Workshop on Nanomaterial Safety: Biomarkers

St.Christoph/Arlberg, February 26th - 29th, 2012

NanoLINEN (NANOtoxicology Link between INdian and European Nations)

http://www.nanolinen.org

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Program

Sunday, February 26th, 2012
Chimney Hall, Hospiz Hotel

7:00 p.m. Get-together – Welcome Reception

Thereafter Dinner at "BRUDERSCHAFTSSTUBE" (Ground-floor)

Monday, February 27th, 2012
Seminar room, Sporthotel, 2nd Floor

10:15 – 12:30 Chair: Karahalil B (Ankara, Turkey), Fuchs D (Innsbruck, Austria)

10:15 Introduction - NewIndigo / nanoLINEN
Karahalil B (Ankara, Turkey)

Introduction - Biomarker Workshop
Fuchs D (Innsbruck, Austria)

10:30 Multidimensional immunoinflammatory monitoring in acute inflammation and septic states
Faist E (Munich, Germany)

11:00 Applications of immune system biomarkers in clinical studies
Fuchs D (Innsbruck, Austria)

11:30 Immune system biomarkers in cardiovascular disease - A clinical perspective
Kaski JC (London, United Kingdom)

12:00 Identification and application of biomarkers in kidney transplantation
Müller TF (Edmonton, Canada)

12:30 – 3:15 p.m. Break

3:15 – 5:15 Chair: Bonassi S (Rome, Italy), Fuith LC (Eisenstadt, Austria)

3:15 Interferon-γ-induced biochemical pathways - friend and foe in tumor immunology
Brandacher G (Baltimore, MD)

3:45 IL28B polymorphism, IP10 and other biomarkers in patients with hepatitis C virus infection
Zoller H (Innsbruck, Austria)

4:15 Pathophysiological networks and new therapeutic approaches in the anemia of chronic disease
Weiss G (Innsbruck, Austria)

4:45 Innate and adaptive immune system in Alzheimer's disease: therapeutic implications
Blasko I (Innsbruck, Austria)

5:15 – 5:40 Coffee Break

5:40 – 6:50 Chair: Debbage P (Innsbruck, Austria)

5:40 *In-vitro* models for the detection of long-term nanoparticle effects
Froehlich E (Graz, Austria)

6:10 Nano versus bulk toxicity: need for critical human biomarkers
Tripathi A, Roy R, Dwivedi PD, Das M (Lucknow, India)

6:30 Influence of TiO_2 nanoparticles *vs.* bulk material on human peripheral blood cells and on myelomonocytic cell line THP-1
Schröcksnadel S, Herlin N, Carriere M, Fuchs D (Innsbruck, Austria; Grenoble & Sarclay, France)

Tuesday, February 28th, 2012:
Seminar room, Sporthotel, 2nd Floor

10:15 – 12:25 Chair: Karakaya A (Ankara, Turkey), Herlin N (Sarclay, France)

10:15 Biomonitoring studies in exposed populations: An epidemiological perspective
Bonassi S (Rome, Italy)

10:45 Biomonitoring of occupational exposure to styrene
Teixeira JP (Porto, Portugal)

11:15 Evidence for short-term hepatotoxicity of gadolinium-bearing albumin-based nanoparticles in a rat model
Debbage P (Innsbruck, Austria)

11:45 Nanoparticle interaction with proteins: Does it co-relate with toxicity?
Dwivedi PD, Roy R, Tripathi A, Das M (Lucknow, India)

12:05 Different patterns of cytotoxicity in titanium dioxide nanoparticle treated cell lines
Engin AB, Karahalil B (Ankara, Turkey)

12:25 – 3:15 p.m. Break

3:15 – 5:15 Chair: Müller TF (Edmonton, Canada), Brandacher G (Baltimore, MD)

3:15 Microarray-based gene expression profiling: possibilities and limitations
Gostner J (Innsbruck, Austria)

3:45 Limitations of LC-tandem mass spectrometry in the clinical laboratory
Seger C (Innsbruck, Austria)

4:15 Neopterin and progress of malignant diseases - a glimpse of host-tumour interaction?
Reibnegger G (Graz, Austria)

4:45 Psychoneuroimmunology of HIV infection
Kurz K, Zangerle R, Weiss G, Fuchs D (Innsbruck, Austria)

5:15 – 5:40 Coffee Break

5:40 – 6:50 Chair: Fröhlich E (Graz, Austria)

5:40 Changes in neopterin levels and tryptophan degradation with silica nanoparticles
Palabiyik SS, Girgin G, Tutkun E, Yilmaz H, Baydar T (Ankara, Turkey)

6:00 Effect of occupational zinc nanoaerosol exposure on neopterin levels and tryptophan degradation
Girgin G, Saraç ES, Baydar T, Aydın A, Şahin G (Ankara, Turkey)

6:20 Evaluation of genotoxicity in infertile men
Coskun E (Ankara, Turkey)

6:40 Closing remarks
Bonassi S (Rome, Italy)

Wednesday, February 29th, 2012
Seminar room, Sporthotel

9:30 nanoLINEN – Business Meeting
Karahalil B (Ankara, Turkey)

ABSTRACTS

Chronic activation of the innate immune system in Alzheimer‘s disease: therapeutic implications

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Signs of an inflammatory process, in particular the activation of brain microglia cells and increased levels of pro-inflammatory cytokines in brain tissue, have been described repeatedly in patients with Alzheimer's disease (AD). 90% of all Alzheimer cases are attributed to sporadic or late onset AD (LOAD). The cause of LOAD remains unclear; although the aggregation of amyloid-beta (Aβ) peptides is regarded as an initial step.
Advanced age represents a major risk factor for the development of LOAD. Aging of the immune system is accompanied by a pro-inflammatory driven reaction. The accumulation of harmful metabolic products, such as Aβ-peptides, accompanied by the decreasing ability of the human body to respond to such aggressors, can potentiate further brain degeneration. This self-reinforcing cycle is assumed to be highly active and pronounced in moderate to severe stages of AD, as cognitive parameters correlate better with inflammatory proteins in more advanced AD-stages compared to less impaired patients.
The significance of anti-inflammatory based approaches in future dementia treatment is supported by preliminary clinical studies with Aβ immunotherapy. Results showed that a decrease in Aβ plaques was accompanied by an increase in microglial activation. The activated microglia is necessary for the degradation of Aβ plaques and restoration of neuronal function; however, its long-term activation may leads to an over-production of inflammatory molecules which further damages the brain tissue.
The potential of anti-inflammatory substances in the treatment of AD has been tested over the past decade. Along with negative results to date, studies with a sub-class of non-steroidal anti-inflammatory drugs (NSAIDs) suggest that treatment effects differ at various stages of the disease.
Given the complexity of AD, it has become increasingly clear that multiple drugs targeting different sites of the pathogenic cascade will be necessary. Since the brain's capacity to repair itself is limited, the use of therapies based on nanoparticles to deliver anti-inflammatory molecules to the brain requires a thorough selection of distribution pathways, as well as the selection of safe carriers.

Biomonitoring studies in exposed populations: An epidemiological perspective
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Exposure assessment is a critical issue in human population studies. Quantitative estimation of health risks mostly depends on exposure characterization and the nature of the dose response relationships. The collection of sparse or inadequate exposure data, the limited understanding of exposure mechanisms, and the insufficient understanding of the exposure-dose-response pathway generate the greatest uncertainties in risk assessment, especially when mixed or multiple exposures are implicated. Particular conditions of uncertainty occur when pooled analyses or retrospective assessment is required. Exposure data collection and characterization in large collaborative studies such as HUMN and ComNet will be discussed. The use of high-throughput techniques for exposure assessment may offer a higher sensitivity approach although a number of possible drawbacks mat reduce the advantage of the novel techniques. Several biomarkers may be used for assessing exposure or its early biological effect. In general biomarkers of genotoxicity are more commonly used, although other possibilities such as immunotoxicity are used in particular

conditions. The use of these biomarkers will be discussed and study findings from a survey of workers exposed to asbestos or man-made mineral fibers will be compared.

Interferon-γ-induced biochemical pathways – friend or foe in tumor immunology?

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Insufficient immunosurveillance is an important aspect in early tumorigenesis and in the pathogenesis of malignant disease. In the later course of cancer, the development of immunodeficiency is considered the major reason for disease progression and death. Within the anti-tumoral host defence reaction, Th1-type cytokine interferon-γ (IFN-γ) is of particular relevance. IFN-γ stimulates several anti-proliferative and thus tumoricidal biochemical pathways in macrophages and other cells and also in tumor cell lines. These include inducible nitric oxide synthase, indoleamine (2, 3)- dioxygenase, an enzyme degrading the essential amino acid tryptophan, and the production of reactive oxygen species and neopterin in human macrophages and dendritic cells. Although the anti-proliferative strategy of the immune system aims to inhibit the growth of malignant cells, it can also affect T-cell response and thus contribute to the development of immunodeficiency. Accelerated degradation of tryptophan and increased production of neopterin were found to parallel the course of malignant diseases. Moreover, a higher degree of these metabolic changes characterizes poor prognosis and is associated with the development of anaemia, weight loss and depressive mood in patients. Available data suggest that immunodeficiency in cancer patients may develop as a long-term side-effect of the antiproliferative and pro-apoptotic mechanisms elicited within Th1-type immune response, and enhanced production of pro-inflammatory cytokine IFN-γ seems to be critically involved. In addition, the interaction between immune system and tumor-tissue can thereby lead to the development of specific, and powerful escape mechanisms of certain tumor cells. The pathological interactions between tumor- and host immune cells within the tumor microenvironment create an immunosuppressive network that activates IFN-γ-induced biochemical pathways that subsequently promotes tumor growth and protect the tumor from immune attack.

In this presentation the pivotal role of IFN-γ-induced biochemical pathways in tumor immunology will be discussed. It may also open some relevant discussion about how the ability of nanoparticles to elicit oxidative stress may interfere with immunosurveillance.

Evaluation of genotoxicity in infertile men

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Today, the relationship between male infertility and genotoxic damage is an important topic to be studied on. It is well known that chemicals causes genotoxic damage in human genome of which sperm chromatin is also a part of that. The genotoxic damage on sperm causes defective sperm function which leads to male infertility. Among all the molecular fertility biomarkers, the evaluation of sperm DNA damage is one of the most promising. Therefore, it is important to assess the level of genotoxic damage of the sperm chromatin with reliable biomarkers. Not only the DNA damage on germ cells, but also the accumulated DNA damage in somatic cells is also thought to effect on infertility. By applying the conventional biomarkers of genetic damage on somatic cells, early effects may be highlighted; thus genetic biomonitoring allows detecting adverse effects of mutagens. However, such an approach has not been widely used for developing strategies in risk assessment and disease prevention and no direct information can be drawn on germ cells. Therefore, it seems to be important to extrapolate the findings gathered in somatic cells to germ cells.

For this purpose, we have evaluated the sperm chromatin integrity using one of the most commonly used cytogenetic biomarker, Comet Assay, on sperm samples of 82 infertile men and 63 healthy controls. As well as assessing the DNA integrity on sperm cells, we have evaluated the genetic damage on somatic cells, lymphocytes, from the same subjects to compare the possible relation of somatic-genetic interaction. All those results are compared with morphological and functional sperm parameters, which can be thought as the phenotype of sperm dysfunction and gives us the opportunity to search for the genotype-phenotype relationship of male infertility.

Evidence for short-term hepatotoxicity of gadolinium-bearing albumin-based nanoparticles in a rat model

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Nanotoxicology routinely focuses on toxicity in cell cultures. Longer-term effects occurring in intact living animals take place in complex tissue environments with numerous different cell types and compartmentalized by blood-tissue barriers of varying degrees of exclusivity. We report here results obtained from albumin-based nanoparticles which exhibit no toxicity in cell culture testing, but which exert striking mid-term toxic effects in living animals. Albumin-based nanoparticles prepared as described earlier provided blood-pool imaging in MRI. Quantitative MR measurements and imaging were carried out at time-points from 1 hour to 6 weeks after particle injection. Parallel to the MR studies, atomic absorption spectrometry (AAS) was used to assess gadolinium in the rats' organs, and light microscopy of aldehyde-fixed, wax-embedded organs was carried out.

Intravenously applied nanoparticles remained visible in MR images for 3-4 hours, their blood concentration falling steadily until the MRI detection limit was reached. Simultaneously, gadolinium levels in the liver increased steadily, peaking between 6-24 hours. At 2-3 days after injection, quantitative MRI measurements of gadolinium in the organs diverged strongly from the AAS measurements, indicating major physical changes occurring within the liver tissues. During this interval, light microscopy showed heavy losses of hepatocytes. Several organs, including liver, spleen, and bone marrow, contain specialized vascular beds with tightly-controlled, relatively permeable blood-tissue barriers. In the liver, the blood-tissue barrier is reduced to zero for macromolecules and nanoparticles. Intravascular injection of our nanoparticles therefore allowed them direct access to the hepatocytes. At 2-3 days after nanoparticle injection, striking changes in liver magnetic resonance properties indicated major alterations in tissue structure, which we found to comprise heavy losses of hepatocytes. The occurrence of similar toxic effects should be considered as a possible consequence of applying nanoparticles "targeted" by enhanced permeability and retention ("EPR"). In principle, any organ containing the relatively permeable "sinusoidal capillary" type of microvessels could be affected.

Nanoparticle interaction with proteins: Does it co-relate with toxicity?

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The novel applications of nanoparticles have enhanced the commercial market growth exponentially. As a result global market is flooded with nanotechnology driven products, however, the potential hazards of nano-materials have only recently gained prominence. Since human body is constantly exposed to the environment, the primary routes of exposure being inhalation, oral or dermal making lungs, gastro-intestinal tract and skin as the direct interface for the interaction of nanoparticles. Due to their small size, nanoparticles can invade through the epithelial barrier, and finally enter into the blood and lymphatic circulations. Further, the bio-distribution of nanoparticles to various target organs would ultimately depend upon their interaction with the proteins present in serum and other biological interfaces. A

thorough characterization of proteins and other biological entities binding or adsorbing with nanomaterials is extremely relevant with respect to their uptake, bio-distribution and toxicity. We have observed that there was greater binding of ZnO nanoparticles with serum proteins vis a vis bulk ZnO. The results also showed differential protein binding profile for the nano and bulk particles. Interestingly, ZnO nanoparticles exhibited a dose dependent increase in the adsorption of IgG, which could influence its uptake and disposal from the circulation. The specific protein binding profile could be a function of the specific nanoparticle, depending on the charge, zeta potential, surface topography and electronic structure of the particle in question. A comparative analysis of protein binding profile and its co-relation with the particle toxicity would certainly help to identify key immunotoxicity markers, which could help in extrapolation to human scenario.

Different patterns of cytotoxicity in titanium dioxide nanoparticle treated cell lines

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Titanium is extensively used for a wide range of implanted medical devices due to its advantageous combination of physico-chemical and biological properties. Nano-size titanium dioxide (TiO_2) is also used in a variety of consumer products. Such widespread use and its potential entry through various routes of the body suggest that TiO_2 could pose an exposure risk to humans. Nano-size particles enter systemic circulation, accumulate and damage tissues that are especially sensitive to oxidative stress. Nanoparticle-induced oxidative stress and pro-inflammatory responses are well correlated not only with their surface area but also with the internalized amount of NPs. We hypothesized that TiO_2 nanoparticles can exert diverse cytotoxic effects on various human cell types especially neural cell lines. In order to test our hypothesis, putative cytotoxic effects of oxidative stress due to TiO_2 nanoparticles exposure on IM9, U937 and SHSY5Y (human neuroblastoma cells) were investigated with N-acetyl cysteine (NAC), neopterin and dexamethasone pre-treated cell cultures. IM9, U937 and SHSY5Y cells were exposed to different concentrations of 25 and 60 nm diameter TiO_2 nanoparticles in three different time periods. To determine toxicity levels of nanoparticles, cell viability was estimated by MTT test. Concentration of cells was assessed by counting trypan blue stained cells with a heamo-cytometer. Toxicity of 25nm TiO_2 particles was significantly increased by adding fetal bovine serum in SHYS5Y and U937 cell lines culture medium. Concentration-dependent toxicity of 60 nm TiO_2 particles was weakly increased by adding fetal bovine serum to SHYS5Y, IM9 and U937 cell culture media. NAC pre-treatment induced significant protection for SHYS5Y cell exposed to 25 nm and 60 nm of TiO_2 nanoparticles after a 24 h incubation period. Ten µM neopterin pre-treated SHYS5Y cells displayed significant increases in viability after 24 h exposure to 25 nm TiO_2 nanoparticles. Exposure of dexamethasone pre-treated U937 cells to both 25 nm and 60 nm of TiO_2 nanoparticles induced a slight increase in cell survival at 100 µg/ml particle concentration. Our study demonstrated that exposure of human neural cells (SHYS5Y) to TiO_2 nanoparticles for 24 hours regularly induced reduction of cell viability. We also found similar dose-related effects of TiO_2 nanoparticles in reducing cell survival in IM9 cells. This study clearly indicated that fetal bovine serum is an effective dispersing agent for TiO_2 nanoparticles and increased TiO_2 toxicity in all cell lines. NAC significantly protected the viability of SHYS5Y cell against the putative cytotoxic effects of TiO_2 nanoparticles. Ten µM neopterin, provided significant protection for 25nm TiO_2 nanoparticles exposed SHYS5Y cells.

Multidimensional immunoinflammatory monitoring in acute inflammation and septic states

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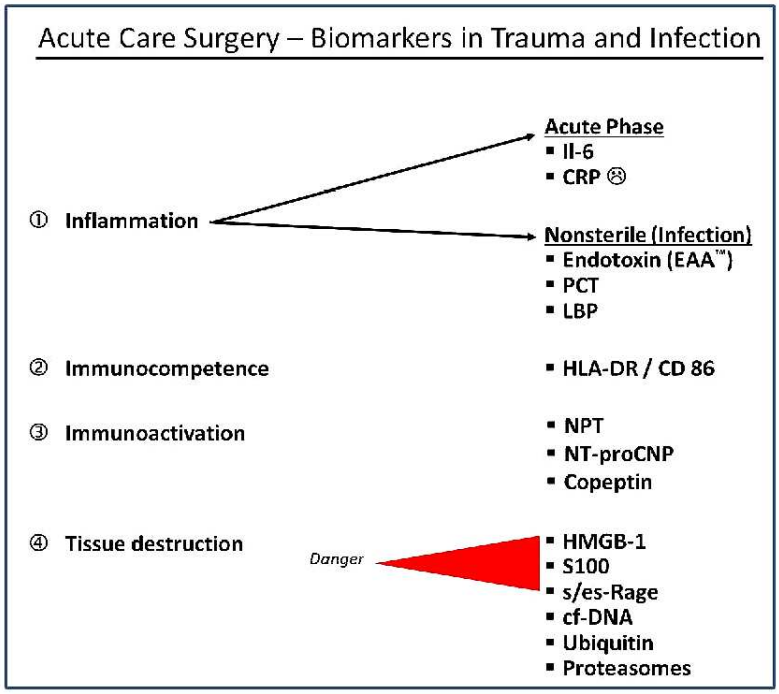
Damage- as well as pathogen-associated molecular patterns (DAMPs / Alarmins / PAMPs) are biological molecular structures that initiate the immunoinflammatory response after tissue injury. The quantity of

circulating alarm signals correlates directly with the extent of tissue destruction and thus, has a major impact on the clinical outcome of the traumatized individual.
Septic organ dysfunction represents the crucial consequence of trauma-induced immunoparalysis. Optimization of biomonitoring of immunosuppressed trauma and sepsis victims constitutes a precondition to achieve a better outcome of these patients. We have recently developed a useful multidimensional biomarker panel that comprises markers of sterile/non-sterile inflammation (1), immunocompetence (2), immunoactivation (3) as well as indicators of traumatic cell necrosis (4).
Immunoprofiling of tissue injury includes the Ubiquitin proteasome complex cf-DNA, S-100 and HMGB-1 – the prototypical alarmin marker. Circulating lipopolysaccharide (LPS; PAMPs family) was assessed with the novel Endotoxin Activity Assay (EAA™). Crucial results of this investigation included: Plasma HMGB-1 represents a most rapid marker of cellular destruction via passive release of these molecules.
NT-proCNP, indicating endothelial injury, appears to be an excellent indicator for the development of posttraumatic sepsis. Also, copeptin, part of the pro-hormon of vasopressin (ADH) has been found in our studies to be an excellent indicator of acute inflammation. Posttraumatic neopterin concentrations are reflecting the degree of immune activation as well as the incidence of organ failure. High endotoxin levels are detectible regularly following major burn trauma, indicating the major role of LPS for the high incidence of sepsis.



We conclude from our still ongoing work on immunomonitoring that the posttraumatic demonstration of cellular damage associated with uprising sterile vs. non-sterile inflammation seems to be mandatory for adequate grading of the degree of injury. Optimized characterization of DAMPs- and PAMPs-specific contribution to the overall degree of organ dysfunction should be able to strengthen the fine-tuning of supportive therapeutic care for the critically ill patient. The combination of the most informative and robust biomarkers within one complex panel should provide a powerful tool for better visualization of the clinical course and the prediction of outcome in critical surgical illness.

***In-vitro* models for the detection of long-term nanoparticle effects**

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Nanoparticles (NPs) are regarded as the leading technology of the 21st century and are increasingly used in the industrial, consumer and medical sectors. It has been shown that NPs can penetrate the external and internal barriers of the human body and enter cells and organs. Once taken up, NPs persist in the organism for several days or even months. This persistence may cause adverse health effects by interference with normal cell and organ function. To identify adverse cellular effects of NPs, we studied the consequences of accumulation in cellular organelles and long-term cytotoxicity upon repeated exposure. Negatively charged polystyrene particles in different sizes were used as model particles and applied only low, not acutely cytotoxic concentrations. Lysosomes, as the most likely organelles for accumulation of NPs, were studied using various assays for lysosomal integrity, pH and enzymatic function. For chronic cytotoxicity testing we used the benchtop bioreactor BioLevitator®, where cells are cultured on microspheres and a constant cell population can be repeatedly exposed to NPs.
Polystyrene particles ≤200 nm showed the maximal rate of co-localization with lysosomes within 4h, larger beads only after 24h. The accumulation in the lysosomes did not impair lysosomal integrity or increase intralysosomal pH (Fig. 1) but slightly reduced the activity of lysosomal enzymes. In contrast to the rather small effects on lysosomes after one single exposure, significant decreases in cell numbers were detected when cells were repeatedly exposed to polystyrene particles for up to 28 days. The data show that accumulation of polystyrene particles in lysosomes after one single exposure has only a small effect on lysosomal physiology. Repeated exposure over a longer time period, however, markedly decreased cell numbers.

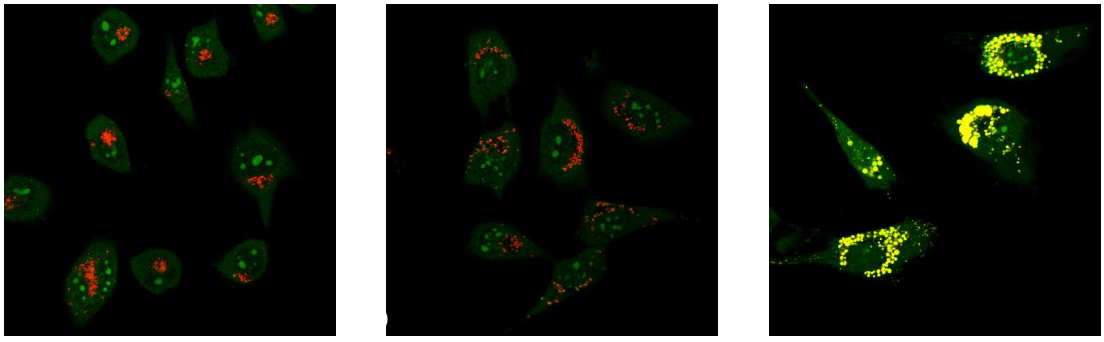

Fig. 1: Acridine orange staining as marker for lysosomal pH. In control cells (a) and in cells exposed to 20 µg/ml 20 nm polystyrene particles (b) lysosomes can be discerned as fine, red fluorescent dots, whereas the swollen lysosomes induced by exposure to the lysosomotropic agent chloroquine (c) are seen as coarse yellow dots.

Applications of immune system biomarkers in clinical studies

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A variety of human diseases goes along with features of an activated immune system, in some of which, if not in all of them, the immune system is directly involved in the pathogenesis. These include most obviously transplant rejection and autoimmune syndromes, as well as infections, malignant diseases, cardiovascular disorders and neurodegenerative processes but also allergy and asthma. For laboratory diagnostics purposes several biomarkers of disease activity have been developed. These include the measurement of cell subpopulations in whole blood and tissue, expression of cytokines, production of cytokines and biomarkers of immune activation, and *in vitro* systems to detect the ability of cells to spontaneously release cytokines or to express certain cell surface markers upon stimulation with mitogens, antigens or cytokines. Most of these assays are well introduced for scientific research purposes and do provide reliable results but the performance of assays often lacks sufficient between-run and

between-lab stability and are thus not generally acceptable as standard laboratory tools. In addition, assays for cytokines are often of insufficient sensitivity to define a reasonable normal range in patients, and also the reproducibility of results is unsatisfactory and influenced by the low stability of most cytokines in solution. The measurement of soluble cytokine receptors like soluble tumor necrosis factor α or interleukin-2 is usually more robust and of advantage especially in clinical studies. The same holds true for other soluble markers of immune activation such as ß2-microglubulin or neopterin, and high sensitivity C-reactive protein (CRP) as indicator of inflammatory processes. All these latter variables turned out to be of greater laboratory diagnostic value because of good performance characteristics of the assays –mostly ELISA- employed and are well suited for large scale applications. Neopterin is a well characterized product of human monocyte-derived macrophages and dendritic cells which reflects the extent of Th1-type immune response because Th1-type cytokine interferon-γ (IFN-γ) is the primary inducer of neopterin production by the enzyme GTP-cylcohydrolase I. Neopterin concentrations correlate strongly with the rate of tryptophan breakdown indicated by the kynurenine to tryptophan ratio (Kyn/Trp) which is achieved by the enzyme indoleamine-2,3-dioxygenase (IDO). IDO is also induced by IFN-γ and can thus be employed as another indicator of Th1-type immune response. However, thus far no immunoassays are available to utilize the large scale application of Kyn/Trp measurements, and at the moment HPLC is still the method of choice to do so. As laboratory diagnostic tools the measurements of neopterin and/or Kyn/Trp are superior to the monitoring of single cytokine concentrations, because these read-outs reflect the net effect of various positive and negative regulators of monocyte-derived macrophages and DC within the Th1-type immune response. Especially for in vitro applications neopterin and Kyn/Trp are of particular usefulness because other than, e .g., cytokine expression levels the marker concentrations are accumulating and less influenced by transient changes of cytokine expression profiles. Neopterin and Kyn/Trp turned out as significant predictors of disease specific and total mortality in patients with inflammatory diseases such as coronary heart diseases [1-3].

Oxidative stress is a major regulator of immune response because several especially pro-inflammatory cytokine cascades can be induced by oxidative stress. Thus, any influence of nanoparticles on cellular redox systems will impact on the inflammation status and cytokine production rates. Therefore, the measurement of neopterin and/or Kyn/Trp appears very suitable for epidemiologic studies to eventually demonstrate an effect of nanoparticles to trigger inflammatory disease.

[1] Kaski JC, et al. Elevated serum neopterin levels and adverse cardiac events at 6 months follow-up in Mediterranean patients with non-ST-segment elevation acute coronary syndrome. Atherosclerosis 2008;201:176-83.

[2] Grammer TB, et al. Neopterin as a predictor of total and cardiovascular mortality in individuals undergoing angiography in the Ludwigshafen Risk and Cardiovascular Health study. Clin Chem 2009;55:1135-46.

[3] Pedersen ER, et al. Systemic markers of interferon-γ-mediated immune activation and long-term prognosis in patients with stable coronary artery disease. Arterioscler Thromb Vasc Biol 2011;31:698-704.

Effect of occupational zinc nanoaerosol exposure on neopterin levels and tryptophan degradation

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Hot dip galvanization is the process of coating materials with zinc as a protective coat in order to protect the metal items from corrosion. The greatest risk in hot dip galvanization process is the zinc oxide aerosol fume rising from bath-tube surface in nano dimensions. In the present study, it was evaluated whether inhalation of zinc oxide nanoaerosols cause alteration in neopterin levels, a cellular immunity biomarker, and tryptophan degradation. Sixty-two males who worked in galvanization process and 23 male office personnel as a control group were included in this study. Urinary neopterin, creatinine, serum tryptophan

and kynurenine levels were detected by HPLC. Serum neopterin levels were analyzed by ELISA (IBL, Germany). Serum and urinary zinc levels were measured by atomic absorption spectroscopy. Urinary neopterin results were expressed as μmol neopterin/mol creatinine and tryptophan degradation was presented as kynurenine to tryptophan ratio. Urinary neopterin levels, serum neopterin levels and serum zinc levels of the worker group were statistically higher than the control group (all $p < 0.05$). Urinary neopterin levels were found to be correlated with kynurenine/tryptophan and serum zinc levels (both, $p < 0.05$). Our data indicates cellular immune activation by zinc nanoparticle exposure as shown by elevated urinary neopterin levels and tryptophan degradation. Additionally, it was estimated that neopterin may be used as a biomarker in early diagnosis of potential exposure to occupational zinc and nano-scale particles.

Microarray-based gene expression profiling: possibilities and limitations

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To-date, transcriptional profiling is one of the most frequently used large-scale data acquisition methodology in basic and clinical research. The possibility to simultaneously measure the expression of thousands of genes has made possible a variety of approaches to analyze biological samples. The analysis of global transcriptional signatures of cells and tissues may provide an unbiased insight into regulatory processes and might be helpful in deciphering complex molecular interaction networks.

Different secondary data analysis strategies are available to extract transcript subsets, that are determinants of cellular status. Computational methods to uncover groups and patterns of co-regulation or causal relationships are e.g. principal component analysis (PCA), clustering, classification and functional enrichment strategies as well as pathway and network analysis.

Microarray technology facilitates the identification of genes involved in human diseases by providing disease specific gene activity information, however, direct and indirect regulatory effects cannot be distinguished, and in some cases, these discrepancies may interfere with the deduced biological information. Furthermore, the application of large-scale data acquisition technologies is always limited, as they consume a considerable amount of temporal and financial resources. A rigorous experimental design is necessary to avoid pitfalls in the use of microarray data e.g. by generating datasets that are too inhomogeneous to deal with or by choosing inadequate experimental parameters. The application of fractional factorial designs and statistical methods, which consider the need for replicating experiments and resources, may reduce experimental efforts.

The application of gene expression profiling as a diagnostic tool for clinical decision-making, e.g., as it has been reported for the application of predictive signatures of disease prognosis, represents a small benefit (in relation to the effort) of this technology. A much greater value have microarray-based expression studies in their potential to allow a more precise characterization of the molecular mechanisms involved in a pathology, thus, they can provide highly valuable information for the discovery of new therapeutic targets or biomarkers.

Many nanotoxicological studies have focused on endpoints such as viability, cell stress, production of reactive oxygen species or immunological parameters. The application of large-scale data aquisition strategies such as transcriptional profiling may be helpful in the identification of new informative targets, to build up a predictive model for nanoparticle activity in a cellular environment. By identifiying core mediators of action, both *in vitro* and *in vivo* microarray studies could promote the development of diagnostic tools for the testing of nanoparticle toxicology in human populations.

Biomarkers of inflammation and cardiovascular disease – a clinical perspective

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Autoimmune disorders are associated with a significant increase in the incidence of cardiovascular disease (CVD) and cardiovascular mortality. Although an increased risk is most well established for

patients with rheumatoid arthritis and systemic lupus erythematosus, some recent evidence suggests an increased risk also exists for patients with ankylosing spondylitis, psoriasis, and psoriatic arthritis Cardiovascular disease (CVD) is a major cause of death in these patients, predominantly as a result of accelerated atherosclerosis. RA and SLE patients also have an increased prevalence of subclinical vascular disease, as assessed by the presence of carotid artery plaque, increased carotid intima–media thickness and coronary arterial calcification However, an increased prevalence of subclinical atherosclerosis in patients with systemic sclerosis, ankylosing spondylitis and Sjögren's syndrome is still debated, mainly because most studies have used small samples sizes.
While both SLE-specific and non-specific autoimmune and inflammatory mechanisms have been proposed to play a prominent role in the induction of premature vascular damage in rheumatic disorders, the exact etiology is not completely understood. Traditional CV risk factors seem to be less important predictors of CV events than the presence of inflammation. There is increasing evidence that chronic inflammation contributes to accelerated atherogenesis and plays a role in all stages of atherosclerosis (i.e. atherogenesis, atheroma progression, and the development of thrombosis).
T cells are abundant in the atherosclerotic plaques. CD4+ T helper cell (Th) 1 lymphocytes which secrete pro-inflammatory cytokines (TNF-α, IFN-γ, IL-2) predominate over Th2 lymphocytes that produce IL-4, IL-5, and IL-10. T cells can be identified in the fatty streaks. Cytotoxic CD8+ T cells are also present in atheromatous lesions. A subtype of CD4+ T cells lacking the co-stimulatory receptor CD28 (CD4+CD28null T cells) have been suggested as a mechanism for the accelerated atherosclerosis in RA and other conditions. Inflammatory and immunological markers have been proposed as useful markers of risk in clinical practice. This presentation will focus on the clinical role of inflammatory markers for the assessment of cardiovascular risk in different patient populations.

Psychoneuroimmunology of HIV infection

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Human immunodeficiency virus infection is characterized by over-whelming, but ineffective Th1 type immune response directed against the virus. Interferon-γ (IFN-γ), which is produced in large amounts by activated T-lymphocytes and NK cells, is a key cytokine orchestrating cellular immune response. It induces several biochemical pathways, like tryptophan degradation by the enzyme indolamine 2,3-dioxygenase (IDO), the formation of reactive oxygen species and the production of the pteridine neopterin by human monocytes and macrophages. Furthermore, IFN-γ also enforces the production of other pro-inflammatory cytokines, like tumor necrosis factor-α. IFN-γ-mediated pathways appear to be involved in the development of immunodeficiency and also of other "complications" of HIV infection, like neuropsychiatric symptoms or anemia: Tryptophan degradation by the enzyme IDO is strongly increased in HIV-1 infected patients, and has been associated with the development of immunodeficiency and anemia. Furthermore, enhanced tryptophan catabolism has also been linked with cognitive impairment, sleep disturbances and neuropsychiatric symptoms in HIV-infected patients. However, also an altered metabolism of the catecholamines dopamine, adrenalin and noradrenalin has been proposed recently to contribute to the development of neuropsychiatric symptoms or mood disorders like depression: Oxidative stress might impair the function of the enzyme phenylalanine-4-hydroxylase (PAH), which converts phenylalanine to tyrosine and is rate-limiting in the biosynthesis of dopamines. In line with this hypothesis, an accumulation of phenylalanine and a concomitant decrease of tyrosine have been shown in HIV-1 infected patients before therapy. Antiretroviral therapy (ART) is very effective to slow down immune activation, tryptophan degradation by IDO and also increase tyrosine concentrations in parallel with a decrease of phenylalanine levels. Furthermore, ART has also been shown to improve symptoms of depression, indicating that HIV-induced immune activation might contribute to the

development of neuropsychiatric symptoms, while ART-mediated down-regulation of immune activation is effective to reverse or improve such symptoms.
As neuropsychiatric symptoms are also frequently encountered in patients suffering from other chronic diseases characterized by on-going cellular immune response, immune activation is very likely to play a pivotal role in the development of these symptoms. Nanoparticles could be employed to target and image biological structures and inflammatory cells, thus investigating their role in the pathogenesis of HIV and of inflammatory diseases in general.

Identification and application of biomarkers in kidney transplantation

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The objective of this talk will be to give a critical overview about biomarkers in kidney transplantation. The human genome project provided us with high-throughput tools to identify potential diagnostic molecules and therapeutic targets to evaluate organ quality, monitor transplant function, diagnose rejection and other post-transplant complications and predict outcome. However, an established, robust biomarker tool set routinely used in the clinical day-to-day routine of solid organ transplantation is still missing.
In this talk studies using microarray technology for biomarker identification and application to assess donor organ quality in kidney transplantation will be used to discuss strengths and weaknesses of the genomic based tools. In particular the diagnostic quality of gene expression data in comparison to histology based, blood and urine derived biomarkers will be analyzed. Examples for the unique strengths of transcriptome measurements to assess changes in metabolism, measure continuity of changes from healthy to severely sick, quantitate inflammatory burden, detect stereotyped responses or understand biological pathways will be given.
However, weaknesses of the genomic approach and reasons why it is still not used in the clinical routine will be outlined as well. In particular the lack of clinical utility, the problem of the 'soft' gold standard and reference values, the often poor clinical phenotyping, the insufficient follow up and individualized approach will be highlighted.
In the last part of the talk an outlook will be given indicating key requirements to achieve the goal of personalized medicine. In particular the need for a systems biology approach, integrating clinical, pathology, imaging and genome data for useful individualized patient management will be outlined. An example for improving the measurement of kidney transplant function will be given.
Overall this talk will try to summarize lessons learnt from using the new technology of microarray gene chips to identify and apply biomarkers in a large scale study of kidney transplants.

Changes in neopterin levels and tryptophan degradation with silica nanoparticles

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With the development and wide use of nanomaterials in recent years, human exposure to nanoparticles has become unavoidable. Concerns and fears that have been expressed regarding potential risks of nanomaterials to worker's safety, human health, and environmental contamination caused growing interest in nanotoxicology. Nanoparticles can translocate from the entry portals into the circulatory and lymphatic systems, ultimately to body tissues and organs, then interact with cellular or subcellular structures. Studies in animal or cultured cell models have reported that nanoparticles could cause pulmonary inflammation and extrapulmonary toxicity. Occupational exposure to silica, the main constituent of sand and granite, leads disabling pulmonary fibrosis called silicosis. Toxic outcomes to silica exposure are likely to be mediated mainly activation of macrophages. Toxicological studies about

silica nanoparticles have reported that they increase production of reactive oxygen species (ROS) and induce pro-inflammatory and inflammatory responses. The aim of this study is to investigate neopterin levels as a potential exposure biomarker and to evaluate tryptophan degradation in silica exposed workers. Fifty-three silica workers, who were hospitalized in the Occupational Diseases Hospital, were included in this study while 22 healthy subjects recruited for the study as a non-exposed (control) group. Mean urinary neopterin levels (± SEM) of the workers were 176 ± 20 μmol/mol creatinine while control group had 139 ± 6 μmol/mol creatinine. However, the difference was not significant ($p > 0.05$). Kynurenine to tryptophan ratio of exposed and control groups were 39 ± 1.5 μmol/mmol and 30 ± 1.7 μmol/mmol, respectively. Kynurenine/tryptophan was statistically higher in silica workers ($p < 0.05$). It may be concluded that neopterin may play a role in the prediction of some disorders related to occupational exposure to silica particles. Since alterations in kynurenine pathway may lead to increased formation of neuroactive metabolites. Further studies are needed to explain the toxicity mechanisms of occupational exposures. A multidisciplinary approach should be considered in order to explain detailed mechanisms of nanomaterials.

Neopterin and progress of malignant diseases – a glimpse of host–tumour interaction?

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The discovery of strongly fluorescing compounds in urine samples from cancer patients by H Wachter and colleagues in the late 1960s paved the way to identifying 10 years later neopterin as the most important of these substances. Subsequently, neopterin could be established as a useful non-invasive marker for the activation of cell-mediated immune mechanisms. While it became evident that neopterin is not a classical tumour marker, scientific research concerning its behaviour in a broad variety of malignant diseases nevertheless remained active: unlike tumour markers in the usual sense of the word neopterin is not produced by tumour cells; rather, it represents a means for studying the interaction between the host's immune system with the malignant process.

The initial research studies were focussing upon the diagnostic features like sensitivity and specificity of neopterin in a broad spectrum of solid as well as of haematological malignancies of various stages and grades. Shortly, the diagnostic reliability of neopterin concentrations turned out to be as variable as the diseases studied: in most haematological cancers, very strongly elevated neopterin concentrations and diagnostic sensitivities up to 100% were detected, while in, e.g., breast cancer even in strongly progressed stages mean neopterin concentrations were, if at all, only slightly raised.

Somewhat unexpectedly, later studies revealed that neopterin concentrations in urine and serum of patients suffering from quite diverse malignancies carry significant and clinically relevant predictive information regarding the subsequent progress of disease: high concentrations of neopterin are invariably correlated with a worse prognosis. Notably, the predictive power of neopterin can even exceed that of classical and specific markers. In multivariate settings it was shown that the predictive information provided by neopterin concentrations is statistically independent from many other potential prognostic variables.

In several studies it was demonstrated that there is a good correlation between certain direct immunological activity measures like concentrations of interferon gamma, interleukins and the like. A distinct advantage of neopterin in contrast to substances like interferon gamma is its considerable chemical stability making it a robust and reliable marker for clinical practical use.

Summarizing the findings of numerous investigations we can safely conclude that the concentrations of neopterin in body fluids of cancer patients carry significant information about the reaction of the host's immune system with the malignant cancer. The strong heterogeneity of findings in diverse tumour types appears to reflect the multitude of possible interaction patterns.

Influence of TiO_2 nanoparticles *vs.* bulk material on human peripheral blood cells

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Nanomaterials have been increasingly produced and used within the last years and consequently the potential exposure of humans to nanoparticles has been rising as well. Because of their small size (1-100nm) the physicochemical properties of nanomaterials may be different from bulk materials and may pose a threat to human health. Only little is known about the effects of nanoparticles on the human body and therefore it seems to be important to investigate possible harmful effects of nanomaterials on vital biochemical processes like the immune response.

The pro-inflammatory cytokine interferon (IFN)-γ is released in the course of cellular immune response mediated by Th1-type T-helper cells and induces the expression of enzymes GTP-cylcohydrolase I (GCH) and indoleamine-2,3-dioxygenase (IDO) in human macrophages and dendritic cells. GCH induces the production of neopterin, while IDO catalyses the degradation of the essential amino acid tryptophan to kynurenine. Both biochemical processes are closely related to the course of diseases like infections, atherogenesis and neurodegeneration. In this study, we investigated the effects of TiO_2 bulk material and nanoparticles on Th1-type immune response using the *in vitro* model of peripheral blood mononuclear cells (PBMC). Upon stimulation of PBMC with 10µg/ml phytohemagglutinin (PHA), neopterin formation and tryptophan breakdown increase significantly.

PBMC were exposed to rising doses of bulk TiO_2, OCTi60 (10 nm diameter) and commercial P25 (25 nm diameter) TiO_2 nanoparticles and were stimulated or not with PHA. While P25 TiO_2 nanoparticles had only little influence on neopterin formation, bulk material and OCTi60 TiO_2 nanoparticles increased neopterin production in the supernatants of unstimulated and stimulated cells significantly, the effects were stronger in OCTi60 TiO_2 nanoparticle preparations compared to bulk material. No effects of TiO_2 preparations on tryptophan breakdown were determined in unstimulated cells, in stimulated cells a slight increase was found at low concentrations for all preparations. At the highest concentrations used even an inhibitory effect on IDO activity was observed.

In conclusion, OCTi60 TiO_2 nanoparticles and bulk material stimulate the formation of neopterin in PBMC, and there are distinct differences between the preparations. The parallel inhibitory influence on IDO suggests that the net effect of the tested particles would be even stronger pro-inflammatory when the immunosuppressive activity of IDO is absent. However, this study can be considered as pilot only and clearly, for a firm conclusion further experiments are needed.

Limitations of LC-tandem mass spectrometry in the clinical laboratory

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Technical maturation brought liquid chromatography tandem mass spectrometry (LC-MS/MS) into majority of major clinical laboratories worldwide. Especially in therapeutic drug monitoring, endocrinology, and toxicology LC-MS/MS became an indispensable research and routine tool [1-5].

Although well designed LC-MS/MS assays generally outperform immunoassays due to their accuracy, sensitivity, precision, and inherent multiplexing capability, they are not free from analytical problems. Besides limitations in selectivity due to the occurrence of “isobaric” interferences of different origin, sudden and unpredictable ion yield attenuations, often known as “ion suppression effect”, can be considered the Achilles heel of quantitative bio-analytical mass spectrometry [5]. It is – besides the occurrence of isobaric interferences – the major error source in LC-MS/MS. It is compromising the accuracy of an assay and its precision and can easily lead to gross errors in analyte quantification. Individual co-medications or pathologically altered patient specimen are major causes for both

interferences and ion yield fluctuations. Special measures have to be taken to evaluate these accuracy limiting interferences prior to bringing an LC-MS/MS assay into the highly regulated clinical routine environment [6]. Most LC-MS/MS methods used in clinical laboratories are still "home-brewed" laboratory-developed tests operating on very heterogeneous instrument configurations, although commercial IVD-CE certified LC-MS/MS assay kits have become available recently. Consequently, assay heterogeneity and lacking traceability to reference procedures or materials leads to an increased imprecision in proficiency testing as well as to inaccurate result reporting if basic rules of assay validation and "post marketing" surveillance are violated.

The position of LC-MS/MS and its advantages / disadvantages compared to immunoassays will be discussed on the most prominent high throughput application examples – immunosuppressant drug monitoring [2] and 25-OH-Vitamin D serum level assessment. In addition, technical limitations and analytical problems of LC-MS/MS instrumentation will be critically evaluated in the light of technical development.

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Biomonitoring of occupational exposure to styrene

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Styrene is a commercially important chemical widely used in the manufacture of resins, polyesters, and plastics. The highest levels of human exposure to styrene occur in occupational settings, especially during the production of reinforced plastic products, which involve manual lay-up or spray-up operations.

The objective of this work was to study occupational exposure to styrene in a multistage approach, in order to integrate the following end-points studied: styrene in workplace air, mandelic and phenylglyoxylic acids (MA + PGA) in urine, haemoglobin (Hb) adducts, sister-chromatid exchanges (SCE), micronuclei (MN), DNA damage (comet assay) and genotypes of polymorphic genes of some metabolising enzymes. Seventy-five workers from a fibreglass-reinforced plastics factory and seventy-seven unexposed controls took part in the study.

The mean air concentration of styrene in the breathing zone of workers (30.4 ppm) was higher than the threshold limit value of 20 ppm recommended by the ACGIH, and the biological exposure index adopted by the ACGIH for exposure to styrene prior to the next shift (MA + PGA = 400mg/g creatinine) was exceeded, indicating that styrene exposure for this group of workers was higher than recommended. The level of Hb adducts and SCE in exposed workers were significantly increased as compared with controls. The DNA damage was higher among styrene-exposed workers than in controls. No significant differences were observed in the MN. Concerning the effect of the genetic polymorphisms on the different exposure and effect biomarkers studied, we observed the effect of microsomal epoxide hydrolase activity on Hb adducts of highly exposed individuals and on the levels of SCE of exposed workers.

The present results suggest the importance of individual susceptibility factors in modulating genotoxicity, although cautious interpretations are required since the size of the study population limits the power of many of the analyses. Because the effects of these polymorphisms are relatively subtle, and some important alleles are relatively rare, a much larger study population will be necessary to evaluate their effects on biomarkers, especially when gene-gene interactions are considered.

Nano *versus* bulk toxicity: need for critical human biomarkers

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Although nanomaterials are present in biosphere ever since the beginning of Earth, nonetheless human interventions have brought about the advent of several novel engineered nanoparticles with peculiar size, shape and surface properties for various applications. These nanoparticles may interact with the immune components depending on their hydrophobicity, hydrophilicity, chemical composition, shape and catalytic activity. There have been limited studies on the outcome of such interactions and as such there are no specific guidelines of immunotoxicity testing for nano-materials. It has been suggested using animal models or cell cultures that engineered nanoparticles can exacerbate the pre-existing inflammatory condition leading to severe pathological stress, however these results need to be compared with respective bulk material. In our study we have observed that ZnO bulk particles were more potent in instigating the ROS generation and production of proinflammatory cytokines by macrophages when compared to ZnO nanoparticles. On further elaboration, it was observed that there was dose dependent and time dependent increase in the uptake of bulk particles with respect to nanoparticles, quite paradoxical to the earlier notion that the small size attribute of nanoparticles would give them an upper edge to breach the cellular boundary. A detailed mechanistic study revealed the involvement of several receptor based uptake pathways operational in macrophages, and a higher degree of bulk uptake co-related well with the corresponding contribution of phagocytic pathways for them. These preliminary studies clearly warrant a high degree of caution in interpreting the toxicity of nano versus bulk particles, which could be cell type specific. To study the toxicological manifestations, nanoparticles and their interaction with biological entities needs to be thoroughly characterized both outside and within the biological environment. Till date animal models have been used for conventional immunotoxicity assays, but their extrapolation to human systems can always be debated. The lack of appropriate human immunotoxicity biomarkers is a major impediment in the path of epidemiological immunotoxicity studies in nanomaterial exposed populations. Flow cytometry has emerged as a useful tool which is fast replacing the conventional immunotoxicological assays. It is being used for the detection of serum immunoglobulins, multiple cytokines, immunophenotyping and even intracellular signaling molecules. Further, a critical use of microarrays, protein arrays in animal models and exposed population can lead to identification of key biomarkers which can be later employed for high throughput clinical testing with the aid of flow cytometry.

Pathophysiological networks and new therapeutic approaches in the anemia of chronic disease

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Anaemia of chronic disease (ACD), also termed as anaemia of inflammation, is the most frequent anaemia in hospitalised patients and is found with a high frequency in subjects suffering from auto-immune disorders, severe infections and cancer. ACD is an immune-driven disease which is induced by several cytokine-mediated pathways. First, cytokines as well as the acute phase protein hepcidin induce iron retention within cells of the reticuloendothelial system (RES), resulting in an iron-restricted erythropoiesis. This effect may be further aggravated by a inflammation mediated shortening of erythrocyte life span and stimulation of erythrophagocytosis. Second, mainly pro-inflammatory cytokines directly inhibit the proliferation and differentiation of erythroid progenitor cells while at the same time they inhibit the formation and biological activity of the erythropoiesis-stimulating hormone erythropoietin. In addition, several erythropoiesis driven hormones may contribute to ACD by modulating the expression of hepcidin.

While anaemia *per se* causes morbidity due to impaired cardiovascular performance and tissue oxygenation, the development of ACD may harbour also some advantages, especially when infections or cancer underlie ACD. The retention of iron within monocytes and macrophages results in a reduced availability of the metal for invading pathogens which need iron for their growth and proliferation. Furthermore, due to the negative regulatory effects of iron on cell-mediated immune function, iron restriction leads to strengthening of innate immune effector pathways directed against invading pathogens. While this is an effective defence strategy in infection and cancer, ACD has to be considered as a side effect in association with auto-immune disorders. Accordingly, while the therapeutic application of iron for the treatment of ACD is risky and may cause exacerbation of the underlying disease in infection and cancer, treatment of ACD with iron in rheumatic disease may reduce disease activity due to its negative effects on pro-inflammatory immune pathways, a concept which has to be proven clinically in the future. In addition, due to our expanding knowledge on the regulation of iron homeostasis and specifically the elucidation of the complex network underlying the regulation of hepcidin expression, novel therapeutic concepts aim to modify hepcidin formation, thereby counter-acting iron restriction in the RES and mobilising the metal for erythropoiesis.

IL28B polymorphism, IP10 and other biomarkers in patients with hepatitis C virus infection

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Chronic hepatitis C virus infection is a global health problem affecting 2.2 – 3.0 % worldwide (130-170 Million people) and a major cause of liver related morbidity and mortality. Until recently, a combination of pegylated interferon alpha (IFN-a) and ribavirin was the most effective treatment strategies for chronic hepatitis C. After a treatment course of 24 to 48 weeks sustained viral response rates of 20 to 80 % can be achieved with of pegylated IFN-a and ribavirin combination therapy. Treatment response is determined by viral factors including genotype, viral load and quasispecies diversity as well as host factors such as age, sex, ethinicity and body mass index.

Genome wide association studies on genetic factors that are associated with favorable treatment response in patients infected with the difficult to treat HCV genotype 1 have identified polymorphisms near the IL28B gene. IL28B is a lambda type interferon (IFN-λ) that activates transcription of 'interferon stimulated genes (ISGs)' through a complex of IFN-λ-receptor 1 and IL10-receptor. Expression array studies suggest in treatment naïve patients suggest, that patients with favorable IL28B associated genotypes (e.g. rs12979860 'CC' genotype) have lower pre-treatment hepatic ISG expression than unfavorable genotypes. In patients with the CC genotype treatment with pegylated interferon is thus a stronger inductor of ISG expression and hence associated with higher response rates.

We studied kynurenine to tryptophan ratios (Kyn/Trp) as a surrogate parameter of indoleamine 2,3-dioxygenase (IDO) activity. IDO is a known interferon stimulated gene and our preliminary results in 25 HCV infected patients shows that its activity correlates with IL28B genotype with higher Kyn/Trp in patients with the favorable IL28B genotype 'CC'.

In conclusion, in patients with chronic HCV infection, IL28B genotype determines regulation and expression of interferon-stimulated genes, but in contrast to IP10 levels, which are lower in IL28 CC patients, IDO activity might be under control of different regulatory mechanisms. If surrogate parameters of IDO activity are predictors of treatment outcomes and may therefore assist the selection of patients for IFN based therapies of chronic HCV is currently under investigation.

All authors gratefully acknowledge the funding from the New INDIGO programme (NanoLINEN project).

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