuvant chemoradiation. These parameters were correlated with the calculated volume of irradiated small bowel. Final results of these analyses will be presented.