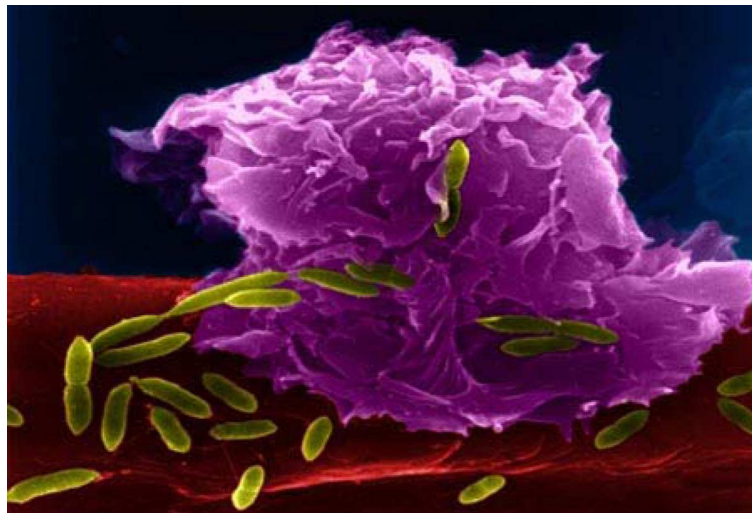


2nd CIIT SCIENCE DAY

June 30th, 2011

Abstract book



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WELCOME

In 2009, the “Comprehensive Center for Infection, Immunity, and Transplantation (CIIT) (also visit: http://www.i-med.ac.at/innere_medizin/innere_medizin_1/CIIT) was established at the Medical University of Innsbruck. The CIIT is coordinated and organized by a speakers’ team from different disciplines and aims to promote and optimize the interdisciplinary collaboration and interactions in terms of clinical practice, science and teaching in these fields of interest at the Medical University of Innsbruck.

Therefore, a series of lectures and case studies has been established to promote this interdisciplinary exchange. Within the CIIT colloquium local and external speakers present and discuss new interesting research topics in infection, immunity and transplantation. The Grand Rounds are clinically orientated and focus on the presentation and discussion of interesting clinical cases.

The CIIT Science Days at the MUI bring together allocated researchers with a scientific focus in infection, immunity and transplantation and will thus provide an overview of current scientific topics and projects in the respective research areas. To this aim actual research projects or studies will be presented by the authors during guided poster tours. In the 1st Science Day in 2010, 85 posters from 46 different research teams were presented. For the 2nd Science Day in 2011, 59 abstracts from 34 different research teams have been submitted, which will guarantee a broad and fruitful scientific exchange.

In addition, we are very happy that Prof. Esther von Stebut-Borschitz from the Department of Dermatology at the Johannes-Gutenberg University, Mainz, who is a well recognized European researcher in innate immunity and host-pathogen interaction, has agreed to give the keynote lecture at this 2nd Science Day.

Finally, I would like to thank all the people who were involved in the organization of this 2nd Science Day, specifically Thomas Sonnweber, Patrizia Stoitzner, Patrizia Nössing, Susanne Rofner, Bettina Sartori, Michaela Lackner, Susanne Perkhofer, Markus Schrettl as well as the poster moderators. We are also most grateful to our sponsors who provided significant financial support. They are acknowledged on the back of this abstracts book. I wish all participants and guests a stimulating and interesting meeting and a fruitful scientific exchange.

CIIT speakers team

Program

LOCATION: großer Hörsaal and Seminarräume MZA (Medizinzentrum Anichstraße)

Großer Hörsaal:

14.00-14.15 Introducing words: Univ.-Prof. Dr. G. Weiss (CIIT-speaker)
Opening: Vice-rector Univ.-Prof. Dr. G Sperk

Seminarräume 1/2: Moderated Poster sessions

14.15-15.30 Poster session I (Dendritic cells, bacterial infection, fungal infection)

15.45-17.00 Poster session II (Various diseases, immunity, transplantation and viral infection)

Seminarraum 3

17.00-18.00 Keynote Lecture: Prof. Dr. med. Esther von Stebut-Borschitz,
Johannes-Gutenberg University, Mainz, Germany
"Role of Th subsets in cutaneous leishmaniasis"

18.00-20.00 Poster discussions with light buffet and drinks

Moderated poster sessions

	Topic	Poster first authors	Moderator
14.15-15.30 Poster session I	Dendritic cells	Banki Z, Elentner A, Flacher V, Gredler V, Lutz-Nicaldoni C, Martinz V, Scheffler J, Sparber F, Tripp CH, Posch W	Nikolaus Romani
	Bacterial infection	Berkthold M, Eitzinger C, Fritsche G, Khalid S, Mair SM, Mitterstiller AM, Nairz M, Poolpol K, Tancevski I	Reinhard Würzner
	Fungal infection	Blatzer M, Eigentler A, Gsaller F, Hegedus N, Jöchl C, Petrik M, Rambach G, Schafferer L, Speth C	Hubertus Haas
15.45-17.00 Poster session II	Various diseases	Brunner J 2x, Hofer J, Lengauer S, Mader S, Mühlberger N, Ratzinger G, Sonnweber T 2x, Theurl I	Günter Weiss
	Immunity	Hinterleitner R, Kern G, Ottina E, Perret R, Schröcksnadel S, Schroll A, Tymoszuk P, Wallner S, Wieggers GJ, Wöss C	Doris Wilflingseder
	Transplantation and viral infection	Ashraf MI, Cardini B, Dold C, Egerer L, Fabritius C, Griesmaier E, Kleines M, Kunert K, Neubauer V, Sroczynski G, Trojer R	Katja Kotsch

Coffee will be available throughout the poster sessions.

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Dendritic Cells
Posterwalk by Nikolaus Romani

Targeting viral antigens to CD11c on dendritic cells induces retrovirus-specific T cell responses

Z. Bánki¹, A. Ejaz¹, G. Huber¹, C.G. Ammann², V. Oberhauser¹, S. Lengauer¹, S. Schimmer³, U. Dittmer³, D. von Laer¹, H. Stoiber¹

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Background: Dendritic cells (DC) represent the most potent antigen presenting cells to induce efficient cytotoxic T lymphocyte (CTL) response against viral infections. Delivering antigens (Ag) to receptors on DCs is intensively studied as promising tool to induce antitumor and antiviral immune response by DCs.

Methods: Here we investigated the potential of CD11c-specific single-chain fragments (scFv) fused to immuno-dominant peptide of Friend virus for the induction of virus-specific T cell response by DCs.

Results: CD11c-specific scFv selectively targeted viral antigens to DCs and thereby significantly improved the activation of virus-specific T cells *in vitro*.

Conclusions: DCs loaded with viral Ag targeted to CD11c provided improved rejection of FV-derived tumors and more importantly efficiently primed virus-specific CTL response *in vivo*. Since the induction of strong virus-specific T cell responses is critical in viral infections, CD11c targeted protein vaccines triggering cellular immunity might provide alternatives beside other vaccination strategies.

Pregnane X Receptor (PXR) links xenobiotic metabolism to the cutaneous immune response

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Background: The pregnane X receptor (PXR) is a member of the nuclear receptor superfamily that regulates the transcription of genes involved in all phases of endo- and xeno-biotic metabolism and thus prevents toxic accumulation of metabolites within cells. While skin is the largest metabolic organ of the body, the role of PXR in cutaneous immunity was never investigated.

Methods: Contact dermatitis was induced in mice by topical application of TPA or TNCB. When skin inflammation reached its maximum, mice were further treated with topical application of PXR agonists. Ear inflammation was evaluated once a day for 5 days. At the end of the experiment, H&E staining, FACS analysis of dermal inflammatory infiltrates and qPCR were carried out on mouse ears. Alternatively, qPCR was carried out on mouse keratinocytes treated with TNBS and PXR agonists for 48h.

Results: PXR activation ameliorated both irritant and allergic contact dermatitis. Moreover, rifampicin, a well-known activator of human PXR, when applied topically, improved allergic contact dermatitis in mice humanized for PXR (Tg-hPXR) and for PXR and CYP3A4 (Tg-CYP3A4/hPXR). Rifampicin down-regulated the expression of IL-1beta in an hPXR-mediated manner in PXR-humanized epidermis sensitized and challenged with a contact allergen. Moreover, rifampicin down-regulated iNOS and IL-1beta in activated keratinocytes. Notably, Tg-CYP3A4/hPXR mice responded more readily to rifampicin than Tg-hPXR mice, suggesting a link between CYP3A4 and the rifampicin-mediated anti-inflammatory response. Conversely, PXR deficient mice exhibited exaggerated allergic contact dermatitis. Finally, PXR was expressed in lymphocyte-rich infiltrates of various human inflammatory skin disorders.

Conclusion: PXR plays a regulatory role in the cutaneous immune system, and CYP3A4, the key enzyme of drug metabolism regulated by PXR, further modulates PXR-mediated anti-inflammatory activity.

Langerhans Cells and Dermal Langerin+ Dendritic Cells Transport Antibodies Targeting the C-type Lectin DEC-205 in vivo, but are not Essential to Subsequent Cytotoxic Responses

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Antigens deposited in the skin, such as vaccines given subcutaneously, are captured by different cutaneous dendritic cells (DC), but also DC residing in secondary lymphoid organs. We found that intradermal injection of mAb to C-type lectin receptors DEC-205/CD205 and langerin/CD207 resulted in strong and rapid labeling of epidermal Langerhans cells (LC); this implies diffusion of large molecules through the basement membrane into the epidermis. Anti-DEC-205 also targeted langerin+/CD103+ and langerin-/CD103- dermal DC. Following in vivo uptake of ovalbumin-coupled anti-DEC-205, LC isolated by migration from epidermal sheets potently induced proliferation of ovalbumin-specific CD4+ and CD8+ T cells in vitro, suggesting that LC efficiently present receptor-targeted antigens administered in the skin. In vivo, the targeted skin DC migrated through lymphatic vessels in steady state and inflammation. The transport of the targeting mAb to skin-draining lymph nodes was strongly dependent on migrating skin DC, most of which were langerin+. Transport was increased upon topical skin treatment with the TLR7 agonist imiquimod. Participation of dermal langerin+ DC transiently increased upon inflammation. Complete removal of the site where ovalbumin-coupled anti-DEC-205 had been injected substantially decreased endogenous cytotoxic responses against ovalbumin peptide-loaded target cells. Surprisingly, selective ablation of langerin+ DC by means of langerin-diphtheria-toxin receptor knock-in mice did not affect such responses, independently of the adjuvant chosen (imiquimod, poly I:C). Thus, in the context of cutaneous targeting of DC in vivo, langerin+ skin DC play a major role in transport of DEC-205-bound mAb, but appear redundant for the induction of subsequent CD8+ T cell responses.

The Tolerogenic Capacity of ECDI-treated Cells

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Background: Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system. It was shown that dendritic cells (DC) are recruited to MS lesions, where they mature and might contribute to the local activation and expansion of T cells. Uptake of apoptotic cells by DC has been involved in tolerogenesis as well as in immune activation. Treatment of cells with the apoptosis-inducing, chemical cross-linker ethylcarbodiimide (ECDI) was shown to promote tolerance in several animal models of autoimmune diseases.

In the current study we analyze whether uptake of apoptotic autologous blood mononuclear cells (PBMC) affects the activation of antigen-presenting-cells (APC) - monocyte-derived DC and monocytes.

Methods: The efficiency of various inducers of apoptosis was analysed by flow cytometric stainings for annexin. Furthermore, ECDI-treated cells were analyzed by scanning electron microscopy to gain deeper insights into ECDI-induced changes. DC were generated from monocytes in the presence of IL-4 and GM-CSF. Cells were co-cultured either with ECDI-treated-, UV-irradiated-, γ -irradiated- or untreated autologous PBMC. The next day, cells were further stimulated with LPS or a maturation cocktail. Cell activation was analyzed by the expression of cell surface markers and the secretion of various cytokines in cell culture supernatants. The immunostimulatory capacity of apoptotic-cell loaded DC was assessed in allogeneic mixed leukocyte reactions (MLR). Furthermore, monocytes were pre-incubated with γ -irradiated- or ECDI-treated antigen-coupled cells (tetanustoxoid or myelin-proteins), washed and further co-cultured with autologous T cells and tetanustoxoid/myelin-proteins. The proliferation of T cells was measured via dilution of carboxyfluorescein-succinimidyl-ester (CFSE).

Results: We found that different strategies to induce apoptosis had no profound influence on the maturation and activation of DC and monocytes. The uptake of apoptotic cells by DC did not affect their immunostimulatory capacity in allogeneic MLR. However, in autologous MLR, T cells which were co-cultured with monocytes - pre-incubated with ECDI-treated cells - did not proliferate in response to antigens.

Conclusion: Our results indicate a potent immunomodulatory effect of ECDI-treated cells on APC, exclusively in an autologous manner. These results provide further evidence for the importance of APC in antigen specific tolerization with ECDI-treated antigen-coupled cells.

Acknowledgements: The authors are grateful to N. Romani and M. Forstner from the Department of Dermatology and Venereology, Innsbruck Medical University for performing ³H-thymidine assays.

Reinforcement of Cancer Immunotherapy by adoptive transfer of *cb1b*-deficient CD8⁺ T cells combined with a DC vaccine

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Background: Therapeutic efficacy of adoptive T cell therapy (ACT) for cancer in the clinic is limited, mainly due to insufficient *in vivo* activation, expansion and survival of adoptively transferred effector T cells. In addition, transferred tumor-specific T cells encounter suppressive milieu signals exerted from both the tumor and regulatory T cells. Thus, strategies rendering adoptively transferred immune cells resistant to inhibitory environmental cues by the tumor stroma are considered attractive for improvement of current cancer immunotherapy. Gene ablation of *casitas b-lineage lymphoma protooncogene b (cb1b)*, an E3 ubiquitin ligase and negative regulator of T cell activation, was shown to induce potent anti-tumor responses in immunodeficient mice. In this study we aimed to improve ACT by the use of polyclonal hyperreactive *cb1b*-deficient CD8⁺ T cells.

Methods: We tested the sensitivity of *cb1b*-deficient CD8⁺ T cells towards the suppressive effects of TGF- β *in vitro* by applying proliferation assays (³H thymidine uptake) and determination of cytokine expression (IL2, IFN- γ) via Bioplex and FACS analysis. Employing the B16ova mouse melanoma model we investigated the effect of *cb1b*-deficiency in adoptively transferred polyclonal not TCR-specific CD8⁺ T cells. To increase anti-tumor responses we combined ACT with active vaccination with tumor-antigen pulsed dendritic cells (DCs). Tumor growth was measured with a caliper every 2nd day. Number of tumor and lymphnode infiltrating lymphocytes, number of tumorantigen-specific CD8⁺ T cells and IFN- γ expression of infiltrating CD4⁺ and CD8⁺ T cells was analyzed by FACS. Using an *in vivo* killing assay we investigated the cytotoxicity of transferred immune cells.

Results: We provide evidence that *cb1b*^{-/-} CD8⁺ T cells are hyperresponsive to TCR/CD28-stimulation *in vitro* and protected from the negative effects induced by TGF- β . Nevertheless and unexpectedly, ACT of polyclonal, non TCR-transgenic *cb1b*-deficient CTLs into tumor bearing immunocompetent wt mice is not sufficient to reject B16ova or EG7 tumors *in vivo*. However, *cb1b*^{-/-} transferred CD8⁺ T cells can be *in vivo* re-activated by a DC vaccine (SIINFEKL-pulsed DCs). In strict contrast to ACT monotherapy, this approach delays tumor outgrowth and significantly increases survival rates, which is paralleled by an increased CTL infiltration rate to the tumor site as well as enrichment of tumor antigen-specific and IFN- γ -secreting CTLs in the draining lymph nodes. *In vivo* cytolytic activity is increased in tumor bearing mice treated with DCs and *cb1b*-deficient CTLs compared to those treated with DCs plus wt CTLs.

Conclusion: We provide experimental evidence that Cbl-b targeting either by means of genetic inactivation (e.g. siRNA mediated knock down) or pharmacological inhibition of Cbl-b function represents an innovative approach to improve the efficacy of ACT. Activation of the transferred T cells by a DC vaccine induces profound anti-tumor immune responses.

The study is supported by a research grant of the Cumming Foundation (WY, USA).

Effects of Epidermal Barrier Disruption on Vitamin D3-induced Atopic Dermatitis-like Inflammation in Mice

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Background: Repeated high dose topical vitamin D3 (VitD3) induces an atopic dermatitis (AD)-like inflammation by upregulating the expression of thymic stromal lymphopoietin (TSLP) in keratinocytes and requires the presence of epidermal Langerhans cells (LC).

Methods: To test the hypothesis that TSLP-induced skin inflammation is aggravated by skin barrier impairment, Balb/c mice were topically treated with VitD3 after disruption of the epidermal skin barrier by tape stripping and a detailed analysis of the inflammatory response was carried out using light microscopy, ELISA, FACS and qPCR analyses.

Results: Tape stripping prior to topical VitD3 worsened epidermal hyperplasia and inflammatory infiltrates, when compared to controls treated with VitD3 only. Furthermore, tape stripping combined with topical VitD3 resulted in greater cell numbers in skin draining lymph nodes, when compared to control mice treated with VitD3 alone. In contrast, epidermal barrier disruption did not amplify levels of plasma IgE, epidermal TSLP expression, or numbers of activated CD4⁺ T cells, CD4⁺ CD25⁺ FOXP3⁺ regulatory T cells, and dendritic cells (DC) migrated to the skin draining lymph nodes.

Conclusion: These results suggest that abnormal immune reactivity and impaired skin barrier function synergize in aggravating local AD-like inflammation.

Conditional Gene Ablation of the MAP Kinase Adapter Protein p14 in Dendritic Cells induces a Myeloid Proliferative Disorder

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Background: Dendritic cells are key players of the immune system and link innate to adaptive immune responses. Their major task is the uptake and processing of pathogens and subsequent presentation of antigens to T cells. These processes strongly depend on endosomal/lysosomal trafficking. Conditional gene disruption of the adapter protein p14 in mice demonstrates that the late endosomal p14/MP1-MEK1 signaling complex is required to control endosomal traffic and tissue homeostasis (Teis et al., J Cell Biol, 2006).

Methods: To address the molecular function of p14 in dendritic cells, we generated a conditional knock out mouse model, which allows the specific deletion of p14 in CD11c expressing cells. The effects were analyzed in tissue (histological methods, FACS, ELISA) and primary cell culture (Western Blot).

Results: The knock out mice were viable but developed a severe pathological phenotype resembling a myeloid proliferative disorder (MPD) at the age of two to three months. The most obvious morphological symptoms included enlarged lymph nodes and splenomegaly. The structural morphology of these organs was disarranged and massive leukocyte infiltrates were observed, which could further be identified as dendritic cells. Additionally, the mice developed infiltrates of monocytes and activated dendritic cells in skin and liver. These infiltrates were also surrounded by single T cells being known as the direct interaction partners of activated dendritic cells. The bone marrow of the CD11c-p14 knock out mice was hyperplastic, accompanied by an increase of hematopoietic stem cells. Furthermore a MPD characteristic shift from the granulocytic towards the monocytic/dendritic cell lineage, an increase in the T helper cell population and a decrease of the erythrocyte progenitors were observed. In the serum of the CD11c-p14 knock out mice at the age of 1 to 6 months, Flt3-ligand, a specific cytokine inducing conventional dendritic cell differentiation, was significantly elevated. Additionally, its receptor Flt3 showed an increased surface localization on splenic dendritic cells. Similar observations were made in p14 depleted keratinocytes where the degradation of the EGF receptor was severely disturbed leading to an accumulation on the plasma membrane (Teis D. et al., 2006, JCB). The accumulation of the receptor on the cell surface and the enhanced availability of its ligand resulted in an increased downstream signaling of Flt3 shown by the phosphorylation of the mTOR target p70 S6 kinase 1. This pathway downstream of the Flt3 receptor is known to be crucial for dendritic cell differentiation (Sathaliyawala T. et al., 2010, Immunity).

Conclusion: Finally we can conclude that p14 deletion in dendritic cells severely affects their tissue homeostasis and leads to a MPD.

Deletion of the signal transduction molecule p14 under the CD11c promotor impairs the development of murine Langerhans cell network

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Background: Dendritic cells (DC) are important regulators of immunity and tolerance. To fulfill their antigen presenting capacity, DC need to process and distribute incorporated antigen via endosomal sorting to distinct cellular compartments so that they can present it to effector T cells. The extracellular signaling-regulated kinase (ERK) cascade is involved in endosomal sorting processes. Hence, we investigated the role of the adaptor molecule p14, an essential part of the ERK cascade, in the context of DC function.

Methods: We generated a DC specific knock out mouse model by Cre-CD11c-mediated ablation of p14. Phenotypical analysis of the DC populations was carried out by flow cytometry analysis as well as with immunofluorescence microscopy of epidermal sheets and cryostat sections.

Results: The overall numbers of CD11c⁺ DC in spleens and lymph nodes were increased in knock-out compared to wildtype mice. However, within the fraction of migrating DC in the skin-draining lymph nodes we noted greatly diminished numbers of both langerin⁺ CD103⁻ negative migrated Langerhans cells and langerin⁺ CD103⁺ cells (migrated dermal DC). The reduced number of skin DC, especially epidermal Langerhans cells was further confirmed by quantitative and qualitative analysis of the skin of the mice. Investigating the ontogeny of Langerhans cells by analysing the skin of newborn mice, revealed, that Langerhans cells are capable of establishing their epidermal network within 3 days after birth. However, the maintenance and homeostasis of the network seems to be affected in p14 knock-out mice as indicated by a constant loss of Langerhans cells starting within one week after birth.

Conclusion: In summary, our observations identify p14 as an important molecule regulating the homeostasis of the Langerhans cells network. The molecular basis for this phenomenon is currently being investigated.

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Langerin⁺ dendritic cells play a crucial role in susceptibility to squamous cell carcinoma

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Background: Squamous cell carcinoma is one of the most frequent types of skin cancer. Since tumor incidence is increased in immunocompromised individuals we are interested in investigating the role of skin dendritic cells in immunosurveillance of skin tumors.

Methods: Tumors resembling squamous cell carcinoma were induced by a two-stage carcinogenesis model in mice depleted of Langerin⁺ dendritic cells, which represent Langerhans cells and a subset of dermal dendritic cells. Differences in tumor numbers were evaluated and excised tumors were analysed for tumor-infiltrating cell types by FACS and fluorescence microscopy.

Results: First experiments revealed that mice depleted of Langerin⁺ cells developed tumors earlier and in higher numbers than control animals. The numbers of infiltrating T cells were unchanged whereas myeloid-derived suppressor cells (MDSC) increased and natural killer T cells (NKT) decreased in mice lacking Langerin⁺ dendritic cells.

Conclusions: Our observations indicate an important role of Langerin⁺ dendritic cells on tumor onset and early immune responses to skin tumors. The lack of Langerin⁺ dendritic cells shifts the relation of immunologically counteracting cell types towards a tumor promoting milieu. Future experiments will examine the influence of tumor-infiltrating cells on the cytokine milieu and antigen-presenting capability of skin dendritic cells. The expected findings will be important to optimally harness the unique immunogenic properties of skin DC to treat cancer.

IgG-Opsonization of HIV impedes induction of a functional CTL response

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Background: Control of HIV is suggested to depend on potent effector functions of the virus-specific CD8⁺ T cell response. As recently found, complement opsonization of HIV significantly enhanced the capacity of dendritic cells (DCs) to induce efficient CTL responses both *in vitro* and *in vivo*. Following seroconversion the virus is additionally opsonized with specific antibodies, thus resulting in different outcomes with respect to the antigen-presenting capacity due to interactions of IgG-opsonized HIV with Fcγ receptors expressed on DCs.

Methods: *In vitro* prime-boost experiments of naïve CD8⁺ T cells with autologous, differently loaded DCs were performed. The DC-induced CD8⁺ T cell expansion and functionality (IFN-γ secretion, degranulation efficiency, tetramer recognition, antiviral efficiency) were analyzed by FACS. Furthermore, the activation of HIV-specific CD8⁺ T cell clones by the differentially loaded DCs was monitored as well as p21 activation and HLA-class I co-localization.

Results: We here show that IgG opsonization of the virus is associated with a loss of the CTL-stimulatory capacity by DCs as represented by reduced proliferation, low activation of HIV-specific CTL clones and a weak antiviral activity. This impairment in induction of efficient CTLs by DCs exposed to IgG-opsonized HIV was linked to reduced co-localization of the virus with HLA-class I in DCs and up-regulation of the cyclin dependent kinase inhibitor p21 (Cip1/WAF1). Our results illustrate a close correlation between the opsonization pattern of HIV and DC-induced expansion and differentiation of specific CTLs.

Conclusions: Our *in vitro* data provide the first evidence that HIV potently activates DCs at the beginning of infection to generate cellular immune responses in a complement-dependent way, whereas coating of the virus with antibodies seems to preferentially modulate and alter antigen presentation by DCs, to the disadvantage of an efficient CTL response.

Bacterial Infection
Posterwalk by Reinhard Würzner

EHEC-Derived Shiga Toxin 2 Affects Human Plasmatic Coagulation System but not Platelets

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Background: Infection with Enterohemorrhagic *Escherichia coli* (EHEC) is the most important cause for typical hemolytic uremic syndrome (HUS). HUS is defined by renal injury due to damage of renal endothelial cells caused especially by Shiga toxin 2 (Stx2), which is produced by EHEC. This further leads to activation of the coagulation system. Beside this it has been postulated that Stx is able to activate platelets in a direct way. However, there are also hints that the plasmatic coagulation system is affected in HUS. In this study we investigated the effect of Stx2 on platelets and on antithrombin (AT) as a strong inhibitor of the plasmatic coagulation cascade.

Methods: The effect of Stx2 on platelets was investigated by two different experiments. First, aggregometry was performed to show the aggregation inducing capacity of Stx2. Second, in flow cytometry, activation of platelets was shown measuring the expression of two platelet-activation markers, CD62P (P-selectin) and CD63. Concerning AT, ELISA was used to evaluate whether it binds to Stx2. To elucidate the consequences of this binding, a functional assay based on the automated BCS® XP coagulation system (Siemens) was performed to ascertain the inhibiting functions of AT directed against activated coagulation factor II (CF IIa, Thrombin) and X (CF Xa, Stuart-Prower-factor).

Results: Neither in aggregation, nor in the expression of the investigated activation markers CD62P and CD63, a Stx2-induced effect could be observed. However, ELISA revealed a strong binding of AT to Stx2. This binding was confirmed by co-Immunoprecipitation assay. In the functional tests a significant reduction of the anti-factor Xa function of AT was observed at Stx2 concentrations higher than 1.6 ng/ml. However, no significant influence on the anti-FIIa function of AT was detectable.

Conclusion: It appears that platelets are not directly activated by Stx2. However, in this study we show binding of AT to Stx2 which likely directly results in reduced function of AT directed against CF Xa. Therefore we hypothesize, that HUS-associated thrombotic disorders are not only caused by platelet adhesion triggered by Stx2-derived endothelial damage, but may also be mediated through a direct influence of Stx2 via the plasmatic branch of the coagulation system.

N-chlorotaurine, N-chlorodimethyltaurine, and N-dichlorodimethyltaurine are bactericidal against *Pseudomonas aeruginosa* and *Staphylococcus aureus* in a pig cornea infection model

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Background: N-chlorotaurine (NCT), a product of human granulocytes, and its even more stable derivatives N-dichloro- (DC-DMT) and N-monochlorodimethyltaurine (MC-DMT) (NovaBay Pharmaceuticals, Inc.) are promising anti-infective agents for treating conjunctivitis and keratitis. The aim of this study was to investigate whether the microbicidal activity of these active chlorine compounds can also be demonstrated for corneas infected with *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Methods: Corneal discs were punched with a Trepan from eyes from Tyrolean farm pigs. Artificial erosion was created with a hockey knife. Discs were incubated with bacteria and subsequently washed in saline. The different test compounds were applied to the corneas in phosphate buffer at pH 7.1. Discs were homogenized followed by quantitative bacterial cultures or subjected to histological preparation.

Results: In histological sections, not only bacteria attached to the surface, but also accumulations of bacteria in the upper third of the stroma could be seen, if artificial erosion had been performed. All test compounds, 1% (55 mM) NCT, 55 mM DC-DMT, 55 mM MC-DMT, 11 mM NCT+ 37 mM NH₄Cl, or 5.5 mM NCT+ 18.5 mM NH₄Cl, reduced the bacterial counts by approximately 5 log₁₀ after 60 (*P. aeruginosa*) and 120 min (*S. aureus*) incubation. Significant killing started after 5 min incubation and increased continuously with time. Killing of bacteria attached to the surface of the cornea discs with 70% ethanol for 0.5 min led to a reduction of only 0.5-1 log₁₀, indicating that bacteria penetrated into the stroma were inactivated by the test substances.

Conclusions: The results clearly demonstrate that 55mM NCT, MC-DMT, and DC-DMT have the ability to kill *P. aeruginosa* and *S. aureus* in the infected cornea. Addition of ammonium chloride can enhance the activity by formation of monochloramine. Killing kinetics indicate that frequent application of eye drops with chloramines is probably of advantage to cure an eye infection.

Nramp1 induces Lipocalin-2 via enhanced NF-kappaB signalling leading to better killing of *S. typhimurium*

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In mice, the expression of the phagolysosomal protein Nramp1 (natural resistance associated macrophage protein 1, Slc11a1) is of pivotal importance for host resistance to several intracellular pathogens, such as *Salmonellae*, *Mycobacteria* and *Leishmania*. Nramp1 is expressed in phagocytic cells and acts as a transporter for protons, iron and other divalent cations. The expression of Nramp1 is associated with enhanced activity of pro-inflammatory pathways (such as the formation of nitric oxide) as well as down-regulation of the anti-inflammatory cytokine interleukin-10. Lipocalin-2 (Lcn2) is a small antimicrobial peptide, which exerts bacteriostatic effects by depriving bacteria of the essential nutrient iron. Lcn-2 binds and contains iron laden bacterial siderophores, the most important iron source for these microorganisms.

Using RAW264.7 murine macrophages stably transfected with functional (RAW-37) or non-functional (RAW-21) Nramp1, we investigated the influence of Nramp1 expression on Lcn2 production. We found that Nramp1 function leads to up-regulation of both, mRNA and protein levels, of Lcn2 upon stimulation of macrophages with IFN-gamma and LPS. The same findings were observed in peritoneal macrophages taken from Nramp1 resistant or susceptible mice. Upon infection of macrophages with *S. typhimurium* addition of a neutralising anti-Lcn2-antibody abolished the ability of Nramp1-expressing cells to control bacterial growth. Furthermore, Lcn2 (Nramp1 expression) did not affect the growth of intramacrophage *Salmonellae* carrying a mutation of the siderophore enterobactin. Inhibitor experiments and transcription factor activity assays showed that enhanced NFkappaB binding activity leads to upregulation of Lcn2 in Nramp1-functional RAW-37 cells.

Taken together, Nramp1 exerts a novel anti-microbial function via induction of lipocalin-2 production and deprivation of the essential nutrient iron for intracellular pathogens.

Controlling Acute Infection with *Pseudomonas aeruginosa* by Blocking the Interaction with the Complement Regulator Protein fH

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Background: According to the Infectious Disease Society of America, *Pseudomonas aeruginosa* is among the six top-priority dangerous drug-resistant microbes and accounts for 23% of nosocomial infections in intensive care units. Presently, no vaccination is available. Thus alternative approaches to combat this microbe are urgently needed. Among the numerous immune and inflammatory responses upon acute infection of the lung, experiments indicate that the complement system plays a critical role in the protection against *P. aeruginosa*. However this complement-mediated involvement in the immune response does not result in a complete elimination of the microbe, as *P. aeruginosa* acquires and binds fH, a regulator of complement activation (RCA) in fluid phase. By binding of fH on its surface, *P. aeruginosa* interferes with the activation pathways of the complement system and block the induction of the lytic pathway. Therefore, *P. aeruginosa* is protected against complement-mediated lysis (CML). We aim to inhibit the binding of fH to *P. aeruginosa* that should result in the efficient induction of CML, both in vitro and in vivo.

Methods: To investigate our aim we analyzed the binding sites of fH to the microbe by Western blot, ELISA assays and FACS utilizing fH-derived sequences. The bactericidal effect of these fH-derived peptides was analyzed by in vitro lysis assays using normal human serum (NHS) and normal mouse serum (NMS) as a source of complement. To have a closer look at the in vivo situation C57BL/6 mice were inoculated with *P.aeruginosa*, pre-incubated with either fH-derived peptides or PBS. Mice were sacrificed and bacterial load in the lungs was estimated.

Results: Western blot analyses revealed fH-derived peptides to be responsible for binding of fH to *P.aeruginosa*, which was also confirmed by ELISA and FACS. Treatment of *P.aeruginosa* with these peptides resulted in significantly higher complement mediated bactericidal effect of serum. First mouse experiment has confirmed that CML can be induced in vivo resulting in a significant reduction of the bacterial titers upon acute infection of the lung.

Conclusion: Based on these results we can conclude that fH-derived peptides may provide a therapeutic means to enhance the immune response against *P. aeruginosa*, which contributes to the clearance of the pathogen by the immune system in infected patients

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

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Background: Iron overload can adversely influence the course of infection by increasing microbial replication and suppressing anti-microbial immune effector pathways. Recently, we have shown that the calcium channel blocker nifedipine can mobilize tissue iron in mouse models of iron overload. We therefore investigated whether nifedipine treatment affects the course of infection with intracellular bacteria by modulating iron homeostasis.

Methods: RAW 264.7 cells were infected with *Salmonella typhimurium* (S.t_m) or *Chlamydia pneumoniae* and stimulated with varying concentrations of nifedipine and/or EGTA.

C57BL/6 mice were infected intraperitoneally with S.t_m and subsequently injected nifedipine for three consecutive days. Bacterial counts in livers and spleens as well as protein expression were determined by means of Western blot analysis.

Results: Nifedipine treated, S. t_m infected RAW cells showed a significant reduction in bacterial loads. Nifedipine seems to act on a calcium independent pathway, upregulating the iron exporter ferroportin 1. A reduction of intracellular bacteria could be also shown for *Chlamydia pneumoniae*. Mice which were intraperitoneally infected and treated with nifedipine for 3 consecutive days, showed a significant prolonged survival. We found a significant reduction in bacterial loads of livers and spleens from nifedipine treated animals. Protein expression in the spleen, ferroportin upregulation and ferritin downregulation, were in concordance with the in vitro data.

Conclusion: Our study provides evidence that nifedipine affects the course of systemic infection with *Salmonella* as a result of macrophage iron mobilization via ferroportin, which restricts availability of the metal to intracellular pathogens and leads to life prolongation. Nifedipine may be a promising adjunctive therapy for the treatment of infections with intracellular pathogens.

Role of HO-1 and Hif1 α in regulating macrophage iron homeostasis and innate immune response

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Background: Macrophages play an essential role in innate immune which often takes place in hypoxic microenvironments of infected tissue. Inflammatory states are associated with changes in body iron homeostasis. The main systemic response is a rapid fall in plasma iron concentration accompanied by iron sequestration within macrophages. Recently hypoxia-inducible transcription factor 1 (Hif1 α) has been found to regulate macrophage and neutrophil innate immune responses. Similar to Hif1 α , heme oxygenase-1 (HO-1) is involved in stress response, iron homeostasis and host pathogen interactions. In case of the HO-1 most of the physiological functions have been associated with the degrading products of the heme catabolism: ferric iron, biliverdin and carbon monoxide. The protective properties of Hif1 α and HO-1 have been studied in a variety of inflammatory models, however the molecular mechanisms or mode of function in disease, iron homeostasis and immune response remain largely unknown.

Methods/Results: Using RAW264.7 murine macrophages and siRNA knock down of target genes, we investigated the impact of Hif1 α and HO-1 functionality on the expression of iron regulatory molecules. We found that the absence of both genes leads to up-regulation of the iron exporter ferroportin-1 (Fpn1) in both mRNA and protein level. To study the role of Hif1 α and HO-1 in macrophages in *Salmonella enterica* serovar Thyphimurium infection model we found that siRNA technology did not provide reproducible results due to large variations in gene knock down efficiency. Therefore, a lenti-virus based, tetracycline inducible shRNA expression system (under control of TetR-T2A-GFP promoter) has been adapted for HO-1 and Hif1 α in the macrophage cell line RAW264.7. The expression of TetR prevents expression of the shRNA and can be induced by using doxycycline. This technique results in stable and reproducible gene knock down in infected macrophages it is a reliable tool to study the function of specific genes in host pathogen interactions.

Conclusion: Our data suggest a role for HO-1 and Hif1 α in the regulation of iron homeostasis in macrophages. Relevance of the impact HO-1 and Hif1 α on macrophage iron homeostasis and immune control will be studied by using the newly generated shRNA macrophage cell lines and the *Salmonella enterica* serovar Thyphimurium infection model.

The Role of iNOS in Iron Homeostasis and *Salmonella* Infection

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Background: Iron homeostasis and nitric oxide (NO) biology are closely connected to each other since the transcription of inducible NO synthase (iNOS) is controlled by iron while the post-transcriptional control of iron homeostasis via iron regulatory proteins (IRPs) is affected by NO since this labile molecule stimulates the binding affinity of IRPs to its target iron responsive elements (IREs) which links maintenance of iron homeostasis to optimal formation of NO for host defence.

Methods: We studied the effects of the NO donor Nor-5 on the expression of iron metabolic genes in macrophage and evaluated cellular iron homeostasis in *iNOS*^{+/+} and *iNOS*^{-/-} macrophages.

Results: *iNOS* disruption led to significant accumulation of iron in peritoneal macrophages which was paralleled by a significantly decreased ferroportin-1 (Fpn-1) mRNA expression in these cells. The cause-effect relationship between NO and Fpn-1 expression was underscored by the observation that the pharmacological NO donor Nor-5 increased Fpn-1 expression in peritoneal macrophages by a transcriptional mechanism. In addition, peritoneal macrophages from *iNOS*^{-/-} mice showed reduced TNF and IL-12p35 expression following infection with the intracellular pathogen *Salmonella* Typhimurium. While *Salmonella*-infected *iNOS*^{-/-} macrophages displayed increased bacterial load, addition of the iron chelator desferasirox as well as over-expression of Fpn-1 abrogated the differences observed between *iNOS*^{-/-} and *iNOS*^{+/+} macrophages and restored TNF and IL-12p35 production in *iNOS*^{-/-} cells.

Conclusion: Our results demonstrate that NO is a central regulator of iron homeostasis and that its reduction results in an increased iron accumulation in macrophages which can be traced back to down-regulation of Fpn-1 expression due to a transcriptional mechanism. The accumulation of iron in *iNOS*^{-/-} macrophages reduces the expression of M1-type innate host response mechanisms which may partly underlie the impaired immune response of *iNOS*^{-/-} mice.

Binding of Shiga Toxin to Factor H–Related Protein 1

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Background: Typical hemolytic uremic syndrome (HUS), an acute renal disease, is mainly caused by infections with enterohemorrhagic *E.coli* (EHEC) strains. Shiga toxin 2 (Stx2) is a major virulence of EHEC. Recently, an involvement of Stx2 in complement activation and its binding to complement regulatory protein factor H has been described. The aim of our study was to investigate the binding of Stx2 to another member of the FH family, factor H-related Protein 1 (FHR1).

Methods: The experiments were performed by ELISA. Stx2 was immobilized onto microtiter plates. After blocking, FH or FHR1 were introduced, and the bound proteins were detected with polyclonal Factor H antiserum and a secondary anti-sheep IgG conjugated with alkaline phosphatase. The reaction was developed with the chromogen substrate 4-nitrophenylphosphate, and absorbance was measured at dual wavelengths of 415 and 490 nm.

Results: Stx2 does not only bind to FH, but also to FHR1 in a dose dependent manner and, in addition, FHR1 binding appears to be more pronounced than FH binding. FH binding is dose-dependently decreased in the presence of increasing concentrations of FHR1. Stx2 binds to the short consensus repeats (SCRs) 3-5 of FHR1, resembling SCRs 18-20 of FH, while it does not bind to SCRs 1-2 resembling SCRs 6-7 of FH. Two allotypes of FHR1 (FHR1*A and FHR1*B) also bind to Stx2 in dose dependent manner and it appears that Stx2 binds better to FHR1*A, which is found less frequently in atypical HUS, than to FHR1*B. In addition, it is the Stx beta subunit which binds to both FH and FHR1.

Conclusion: In addition to FH, Stx2 can also bind to FHR1 in which SCRs 3-5 represent the binding site. Both proteins compete for binding to Stx2. Stx2 binds to FHR1*A better than FHR1*B, suggesting that this may give some protection against typical HUS. In addition, the Stx beta subunit which is responsible for binding to receptors on host cells, is the region of the toxin that binds to FH and FHR1.

Fibrates attenuate sepsis by enhancing neutrophil influx to the site of infection

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Background: Sepsis is a systemic inflammatory condition following bacterial infection with a high mortality rate and limited therapeutic options. Fibrates are a class of hypolipidemic drugs used to treat hypertriglyceridemia. However, their effects on cardiovascular outcome remain uncertain, restricting their use in the clinical routine. As previous studies indicated pleiotropic anti-inflammatory properties for fibrates, here we aimed to investigate the effect of fibrate treatment on sepsis.

Methods: Sepsis was induced in mice by intraperitoneal injection of salmonella typhimurium, after which the animals were fed chow supplemented with 0.2 % (wt/wt) fenofibrate.

Results: Here we show that fenofibrate treatment significantly reduces mortality in mice with experimental sepsis. Fibrate-treated mice displayed markedly increased neutrophil influx into the peritoneal cavity as soon as after 4 hours and more efficient bacterial clearance than untreated mice. Fibrates reduced the systemic proinflammatory response, and induced a TH1 to TH2 shift. The TH2 shift is likely to be secondary to early control of infection, as fibrates did not alter T cell polarization in vitro. The chemokine receptor CXCR2 is known to be crucial for recruitment of neutrophils to the site of infection. Activation of Toll-like receptors in neutrophils with LPS was previously found to downregulate CXCR2 expression and to impair neutrophil migration. We show here that fibrates prevent the downregulation of CXCR2 and inhibition of chemotaxis induced by LPS in murine neutrophils.

Conclusion: Our results indicate a novel mechanism of action of fibrates and suggest a therapeutic potential for this hypolipidemic drug class in sepsis.

Fungal Infection
Posterwalk by Hubertus Haas

The metalloreductase FreB is involved in adaptation of *Aspergillus fumigatus* to iron starvation

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Background: *Aspergillus fumigatus* employs two high-affinity iron uptake mechanisms, siderophore-mediated iron uptake and reductive iron assimilation (RIA). Genetic inactivation of siderophore biosynthesis, but not RIA, attenuates virulence of *A. fumigatus* in a murine aspergillosis model. Nevertheless, several lines of evidence suggest that RIA also plays a role during infection: (i) elimination of extracellular siderophores causes only partial attenuation of virulence, (ii) during initiation of murine infection both the siderophore system and RIA are transcriptionally induced, and (iii) mutants lacking both RIA and the siderophore system are unable to grow unless supplemented with siderophores or extremely high iron concentrations. The *A. fumigatus* genome encodes 15 putative metalloreductases (MR) but the ferrireductases involved in RIA remained elusive so far.

Methods: Combining phylogenetic analysis, genome-wide expression profiling, and gene deletion analysis with subsequent phenotyping and biochemical analysis was employed to identify the MR involved in RIA in *A. fumigatus*.

Results: Expression of the MR AFUA_1G17270, termed FreB, was found to be transcriptionally repressed by iron via SreA, a repressor of iron acquisition during iron sufficiency, and upregulated during initiation of infection in a murine model of invasive pulmonary aspergillosis. FreB-inactivation by gene deletion was phenotypically largely inconspicuous unless combined with inactivation of the siderophore system, which then decreased growth rate, surface ferrireductase activity and oxidative stress resistance during iron starvation. This study also revealed that the copper-independent siderophore system increases resistance of *A. fumigatus* to copper starvation due to copper-dependence of RIA.

Conclusion: This study identified the first ferrireductase involved in RIA in a filamentous fungus and underlined the partial redundancy of siderophore-mediated iron uptake and RIA. The MR FreB is a heme-iron-dependent enzyme and therefore its upregulation during iron starvation demonstrates metabolic prioritization of available iron during this condition.

The apoptosis inducing factor (AIF) - like mitochondrial oxidoreductase mediates resistance towards the antifungal protein PAF in *Aspergillus fumigatus*

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Background: The antifungal protein PAF of *Penicillium chrysogenum* inhibits the growth of the opportunistic human pathogen *A. fumigatus*. It induces the formation of reactive oxygen species and triggers programmed cell death. A genome wide expression analysis of *A. fumigatus* exposed to PAF indicated that – among other stress responsive genes – the AIF like mitochondrial oxidoreductase gene was induced. AIF is a highly conserved mitochondrial membrane protein and – unlike in *Aspergillus* sp. – the main mediator of caspase independent programmed cell death in mammalian and yeast cells. However, in *A. nidulans* the AIF plays an anti-apoptotic role by regulating the production of reactive oxygen species (ROS) through its putative oxidoreductase and peroxide scavenging activities and has a function in the electron transport in Complex I of the mitochondrial respiratory chain. We therefore were interested in elucidating the role of the *aifA* gene in the PAF response of *A. fumigatus* and characterized an *aifA* deletion strain.

Methods: We tested the susceptibility of a $\Delta aifA$ strain compared to *A. fumigatus* wild-type towards PAF by growth inhibition assays. We further tested the mitochondrial activity in both strains exposed to PAF by determining the oxygen consumption and the intracellular ATP production. Finally, we performed fluorescence stainings in both strains to address the question whether the mitochondria are the primary ROS producing organelles in response to PAF.

Results: We could demonstrate that the *A. fumigatus aifA* deletion mutant was hypersensitive to the antifungal protein PAF compared to the wild-type. Instead, both strains showed comparable sensitivity when treated with the non-primary oxidative stress inducing agents hydroxyurea and 4-nitroquinoline-oxide. Congruently with the oxidative stress inducing function of PAF, the amount of ROS specific signals in PAF treated *A. fumigatus* wild-type increased compared to the untreated control. However, these signals did not co-localize with mitochondria. We could further show that the intracellular ATP level significantly decreased only 15 minutes after PAF exposure whereby the decrease was PAF concentration dependent and more prominent in the *aifA* deletion mutant than in *A. fumigatus* wild-type. Similarly, the oxygen consumption decreased rapidly in the presence of PAF and the $\Delta aifA$ mutant strain was more affected than the wild-type strain underscoring the function of AIF in an efficient electron transport in the mitochondrial Complex I.

Conclusion: The results of this study strengthen the antifungal protein PAF to act as a primary oxidative stress inducing agent in *A. fumigatus* that affects mitochondrial function at an early time point. Our data further indicate that the AifA plays an important role in antagonizing the toxicity of PAF and protecting mitochondria from ROS which is produced outside of these organelles.

The interplay of vacuolar and siderophore-mediated iron storage in the opportunistic fungal pathogen *A. fumigatus*

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Background: Due to its essential role in a wide variety of cellular processes, iron is an essential element for all eukaryotes but its excess is deleterious. As microorganisms lack iron excretory mechanisms, iron homeostasis results from tight regulation of iron acquisition and iron storage to ensure sufficient iron supply and to prevent iron toxicity. *Aspergillus fumigatus* produces the extracellular siderophores (low-molecular mass iron chelators) triacetylfusarinine C (TAFC) and fusarinine C (FSC) for iron uptake and the intracellular siderophores ferricrocin (FC) and hydroxyferricrocin for distribution and storage of iron. On the one hand, siderophore biosynthesis is important for adaptation to iron starvation and therefore crucial for virulence. On the other hand, intracellular iron excess has been shown to increase the content of FC-chelated iron and the expression of AFUA_4g12530, termed CccA, which shows similarity to the vacuolar iron importer Ccc1 of *Saccharomyces cerevisiae*. These data indicate a role of both the vacuole and FC in iron detoxification.

Methods: The function of *A. fumigatus* CccA and the interplay of vacuolar and FC-mediated iron storage was characterized by analyzing the consequences of deletion or overexpression of *cccA* in various genetic backgrounds.

Results: Green fluorescence protein-tagging confirmed localization of CccA in the vacuolar membrane of *A. fumigatus*. During high iron conditions but not iron starvation, genetic inactivation of CccA impaired growth in various genetic backgrounds, in particular in combination with derepressed iron uptake due to deficiency in the iron regulator SreA. In contrast, overproduction of CccA increased iron resistance. Inactivation of FC biosynthesis did not affect iron resistance. In contrast to *S. cerevisiae*, *A. fumigatus* appears to lack mechanisms to recycle vacuolar-stored iron. Uptake of ferric TAFC and FSC is followed by hydrolysis of the siderophore backbone. The iron is transferred into the metabolism, chelated by FC or transported into the vacuole and TAFC/FSC breakdown products are recycled. Lack of FC, CccA and in particular both, increased the cellular content of iron chelated by FSC/TAFC breakdown products. These data indicate that the transfer of iron precedes recycling of FSC/TAFC degradation products, which might represent another iron detoxifying mechanism.

Conclusions: Taken together, these data indicate that vacuolar rather than FC-mediated iron storage is the major iron detoxifying mechanism of *A. fumigatus*.

The *Penicillium chrysogenum* Conidiogenesis is Modulated by the *paf* Gene Product

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Background: The growth of *Penicillium* species can cause severe health problems in humans as they infest plants and fruits and produce unfavourable allergens and highly toxic mycotoxins. Importantly, the exposure to conidia is a major factor of the sensitization of atopic patients. Especially allergic and/or asthmatic patients suffer from exposure to this airborne fungus. Among *Aspergillus*, *Cladosporium* and *Alternaria*, *Penicillium chrysogenum* is the most prevalent species indoors as well as outdoors during all seasons and may cause rhinitis and severe asthma¹. Although *Penicillium* has such a high prevalence in our life, the regulation of conidiogenesis is less well studied than in other filamentous fungi.

P. chrysogenum abundantly secretes a low molecular weight, highly basic and cysteine-rich antifungal protein PAF (*Penicillium* antifungal protein) that inhibits the growth of numerous filamentous ascomycetes, thus conferring an ecological advantage for the producing organism in a highly competitive microbial ecosystem^{2,3}. The expression of the *paf* orthologous gene *afp* in *A. giganteus* surface cultures is developmentally regulated⁴, which raised the interesting questions if the expression of *paf* is associated with asexual development of *P. chrysogenum*, and whether the production of the protein can influence the mitospore development of the producing organism.

Methods: We examined the transcriptional profile of *paf* in *P. chrysogenum* surface cultures with Northern analysis. In parallel asexual development of the cultures was monitored, applying developmental markers such as conidia production and transcriptional analysis of the developmentally expressed genes *brlA*, *rodA* and *rodB*. Moreover, we deleted the *paf* gene in *P. chrysogenum* Q176 strain and examined the mitospore development in the Δpaf strain.

Results: Transcriptional analysis of *P. chrysogenum* surface cultures revealed that the expression of the *paf* gene is spatially and temporally regulated during asexual development. The *paf* deletion strain produced significantly less mitospores and showed a significantly reduced gene expression of *brlA*, *rodA* and *rodB*. Retransformation of the *paf* wild type copy into *P. chrysogenum* Δpaf genome restored the reduced conidia production of the mutant strain.

Conclusion: Our data indicate that PAF enhances conidiation in *P. chrysogenum* by modulating the expression of *brlA*, the central regulatory gene for mitospore development⁵. Importantly, the characterization of PAF as a self-regulation molecule that controls conidiogenesis might allow to develop new approaches to suppress sporulation and thus minimize the risk of exposure and infection with *Penicillium*.

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The CCAAT-binding-complex is Involved in HapX-mediated Gene Regulation in *Aspergillus fumigatus*

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Background: Iron is both, essential for a wide range of cellular processes and toxic in excess since it can catalyze the generation of toxic reactive oxygen species. Therefore all organisms have evolved control systems to maintain the balance between uptake, consumption and storage of iron. In the opportunistic fungal pathogen *A. fumigatus*, the bZIP-type transcription factor HapX mediates adaptation to iron starvation by activation of siderophore biosynthesis and repression of iron-dependent pathways. Consequently, HapX-deficiency results in attenuation of *A. fumigatus* virulence in a mouse model. The *A. nidulans* HapX ortholog has previously been shown to repress iron-dependent pathways by protein-protein interaction with the DNA-binding, heterotrimeric CCAAT-binding complex (CBC). The CBC is conserved in all eukaryotes and believed to participate in regulation of up to 30 % of all genes.

Methods: The role of the CBC in iron regulation in *A. fumigatus* was characterized by comparison of the consequences of genetic inactivation of HapX, the CBC subunit HapC, and both.

Results: HapX-deficiency impaired growth and conidiation on solid media and biomass production in liquid media during iron starvation but not iron sufficiency. In contrast, HapC-deficiency was deleterious during both iron starvation and iron sufficiency, which underlines iron regulation-independent functions of the CBC. Similar to HapX-deficiency, HapC deficiency blocked colony formation from single conidia in the presence of the iron chelator bathophenanthroline disulfonate, decreased production of the extracellular siderophore triacetylfusarinine C, decreased transcript levels of siderophore-biosynthetic genes, and increased transcript levels of genes involved in iron-consuming pathways. Concurrent deficiency in both HapX and HapC phenocopied HapC-deficiency.

Conclusion: These data suggest that in *A. fumigatus* both the activating and repressing functions of HapX require the CBC, most likely via direct protein-protein interaction as shown previously for *A. nidulans*.

⁶⁸Ga-siderophores for imaging of invasive pulmonary aspergillosis

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Background: Invasive pulmonary aspergillosis (IPA) mainly caused by *Aspergillus fumigatus* (*A.f.*), is a major cause of morbidity and mortality in neutropenic patients. Current diagnostic methods lack specificity and/or sensitivity and early detection could be life-saving. We showed that siderophores can be labelled with ⁶⁸Ga and be used for PET imaging of *A.f.* infection in rats. Here we report on the comparison of the most promising ⁶⁸Ga-siderophore candidates.

Methods: Studied siderophores were labelled with ⁶⁸Ga using acetate buffer. Log P, protein binding and stability in different media were determined. *In vitro* uptake was studied in *Aspergillus fumigatus* (*A.f.*), *flavus* (*A.fl.*), *terreus* (*A.t.*), *Candida albicans* (*C.a.*), *Rhizopus oryzae* (*R.o.*), *Fusarium solani* (*F.s.*), *Pseudomonas aeruginosa* (*P.a.*) and *Klebsiella pneumoniae* (*K.p.*) iron-deplete and iron-replete cultures. *In vivo* biodistribution was performed in normal mice and infection model was established using immunosuppressed rats inoculated by *A.f.* Static scans and dynamic μ PET imaging were performed and correlated with *ex vivo* control of lung infection.

Results: ⁶⁸Ga-siderophores can be labelled with high radiochemical purity and specific activity. The most promising siderophores ⁶⁸Ga-Triacetylfusarinine C (TAFC) and ⁶⁸Ga-Ferrioxamine E (FOX E) showed hydrophilic properties, low protein binding and high stability. Both compounds revealed high uptake in *A.f.* iron-deplete cultures and significantly lower uptake in *A.fl.*, *A.t.*, *R.o.*, *F.s.*, whereas in *C.a.* and *P.a.*, *K.p.* negligible uptake was observed. Overall uptake of ⁶⁸Ga-FOX E was higher than of ⁶⁸Ga-TAFC. In normal mice ⁶⁸Ga-TAFC and ⁶⁸Ga-FOX E showed rapid renal excretion with high metabolic stability. In the rat infection model focal lung uptake was detected and increased with severity of the infection, whereas in control rats no uptake in lung was observed. Static scans were performed 30 min p.i. The dynamic imaging showed rapid uptake and no washout from infected lung tissue for the whole imaging period (60 min).

Conclusion: ⁶⁸Ga-FOX E and ⁶⁸Ga-TAFC showed high *in vitro* uptake in fungal species, especially in *A.f.* Both compounds displayed excellent pharmacokinetics, highly selective accumulation in infected lung and correlation with severity of disease in the rat infection model, which makes them promising candidates for sensitive and specific imaging of IPA.

***Aspergillus Fumigatus* Secretes Soluble Factors that induce Complement Deposition on Thrombocytes**

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Background: Invasive aspergillosis is a life-threatening disease with high mortality and lethality rates. Mechanisms of pathogenesis and immune defense are insufficiently clarified, and further studies are an urgent need. A more profound knowledge about interactions between the fungus and innate immunity may help to develop new therapeutic approaches. Various proteins of the complement system interact specifically with thrombocytes to activate them and vice versa. In our studies we aimed to examine the influence of soluble factors secreted by *A. fumigatus* on the deposition of complement proteins on platelets.

Methods: *A. fumigatus* was grown in RPMI medium; culture supernatant was harvested after 2 days. Platelets were incubated with different concentrations of the supernatant and subsequently opsonized with serum. Fluorescent antibodies were used to label deposited complement proteins and the platelet activation marker CD62P (P-selectin). Complement deposition and platelet activation were analyzed by flow cytometry.

Results: *Aspergillus fumigatus*, when grown in cell culture medium, secretes factors that are able to activate human thrombocytes, as shown by appearance of the activation marker CD62P on the platelet surface. As CD62P has been described to serve as a receptor for complement factors, we investigated the deposition of complement on thrombocytes as a consequence of incubation with the fungal supernatant. Our FACS analysis showed a strong opsonization of the thrombocytes with complement factor C3. Incubation times of only 30 minutes were sufficient to increase the appearance of C3 on the platelet surface. In addition, also other complement factors such as C1q and C5 could be detected on the thrombocytes. Kinetic studies showed a perfect correlation between the fungal factor-induced membrane exposure of CD62P and the amount of deposited complement proteins. Furthermore, we could show that even very low volumes of the *A.fumigatus* culture supernatant were sufficient to elicit the effects of thrombocytes activation and complement deposition

Conclusions: The secretion of soluble factors by *A. fumigatus* induces platelet activation and the deposition of complement factors on the platelet surface; these processes may lead on to various possible consequences that need to be further clarified. On one hand, the spreading of the fungus in the host may be facilitated by consumption of complement proteins and probable ingestion of opsonized thrombocytes by phagocytes. On the other hand, the concurrent activation of platelets and the complement system might induce increased cytokine production and attract immune cells to the focus of infection, which could help to limit the fungal dissemination.

The Role of Ornithine Supply in Siderophore Biosynthesis in *A. fumigatus*

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Background: Iron is an essential nutrient required for a wide range of cellular processes. However, excessive iron accumulation is toxic. Iron is highly abundant in the Earth' crust, but its bioavailability is low as its present mainly as insoluble oxyhydroxides. Therefore, microorganisms evolved fine-tuned iron uptake and storage mechanisms, such as the siderophore system. The opportunistic fungal pathogen *Aspergillus fumigatus* produces four siderophores (low-molecular mass iron-specific chelators): extracellular triacetylfusarinine C and fusarinine C for iron uptake, and intracellular ferricrocin and hydroxyferricrocin for storage and distribution of iron. Moreover, siderophores have been shown to play a crucial role in the pathogenicity of this fungus. Previous studies indicated coordination of siderophore biosynthesis with supply of its precursor ornithine.

Methods: The role of mitochondrial ornithine production in siderophore biosynthesis of *Aspergillus fumigatus* was characterized by analysis of the phenotypic consequences of genetic inactivation of the putative mitochondrial ornithine exporter, AmcA (Afu_8g02760).

Results: Consistent with a role in mitochondrial ornithine export, inactivation of AmcA resulted in a decrease in the cellular ornithine content as well as a decrease in extra- and intracellular siderophore production. In the presence of the iron chelator bathophenanthroline disulfonate, which inhibits siderophore-independent iron uptake, AmcA-deficiency decreased conidiation indicating increased iron starvation. In contrast to siderophore production, AmcA-deficiency did not affect the cellular content in polyamines, which are also derived from ornithine via ornithine decarboxylase. Nevertheless, AmcA-deficiency increased the susceptibility of *A. fumigatus* to eflornithine, an inhibitor of ornithine decarboxylase, most likely due to the decreased ornithine pool.

Conclusion: These data indicate that siderophore biosynthesis is mainly fueled by mitochondrial production of ornithine, rather than by conversion of arginine to ornithine in the cytoplasm. The transcriptional upregulation of AmcA during iron starvation underlines the coordination of siderophore biosynthesis and its precursor supply. Moreover, this study indicates a cellular prioritization of ornithine flux into biosynthesis of polyamines compared to siderophores emphasizing the essentiality of polyamines.

***Aspergillus fumigatus* secretes factors that strongly activate human thrombocytes**

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Background: *Aspergillus fumigatus* is the predominant inducer of invasive aspergillosis, a life-threatening disease for immunocompromised patients. Platelets represent a part of the innate immunity and thus might participate in the antifungal immune defense, but also contribute to inflammation and thrombosis. We studied the hypothesis that *A.fumigatus* secretes soluble factors that modify activity and functionality of thrombocytes.

Methods: *A. fumigatus* was grown for 2 days in medium. The supernatant was harvested and different volumes were added to freshly isolated human platelets. To identify relevant signal transduction pathways various inhibitors were added to the sample. After the incubation activation of thrombocytes was quantified by FACS analysis of the marker CD62P and annexin binding. Furthermore platelet aggregation was studied by aggregometry.

Results: Even minimal volumes of the fungal culture supernatant were capable to potently stimulate the platelets, inducing high expression of CD62P on the surface. Furthermore the secreted fungal factors increased annexin binding of the platelets and induced significant thrombocyte aggregation, even after few minutes of incubation. Experiments using different signal transduction inhibitors indicated that calcium release, PI-3 kinase and the protein tyrosine kinase Syk participated in the signal transduction pathways leading to the stimulation of thrombocytes. Two active components in the fungal culture supernatant could be identified. First, the role of a fungal serine protease was confirmed by use of serine protease inhibitors, which partly eliminated the thrombocyte-stimulating capacity of the *A. fumigatus* supernatant. Second, the mycotoxin gliotoxin seems to play a role, since an *A. fumigatus* mutant unable to synthesize this mycotoxin does not stimulate the thrombocytes to an large extent. Furthermore, the effect of the fungal supernatant could be mimicked by purified gliotoxin. Preliminary experiments with glutathione, a reducing compound that inactivates gliotoxin, suggest the possibility to counteract the action of the mycotoxin and thus to reduce the danger of excessive platelet activation during invasive aspergillosis.

Conclusion: Secreted fungal factors such as proteases and mycotoxins might participate in thrombocyte activation during invasive aspergillosis. Putative consequences could be a platelet-driven antimicrobial response but also, on the other hand, thrombosis and thrombocytopenia.

Various Diseases
Posterwalk by Günter Weiss

Atypical x-linked chronic granulomatous disease in a 10 year old boy

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Background: Introduction:Chronic granulomatous disease (CGD) is an inherited phagocyte disorder caused by mutations in nicotinamide dinucleotide phosphate (NADPH) oxidase subunits. Phagocytic cells of CGD patients are unable to produce superoxide anions and their efficiency in bacterial killing is significantly impaired. It results in a susceptibility to infections of catalase-positive bacteria and fungi (especially *Aspergillus* species), Severe and recurrent skin infections and lymph node abscesses are the most common manifestations of CGD during early childhood. Intra-hepatic abscesses occur later on.

Case report: We report a case of a ten year old boy who was diagnosed as a juvenile sarkoidosis while presenting with cervical and pulmonary lymphadenopathy and high angiotensin converting enzyme. In November 2001 he was hospitalized for septic fever, and ultrasonographic evidence of liver abscess. Surgical treatment revealed a liver abscess in the right lobe.

Methods: Autosomal recessive CGD was diagnosed based on partial lack of superoxide anion production by phagocytes. The dihydrorhodamine 123 (DHR) assay established X-linked gp91(phox) mutation.

Conclusion: An untypical, partial CGD should considered by lymphadenopathy going along with good clinical state.

Early Neuroborreliosis in children is a diagnostic challenge

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Background: Early neuroborreliosis is the second most common manifestation of acute Lyme disease in Europe after erythema migrans. There exists no specific pediatric case definition of neuroborreliosis. The aim of our study is to investigate the role of Lyme disease in the etiology of acute peripheral facial palsy in children.

Patients and Methods: Our patient cohort consisted of acute peripheral facial palsy cases, who were referred to the Department of Pediatrics, Medical University of Innsbruck, Tyrol, Austria, from January 2002 to December 2006. The diagnosis of neuroborreliosis is based on the adapted criteria of the German Neurology Association (table 1).

Results: Lyme disease was the most common cause of facial palsy in Tyrolean children between 2002 and 2006 (43.3%). 7 cases were classified as possible neuroborreliosis, 17 cases as probable and 5 cases as confirmed neuroborreliosis.

Conclusions: Our study is the first assessing pediatric neuroborreliosis and discussing diagnostic guidelines for this frequent manifestation of Lyme disease in children. Clear cut diagnostic criteria defining pediatric neuroborreliosis, are urgently needed to ensure a high degree of diagnostic safety. Based on our current data from the endemic region of Tyrol we suggest a clinical algorithm for the management of children with acute peripheral facial palsy due to Lyme disease.

Renal Transplantation in Factor H Antibody positive aHUS Patients: Results from the International Innsbruck HUS-Net Registry

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Introduction: Antibodies against complement factor H (CFH Ab) have been detected as cause for atypical haemolytic uremic syndrome (aHUS). At present, evidence based therapy recommendations are missing. Many of the patients develop end stage renal disease (ESRD), however, outcome after renal transplantation can be impaired due to the risk of recurrence.

Methods: We report on 16 patients with FH Ab associated aHUS from the Innsbruck HUS-Net registry (www.hus-online.at). Patients were followed from the acute phase over a period of 1 year with recording patient's therapy and the clinical course.

Results: Patients showed a median age of 7 years at disease onset. Within the follow up period of 1 year 25% of the patients developed chronic renal insufficiency, 33% suffered from ESRD, and 67% showed at least one episode of recurrence. Without any plasmatherapy and/or immunosuppression 2/2 ESRD-patients (both on Dialysis) showed disease recurrence, 6/7 patients recurred under plasmatherapy alone and only 2/7 patients, who were treated with plasmatherapy followed by immunosuppression, developed recurrences.

Three patients were transplanted, one patient with a graft of a deceased donor, 2 patients with grafts of living related donors. As induction therapy either ATG or Basiliximab was administered. In one patient, plasmapheresis was performed pre- and 7 days post- transplantation. Continuous immunosuppression with Tacrolimus, MMF and Steroids. 10 months after transplantation complement levels (C3, terminal complement complex) are normal in all three transplanted patients and the FH-Ab titers are in the low range (Cut off <100 AU/ml; low range <500 AU/ml). None of the patients showed disease recurrence.

Conclusion: CFH Ab positive patients are a distinct pathogenetic aHUS subgroup, mainly seen in pediatric patients. Testing for CFH Ab as soon as possible after diagnosis of an aHUS is mandatory since prognosis and recommended therapy is different in positive tested individuals. Based on our results and also published data plasmatherapy should be used for bridging the period of analysis followed by a maintenance therapy with immunosuppressive agents plus/minus intravenous immunoglobulins.

Regarding the management after renal transplantation the reduction of CFH Ab titers well-timed before and an adequate (possibly more intense as usual) maintenance immunosuppression are major principles. Perioperative plasmapheresis may be performed to further insure a recurrence-free postoperative course.

Enhancement of complement-dependent cytotoxicity improves Rituximab based B-CLL therapy

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Background: The monoclonal antibody (MAb) Rituximab introduced a profound shift in the therapeutic landscape of chronic lymphocytic leukemia (CLL). Phase III clinical trials clearly indicate that the addition of rituximab to fludarabine-based chemotherapy improves complete response rates and prolongs progression-free survival.

Despite the outstanding potency of mAb, their clinical efficacy in human cancer is far from optimal and several patients treated with Rituximab do not respond to therapy. The effector mechanism of Rituximab is mainly antibody-mediated cellular cytotoxicity (ADCC). In addition, the binding of mAbs to tumor cells is thought to induce complement (C) activation which may further contribute to the destruction of tumor cells by complement-dependent cytotoxicity (CDC). However these effector functions are limited, as Rituximab is not very efficient in the induction of complement activation and tumors, similar to normal cells, are protected from C-induced damage by regulators of C activation (RCAs), which are over-expressed by certain tumors. Among these RCAs are proteins in fluid phase, like factor H (fH). A common motif of many RCAs is a repeat of about 60 amino acids (aa), called short consensus repeats (SCRs). fH is organised in 20 SCR units. SCR 7 and mainly SCR18-20 mediate the binding of fH to negatively charged cell surface structures such as heparan sulfates. Binding of fH to negatively charged host cells contributes to the protection against damage induced by the host's own complement. Therefore, blocking of the fH binding to CLL cells by SCR18-20 may render the cells increasingly susceptible to CDC in the presence of Rituximab.

Methods: CDC-assays were performed with peripheral blood mononuclear cells (PBMCs) of CLL patients in the presence of normal human serum (NHS), Rituximab, fH-derived protein and/or blocking CD55 and CD59 antibodies. Survival rates were estimated by FACS analysis capturing the Propidium iodide (PI) negative cell fractions.

Results: In complement-based lysis assays CLL cells from therapy naive patients responded different to Rituximab induced CDC. In one group up to 40% of the cells were killed by complement (CDC responder) while the second group showed no or minor CDC (CDC non-responder). In both groups, SCR18-20 boosted CDC. The killing of the cells was specific as CD3-positive cells were not affected. Irresponsiveness to CDC was independent on the expression of the membrane-anchored RCAs CD55 or CD59, although blocking of these RCAs further boosted CDC.

Conclusion: The addition of SCR18-20 showed efficacy in both CDC responder and non-responder and improved the selective killing of CLL cells significantly. SCR18-20 may complement Rituximab based treatment of CLL patients to further optimize tumor therapy.

Complement Activating Antibodies to MOG and AQP4 in CNS demyelinating diseases

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Background: Autoantibodies targeting aquaporin-4 (AQP4) are important biological markers and pathogenic factors in Neuromyelitis optica spectrum disorders (NMOSD). Recent studies indicated a role for anti-myelin oligodendrocyte glycoprotein (MOG) autoimmune responses in animal models of NMOSD. Although high titer autoantibodies to human native MOG were detected in pediatric acute disseminated encephalomyelitis (ADEM) and multiple sclerosis (MS) patients, the role of anti-MOG antibodies in NMOSD remains unresolved.

Methods: Therefore, we analyzed serum samples from patients with NMOSD (n=69), ADEM (n=33), clinically isolated syndrome (16), MS (65), other neurological diseases (OND, n=24), systemic lupus erythematosus (SLE, n=26) and 47 healthy controls for antibodies to MOG and AQP4 via immunofluorescence assay. Furthermore, we investigated their ability to induce complement mediated cytotoxicity (CDC).

Results: Anti-AQP4 IgG were detected in 54 patients (78%) with NMOSD, but not in other diseases and controls. In contrast, we detected high titer anti-MOG antibodies in 14 patients with ADEM (42%, median titer 1:2,560, range 1:160-1:20,480), MS (n=2, both 1:160), CIS (n=2, 1:640 and 1: 5,120), SLE (n=2, 1:160 and 1:320), one OND (1:640) and 4 of 15 NMO-IgG seronegative NMOSD patients (median titer 1:2,560, range 1:1,280-1:5,120), but not in anti-AQP4 antibody positive patients. Antibodies to MOG and AQP4 were predominantly IgG1 and activated the complement cascade.

Conclusion: Human antibodies to MOG and AQP4 might provide new insights in the pathogenesis of NMO.

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Economic evaluation of vaccines – Specific methodological aspects

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Background: Cost-effectiveness is an increasingly important aspect in reimbursement decisions and price negotiations. Many countries have guidelines for economic evaluation of health technologies. However, these usually lack specific recommendations for the evaluation of vaccines, although vaccines differ from other health technologies in important aspects, which should be considered in a valid economic evaluation. Besides technology-specific particularities, particularities of the national decision context must be taken into account. For example, in Germany the efficiency frontier approach is proposed to derive upper limits of reimbursement within specific disease areas, whereas in the UK a similar willingness-to-pay threshold is proposed for all diseases with few exceptions. Our objective is to present methodological recommendations for the economic evaluation of vaccines, and to review the appropriateness of the German efficiency frontier approach for these evaluations.

Methods: We identify differences between vaccination and other health technologies, and use them to derive methodological recommendations for the economic evaluation of vaccines. Subsequently, we check whether the German efficiency frontier approach meets the identified methodological requirements for vaccine evaluation.

Results: Vaccinations differ from therapeutic technologies in several aspects affecting the methodology of economic evaluation. The main difference are indirect effects caused by herd immunity, that reduces the risk of infections also among unvaccinated individuals, increases the average age at infection and the interval between outbreaks, and reduces natural booster effects. Vaccination may also have indirect effects on following generations, which is rather uncommon with therapeutic technologies. Further important specifics of vaccinations are a considerable time lag between the occurrence of cost and health-benefit, a broad spectrum of vaccination-related health effects that need to be valued and integrated in order to assess the overall health benefit of vaccination, and a paucity of competing technologies. Mostly, the only alternative to vaccination is not to vaccinate. The identified specifics of vaccination warrant specific methods for economic evaluations. Herd immunity effects might require the use of dynamic modeling, a generation-spanning time horizon, and a societal perspective. The diversity of health outcomes argues in favor of cost-utility analysis. Discounting, which strongly affects the efficiency of preventive technologies, should be symmetric for cost and health effects. The paucity of alternative options questions the feasibility of the German efficiency frontier approach. Objections against the use of aggregated health measures pose another problem.

Conclusion: General guidelines for economic evaluation of health technologies are inadequate for the evaluation of vaccines. The German efficiency frontier approach may not always be feasible.

Hydroxychloroquin in der Therapie des Lupus erythematoses

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Hintergrund:

Hydroxychloroquin nimmt einen wichtigen Platz in der Therapie vor allem kutaner Lupusformen ein. Es wirkt immunsuppressiv und antiinflammatorisch, weiters hat es auch antithrombotische und cholesterinsenkende Eigenschaften. Neben gastrointestinalen und hepatischen Nebenwirkungen kann ihr Einsatz durch das Auftreten einer Retinopathie limitiert werden. Weiters gibt es Hinweise, dass Rauchen zu schlechterem Ansprechen führt. Dies könnte eventuell durch eine verschlechterte lysosomale Anreicherung des Medikaments oder durch eine schnellere Metabolisierung bedingt sein.

Methodik:

In einer retrospektiven Untersuchung von 102 Lupuspatienten, die zwischen 1988 und 2009 mit Hydroxychloroquin (Quensyl®, Plaquenil®) behandelt wurden, evaluierten wir Therapieindikationen, Therapiedauer, Ansprechen der verschiedenen Lupusmanifestationen, Nebenwirkungen und Ansprechen bei Rauchern.

Ergebnisse:

Hauterscheinungen und Arthralgien waren die Hauptindikationen, Gerinnungsstörungen eine Nebenindikation. Die Therapiedauer war zumeist länger als 6 Monate, in 20% länger als ein Jahr. Die Therapie mit Hydroxychloroquin führte in über 90% der Patienten zu einer Besserung, drei Viertel der Patienten sprachen innerhalb von 6 Wochen an. Bei Hautmanifestationen des tumiden LE und des chronisch diskoiden Lupus erythematoses verzeichneten wir ein fast 100%iges Ansprechen. Arthralgien besserten sich in 85%. 11% der Patienten klagten über gastrointestinale Nebenwirkungen. 6% entwickelten eine Retinopathie, dies hatte in allen Fällen einen Therapieabbruch zur Folge. 50% unserer Patienten waren Raucher. Während das allgemeine Ansprechen in dieser Patientengruppe überraschenderweise fast gleich hoch war wie bei den nichtrauchenden Patienten, fiel ein verzögertes Ansprechen auf.

Schlussfolgerung:

Hydroxychloroquin ist ein effektives und sicheres Therapeutikum für Haut- und Gelenksmanifestationen des Lupus erythematoses. Hohe Ansprechraten und ein schneller Therapieerfolg stehen geringen Nebenwirkungen gegenüber. Ophthalmologisches Monitoring sollte zu Therapiebeginn und dann alle 6 Monate erfolgen. Rauchen führte in unserem Patientenkollektiv zu langsamerem Ansprechen und sollte deshalb vermieden werden.

Hypoxia Mediated Down-regulation of Heparin is Mediated by A Tissue Growth Factor

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Background: Iron metabolism is altered during hypoxia which may impact on hematopoietic response as well as immunosurveillance during oxygen deprivation.

Methods: Using a standardized hypoxia chamber 23 healthy volunteers were subjected to exercise under hypoxic conditions, being consistent with an altitude of 5600 meters, for six hours. Consecutive in vitro and in vivo experiments, using C57BL/6 mice, were performed using a standardized hypoxia cell culture chamber or a hypoxic chamber adjusted for mice, respectively.

Results: Hypoxia resulted in a highly significant decrease of serum hepcidin concentrations accompanied with elevated serum Epo and tissue growth factor concentrations. Using regression analysis we identified a tissue growth factor, which hasn't been implicated in hepcidin regulation so far, to be an independent regulator of hypoxia mediated hepcidin regulation. The functionality of this tissue growth factor in hepcidin signalling was confirmed using in vitro cell culture experiments and in vivo mouse models.

Conclusions: Hypoxia impacts on hepcidin mediated iron regulation which is at least in part mediated via tissue growth factors. This finding sheds new light on the understanding of the regulation of iron homeostasis during hypoxia.

High Fat Diet Leads to Iron Deficiency Due to Heparin Independent Reduction of Duodenal Iron Absorption in a Murine Animal Model of Obesity

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Background: Obesity is often associated with iron deficiency, however, the underlying mechanisms are not fully understood. Heparin is a key regulator of iron metabolism and has been suggested to be responsible for obesity driven iron deficiency.

Methods: Using an animal model of diet-induced obesity we herein studied changes in iron homeostasis and the expression of iron metabolism genes in various tissues. Ninety C57BL/6 mice were fed with standard (SD) or high fat diets (HFD) for eight weeks, and in addition half of the mice were supplemented with high dietary iron (Fe+) for the last two weeks.

Results: Hepatic and splenic iron contents were significantly lower in HFD fed than in SD fed mice supplemented with iron. While neither hepatic and adipose tissue nor serum heparin concentrations differed significantly between SD and HFD fed mice, dietary iron supplementation resulted in increased heparin expression in SD but not in HFD mice. This was mirrored by a significant reduction of duodenal iron absorption in HFD fed mice. Accordingly, the mRNA expression of the duodenal iron transporters ferroportin 1, Dmt1 and TfR1 were higher in HFD fed mice indicating enterocyte iron deficiency, whereas the mRNA levels of the duodenal iron oxidoreductases DcytB and hephaestin were lower in HFD fed mice. In parallel, the expression of markers of inflammation and adipocytokines in the liver did not vary as a function of dietary fat content.

Conclusions: Our study suggests that HFD results in iron deficiency on the basis of impaired iron absorption which is neither linked to increased heparin expression nor to inflammation. In contrast, discordant expression of duodenal oxidoreductases indicates defective iron transfer across enterocyte membranes.

Pharmacologic Inhibition of Hepcidin Expression reverses Anemia of Chronic Disease in Rats

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Background: Anemia of chronic disease (ACD) is the most frequent anemia in hospitalized patients and is associated with significant morbidity. A major underlying mechanism of ACD is the retention of iron within cells of the reticuloendothelial system (RES), thus making the metal unavailable for efficient erythropoiesis. This reticuloendothelial iron sequestration is primarily mediated by excess levels of the iron regulatory peptide hepcidin down-regulating the functional expression of the only known cellular iron export protein ferroportin resulting in blockade of iron egress from these cells.

Methods/Results: Using a well-established rat model of ACD, we herein provide novel evidence for effective treatment of ACD by blocking endogenous hepcidin production using the small molecule dorsomorphin derivative LDN-193189 or the protein soluble hemojuvelin-Fc (HJV.Fc) to inhibit bone morphogenetic protein-Smad mediated signaling required for effective hepcidin transcription. Pharmacological inhibition of hepcidin expression results in mobilization of iron from the RES, stimulation of erythropoiesis and correction of anemia.

Conclusion: Thus, hepcidin lowering agents are a promising new class of pharmacologic drugs to effectively combat ACD.

Immunity
Posterwalk by Doris Wilflingseder

Augmentation of tumor vaccine efficacy by adoptive transfer of ex vivo synthetic siRNA *cblb*-silenced CD8⁺ T lymphocytes

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The E3 ubiquitin ligase *cblb* is an established non-redundant mediator of the CD28 and CTLA4 costimulation pathways and T cell immune response threshold regulator. Recently, adoptive T cell transfer (ACT) of *cblb*^{-/-} CD8⁺ T cells has been shown to augment dendritic cell (DC) immunization-based cancer immunity in immune-competent recipients [Lutz-Nicoladoni, 2011]. Here we provide experimental evidence that DC-based tumor vaccines together with repeated transfer of synthetic siRNA-mediated *cblb* knockdown CD8⁺ T cells augments anti-tumor effector responses in vivo. Silencing *cblb* expression by ex vivo siRNA oligonucleotide transfection of polyclonal CD8⁺ T cells prior to adoptive transfer, as a *phenocopy* of *cblb* knockout CD8⁺ T cells, increased T cell infiltration at the tumor site and significantly delayed tumor outgrowth and increased survival rates of tumor-bearing mice. Consequences of *cblb* silencing in human CD8⁺ T cells ex vivo were in agreement with those observed for murine cells. Within the concept of personalized medicine and as a proof of concept (POC), these results validate the concept of targeting *cblb* in order to engineer hyper-reactive CD8⁺ T cells and repetitive ACT of ex vivo *cblb* siRNA-silenced CD8⁺ T cells. This may serve as add-on adjuvant suitable to augment the effectiveness of existing cancer immunotherapy regimens in the clinic.

Tacrolimus at Nanomolar Concentrations Induces a Myofibroblast-like Phenotype in Human Kidney Fibroblasts by Ligand-Independent Activation of TGF- β Receptor

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Background: TGF- β is considered a strong inducer of renal interstitial fibrosis, which is a dominant factor in long-term outcome of kidney transplant recipients. Here we demonstrate the TGF- β -like effects of tacrolimus on kidney fibroblasts *in vitro* and the modulatory effect of NAD(P)H-oxidase 4 on this process.

Methods: The human renal fibroblast cell line TK-173 was treated with varying doses of tacrolimus (FK506, Prograf®) for three days. mRNA expression levels for NAD(P)H-oxidase 4, transgelin (a myofibroblast marker), TGF- β 1, tropomyosin 1, and the collagen chain alpha-1(V) were determined by real-time qPCR. NOX4 protein expression and intracellular peroxide concentration were also determined.

Results: Tacrolimus-treated renal fibroblasts showed increased expression of NOX4, transgelin, tropomyosin 1, TGF- β 1, and collagen mRNA. The effect started at low nanomolar levels, and reached saturation at 100-300 nM of tacrolimus. NOX4 up-regulation lead to a 20 % (max.) increase in intracellular hydrogen peroxide levels. TGF- β 1 treatment duplicated the effects of tacrolimus. Specific inhibition of the TGF- β pathway repressed the effects of both tacrolimus and TGF- β 1. Neutralization of extracellular TGF- β by specific antibodies almost completely abolished the reaction to TGF- β 1, but left the response to tacrolimus unchanged. Si-RNA mediated knock-down of NOX4 had little effect on the tacrolimus-induced effects, except that COL5A1 expression was decreased in tacrolimus- treated cells.

Conclusion: Tacrolimus at low nanomolar concentrations had TGF- β -like effects on cultured human renal fibroblasts. The binding of tacrolimus to FK506 binding protein 12 (FKBP12) leads to increased TGF- β receptor activity, even in the complete absence of ligand. This effect was sufficient to induce a myofibroblast-like phenotype and might thereby contribute to the induction of interstitial fibrosis in immunosuppressed kidney transplant patients. NOX4 activity may partially modulate the fibrogenic effect.

Elucidation of the Physiological Role of the Bcl-2 Pro-Survival Homologue A1

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Background: The anti-apoptotic protein A1/Bfl-1 reportedly plays a role in lymphocyte and myeloid cell development and maturation. The physiological role of A1, however, is still unclear because conventional knockout techniques cannot be applied to generate a suitable mouse model.

Methods: In order to overcome this problem we have used an alternative strategy based on RNA interference (RNAi). We chose to generate an inducible as well as a tissue-specific transgenic mouse model to knock-down A1. Therefore, we designed an expression construct encoding a shRNA targeting A1 mRNA in the context of the miR30 micro RNA. In one model, this miR30-A1 sequence was embedded in the 3'UTR of a cDNA encoding the fluorescent marker gene *Venus* transcribed from a modified version of the *Vav*-gene promoter containing lac-repressor (lacI) binding sites (lacO), which is specific for the hematopoietic system and can be regulated by IPTG in the context of lacI. In a second approach the miR30-A1 sequence is expressed in the context of a Tet-*CMV*^{min} promoter followed by *EGFP* cDNA sequence driven by the *ubiquitin* promoter. These *Tet-miR30-A1* mice were crossed with *VavP-tTA* mice in order to drive the expression of the mir30-A1 in all hematopoietic cells. In addition, we generated Hoxb8-myeloid progenitor cell lines that can be differentiated into neutrophils or macrophages *in vitro* from *Tet-miR30-A1* mice to establish an *in vitro* system allowing manipulation of A1.

Results: First results suggest that A1 may be important for thymocytes survival during positive selection and for B cell maturation. Furthermore, the differentiation potential into the granulocytic lineage also seems dependent on A1 availability.

Conclusion: A1/Bfl-1 is a critical survival factor for normal and mature lymphocytes as well as neutrophils and may constitute a novel drug target for the treatment of apoptosis-resistant pathologies like chemoresistant chronic lymphocytic leukemia (CLL) and hematological disorder such as neutropenia.

Vaccine Adjuvants Differentially Regulate the Balance of Antigen-specific Effector T cells to Tregs and Control Tumor Protection

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Background: The balance between antigen-specific effector and regulatory T cells plays a critical role in determining the success of anti-tumor immunity. Little is yet known about how current cancer vaccines affect this balance. We have previously shown that vaccination of HLA-A2⁺ metastatic melanoma patients with Melan-A₂₆₋₃₅(A27L) peptide and CpG-ODN in a mineral oil emulsion expands Melan-A-specific CD8 T cells. This vaccine also significantly reduces the frequency of Foxp3⁺ HLA-DQ6-restricted Melan-A-specific CD4 T cells, while the total Foxp3⁺ CD4 T cell population remains unchanged.

Methods: We are studying the impact of vaccination on antigen-specific effector T cells and Tregs in a mouse model of OVA peptide vaccination. Following adoptive transfer of OT-I and OT-II/Foxp3-eGFP T cells we vaccinated mice with OVA peptides in combination with different adjuvants and monitored the response of the different transferred cell populations. Using this model we could also test the therapeutic effect of the different vaccine formulations in a subcutaneous B16-OVA tumor model.

Results: In agreement with previous reports, we observed that OVA-specific Tregs increase in frequency following subcutaneous vaccination with peptide alone. Moreover, we established that the addition of certain adjuvants, including the TLR7/8 agonist Imiquimod and the *Quillaja saponaria* extract Quil A, further promoted antigen-specific Treg expansion. In contrast, addition of other TLR agonists, such as CpG-ODN and Poly(I:C), preferentially induced the amplification of both CD4 and CD8 OVA-specific effector T cells, resulting in a dramatic increase in the ratio of antigen-specific effector T cells to Tregs. When mice were vaccinated therapeutically following B16.OVA tumor engraftment, we found that induction of high ratios of tumor-specific T_{eff} to Treg was associated with increased tumor protection. Accordingly, the greatest degree of protection was observed in the CpG-adjuvanted vaccine group.

Conclusions: These findings confirm and extend our previous clinical observations that the addition of CpG-ODN to a peptide vaccine leads to a reduction in the frequency of antigen-specific Tregs. Further experiments are now underway to understand the mechanism controlling this regulation of the antigen-specific effector to Treg balance and the relationship to the functional outcome.

Immunomodulatory Effects of Vitamin K antagonist Acenocoumarol (Sintrom)

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Vitamin K-antagonists belong to the group of anticoagulants and are commonly used for the treatment and prevention of deep venous thrombosis, pulmonary embolism, myocardial infarction and stroke. Their antithrombotic effects and the mechanisms involved in blood coagulation are well documented, but little is known about their interaction with the immune system, though inflammation and immune activation are crucially involved in the pathogenesis of atherosclerosis and cardiovascular disease. Within cell-mediated (= Th1-type) immune response pro-inflammatory cytokines especially interferon- γ activate GTP-cyclohydrolase I to form neopterin in human macrophages and dendritic cells. In parallel, indoleamine 2,3-dioxygenase (IDO) is induced and degrades tryptophan (trp) to kynurenine (kyn), IDO enzyme activity can be estimated by calculating the ratio of kyn to trp concentrations (kyn/trp). This in vitro study investigated the effects of vitamin K-antagonist acenocoumarol (Sintrom) on freshly isolated peripheral blood mononuclear cells (PBMC) from healthy blood donors. PBMC were incubated with accelerating doses of acenocoumarol and were either left unstimulated or stimulated with T-cell mitogen phytohaemagglutinin (PHA) after 30 minutes. Concentrations of neopterin, trp and kyn were measured in supernatants after 48 hours of stimulation. IDO-activity and neopterin formation were significantly higher in PHA-stimulated PBMC than in unstimulated cells. Acenocoumarol dose-dependently inhibited trp degradation and neopterin production in parallel and at concentrations as low as 10 $\mu\text{g/ml}$ a significant effect was demonstrated in PHA-stimulated cells, whereas in unstimulated cells no such effect was observed. Though plasma levels of acenocoumarol in treated patients are below the concentrations of our in vitro experiments, our data suggests that the immunomodulatory capacity of acenocoumarol could be part of its positive effects in antithrombotic treatment and prophylaxis.

Neutrophil Gelatinase-Associated Lipocalin (NGAL) promotes Granulocytes Emigration

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Background: Neutrophil gelatinase-associated lipocalin (NGAL) or 24p3 is a 21 kDa protein of lipocalin superfamily produced by different cell types including immune cells, hepatocytes and renal tubular cells. In former studies, two main functions of NGAL have been described. On the one hand, it plays a direct role in iron transport and regulates the levels of important proteins of the iron metabolism. On the other hand, NGAL is able to induce apoptosis in cells containing the 24p3 receptor. In the present study, we investigated the role of Lcn2 in emigration of granulocytes.

Methods: Human neutrophils were obtained by peripheral EDTA-anticoagulated blood of healthy volunteers by Ficoll density gradient centrifugation and mouse neutrophils were obtained by heparin-anticoagulated blood by retroorbital puncture. Neutrophils were preincubated with different concentrations of hIL-8, KC, mLcn2, hLcn2, mLcn2 mAb. Migration assays were performed using a modified 48-well Boyden microchemotaxis chamber. A single intradermal injection of *S.typh.* (300 CFU in 50 µl of saline) was given to wild-type and Lcn2^{-/-}. After 24h, the skin at each injection site was excised and fixed in formalin for histologic analysis.

Results/Conclusion: Herein, we report migration of human as well as murine granulocytes in response to Lcn2 as a chemoattractant. Additionally, we find profound defects of granulocyte migration in Lcn2^{-/-} mice in vitro and in vivo. One intracellular pathway might be the Erk1/2 pathway. To conclude, we found a new role of Lcn2 and could describe one indispensable regulation pathway.

The role of STAT1 in differentiation and recruitment of tumor-associated macrophages

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Background: Tumor-Associated Macrophages (TAMs) represent a major non-neoplastic cell population in a variety of animal and human tumors, and their abundance is correlated with a bad prognosis for the patient and an increased risk of recurrence and metastasis. They are considered as important contributors to the locally immunosuppressive environment of the tumor, support its growth, vascularization and systemic spread. However our previous results, as well as the work of others, indicate that they might play a key role in a successful anti-tumor chemo- and immunotherapy via IFN γ – induced expression of iNOS. In light of these findings, the identification of the hematopoietic TAM precursors and the cytokines, which orchestrate their recruitment and differentiation can have a great practical meaning for the tumor therapy, since a pharmacological interference with TAM accumulation can improve the patient's outcome.

Methods: FVB MMTV Neu Stat1^{+/+} and Stat1^{-/-} mice were used in our experiments. The animals, irrespectively of the Stat1 status develop spontaneously mammary adenocarcinomas with a mean latency of about 6 months. The flow cytometry techniques and ex-vivo functional assays with bone marrow cells and isolated TAMs were applied.

Results: We have investigated the functional properties and the mode of recruitment of TAMs to MMTV Neu tumors in Stat1 deficient and proficient mice. The TAM population can be characterized by the CD11b⁺ CD11c⁺ F4/80⁺ GR-1⁻ surface phenotype and is able to inhibit both CD4 and CD8 T-cell response. Our in vitro experiments and in vivo observations suggest that the precursors for TAMs are the circulating inflammatory monocytes, which need the tumor-derived M-CSF to differentiate. The Stat1^{-/-} TAMs, although functionally and ontogenetically similar to their wildtype counterparts, are significantly less abundant in the tumors, which points towards a role for Stat1 in their recruitment to the tumor bead.

Conclusion: The presented data show, that the tumor-associated macrophages stem from blood inflammatory monocytes, which differentiate into TAMs under the influence of M-CSF secreted by the tumor. This observation may be an indication for development and application of M-CSF or M-CSFR-blocking agents for the tumor therapy. The precise mechanism of Stat1 involvement in TAM recruitment needs further investigation.

Cbl-b is a critical regulator of the Natural Killer (NK) Cell activation threshold

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Background: NK cells play a critical role for cancer immune surveillance, and thus are highly attractive immune cells for adoptive cancer therapies. However, in parallel to the limitations in other adoptive immune cell approaches, the cells are in general transferred to the cancer-associated milieu of immune-suppression or unproductive inflammatory response. Thus, strategies rendering cells resistant to inhibitory environmental cues exerted by TGF- β are considered an attractive means for improvement of cancer immunotherapy. The E3 ubiquitin ligase Cbl-b acts as a threshold regulator by limiting the activation of T cells. As a consequence, T cells lacking Cbl-b are hyperactive and do not need a co-stimulatory signal for proper activation. We here investigate whether Cbl-b expression also affects the NK cell compartment.

Methods: Expression of Cbl-b in highly FACS-sorted murine NK cells was analyzed by western blotting and real time PCR. The functional activity (Cytokine production, cytotoxic activity against YAC-1 targets) of Cbl-b in NK cells was tested using either highly purified NK cells from *cblb*-deficient or wt mice or by knocking down Cbl-b in purified wt NK cells. Moreover, the role of NK cells in the tumor rejecting phenotype of *cblb*-deficient mice was defined by NK depletion experiments.

Results: Our results demonstrate that highly purified human and murine NK cells express high levels of Cbl-b mRNA and protein. We next tested the *in vitro* function of murine and human NK cells. CD3⁻NK1.1⁺-sorted NK cells from *cblb*-deficient animals are hyperactive as shown by increased cytotoxic activity and cytokine production as compared to NK cells sorted from wt littermates. Accordingly, knockdown of *cblb* by means of siRNA in a human NK cell line (NKL) or in primary murine NK cells also increases their cytokine production as well as their cytotoxic potential. We next addressed whether *cblb*-deficient NK cells at least in part mediate the tumor-resistance phenotype of *cblb*-deficient animals. Indeed, in contrast to *cblb*-deficient animals receiving control mAb, *cblb*-deficient animals injected with the NK-depleting anti-NK1.1 mAb showed rapid tumor outgrowth in all NK-depleted animals, whereas (as expected) tumor growth was almost completely prevented in *cblb*-deficient mice receiving the control mAb.

Conclusion: In summary, we provide experimental evidence that depletion of NK cells abrogates the tumor rejecting phenotype in *cblb*-deficient animals suggesting that Cbl-b plays an important role in NK cell biology. Our *in vitro* studies underscore the important role of Cbl-b as negative regulator of the NK cell activation threshold. Our study might set the stage for optimization strategies of adoptive NK or lymphokine-activated killer cell (LAK) adoptive immune cell transfer protocols for cancer treatment by Cbl-b targeting.

Life Span Affects Treg Cell Homeostasis and Function

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Background: It is now recognized that regulatory T-cells (Treg cells) expressing the transcription factor Foxp3 play a key role in the maintenance of immune homeostasis and prevention of autoimmune diseases. Defects in cell death have been reported to facilitate autoimmunity as overexpression of anti-apoptotic Bcl-2 or loss of pro-apoptotic Bim in mice facilitates the appearance of autoimmunity, and associates with disease in humans. While apoptosis of activated effector T-cells is critical in eliminating these cells at the end of an immune response, little is known on how Treg cell numbers are regulated. We therefore investigated how Bcl-2 family proteins impact on apoptosis susceptibility, phenotype and distribution as well as suppressive capacity of Treg cells.

Methods: We crossed *foxp3^{gfp}* (wildtype) with Bim-deficient or *vav-bcl-2* transgenic mice and isolated CD4⁺FoxP3GFP⁺ Treg cells and CD4⁺FoxP3GFP⁻ Tcon cells by cell sorting.

Results: Treg cells appeared on the one hand highly susceptible to cytokine deprivation, histone deacetylase inhibitors and Fas-FasL mediated cell death. On the other hand, they were more resistant to glucocorticoids and the DNA damaging drug etoposide than Tcon cells. Treg cell number was increased in Bim-deficient and *vav-bcl-2* transgenic mice but the expression of Treg cell associated markers foxp3, CD25 and GITR appeared to be reduced. As a consequence, Bim-deficient and *vav-bcl-2* transgenic Treg cells were less potent inhibitors of (wildtype) Tcon cell proliferation in a standard *in vitro* suppression assay.

Conclusion: Bcl-2 family proteins affect Treg cell homeostasis and function and may be a potential target for treatment of autoimmune diseases.

Investigating the Role of BH3-only Proteins in B Cell Development

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Background: B cells undergo many selection processes before becoming mature and immunocompetent. The TNF family ligand B cell-activating factor (BAFF), which binds three receptors: BCMA, TACI and BAFF-R, plays an important role in B cell development and survival. Its absence causes the loss of most mature B cells including transitional type 2 (T2), follicular (FO) and marginal zone (MZ) B cells. This deficit can be partially rescued by overexpression of Bcl2, the founding member of the Bcl2 family of proteins. The survival function of Bcl2 is antagonized by BH3-only proteins, pro-apoptotic members within the same family, such as Bim or Bmf. Similar to overexpression of Bcl2, high-levels of BAFF lead to autoimmune disorders due to the survival of autoreactive B lymphocytes, whereas BAFF loss results in B cell death. Consistently, loss of Bim or Bmf causes lymphadenopathy in mice. Elevated levels of BAFF as well as loss of BH3-only proteins have also been observed in patients suffering from autoimmunity and certain forms of cancer, suggesting a tight connection between BAFF signaling, BH3-only proteins, B cell survival and pathology in mice and men.

Methods: Here, we aim to understand to what extent the biological effects of BAFF are based on the modulation of proapoptotic factors such as Bim or Bmf. Therefore, we crossed *bim*^{-/-} and *bmf*^{-/-} animals with mice that overexpress a TACI-Ig fusion protein, in which BAFF is sequestered and non-functional.

Results: Preliminary results suggest, that the deletion of Bim or Bmf can restore in part the survival of T2, FO and MZ B cells in TACI Ig transgenic mice, which is even more pronounced when both BH3-only proteins are lacking.

Conclusion: We conclude that BAFF acts by modulating the expression and/or function of Bim and Bmf, but the molecular basis and if the surviving B cells are also functional remains to be investigated. Based on these findings we want to define whether well-known cell death regulators of the Bcl-2 family, that are already validated drug-targets, can be used in the treatment of autoimmunity and cancer that associate with deregulated levels of BAFF.

Transplantation and viral infection
Posterwalk by Katja Kotsch

Targeting Intracellular Signaling Pathways For The Prevention Of Ischemia/Reperfusion-Induced Damage During Solid Organ Transplantation

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Background: Excessive production of reactive oxygen species (ROS) is an integral part of the cellular stress response and a major contributing factor to the development of ischemia-reperfusion injury (IRI) during solid organ transplantation. In particular mitochondrially-produced ROS are critical for the initiation and progression of IRI, which restricts the pool of donor organs and results in elaborate follow up treatments. In various *in vivo* (IR) and *in vitro* (hypoxia/reoxygenation, HR) models we observed a consistent pattern in the activation of key intracellular signaling pathways under cellular stress. Most strikingly the use of p38 kinase specific inhibitors prevented mitochondrial ROS production and cell death. Here we defined the contribution of p38 to IR- and HR-induced damage and provide first evidence for a therapeutic benefit of p38 inhibition *in vivo*.

Methods: Rat kidney transplantation and clamping were used for the induction of ischemia reperfusion injury (IRI). Hypoxia/ reoxygenation (H/R) were predominantly analyzed in HL-1 cardiomyocytes and primary MEFs. Intracellular signaling was monitored in tissue or cell lysates by using phosphorylation specific antibodies. Mitochondrial ROS levels were determined by imaging of cells pre-loaded with Mitotracker Red CM-H2XRos. ROS/NOS induced tissue damage and AKI in were visualized by 3-nitrotyrosine and HSP70 specific antibodies, respectively. Serum creatinine and urea were determined routinely at ZIMCL (MUI), whereas serum cystatine-c and serum NGAL concentrations were measured by ELISA. BIRB-796 was used as p38 inhibitor in all experiments shown.

Results: The expression pattern of all p38 isoforms was established in HL-1 cells and siRNA-mediated knockdown of the predominant isoform p38 α reduced ROS production, confirming the critical role of p38. Preliminary data suggested the requirement of MAPKAP kinase 2 (MK2) rather than the transcription factor ATF-2 downstream of p38. As observed in other settings reperfusion following kidney clamping or transplantation was marked by a profound increase in the activity of p38, its upstream kinases MKK3/6 and the effector MK2. Application of the p38 inhibitor BIRB-796 *in vivo* prevented the deterioration of kidney function following ischemia/reperfusion manifest by reduced serum creatinine, urea, cystatine-c and NGAL levels. P38 inhibition also provided protection from AKI and oxidative damage during ischemia reperfusion. Thus the inhibition of p38 during IR or HR prevents early key processes, which are essential for the development of IRI.

Conclusion: Inhibition of p38 signaling during IR and HR may provide a potent strategy for limiting ischemia/reperfusion injury (IRI).

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Knockout of the Neuronal Nitric Oxide Synthase Attenuates Ischemia Reperfusion Injury in a Murine Pancreas Transplantation Model

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Background: Tetrahydrobiopterin (H4B) donor pre-treatment significantly attenuates ischemia reperfusion injury (IRI) related pancreatitis in a murine pancreas transplantation (PTX) model. Since underlying mechanisms of tetrahydrobiopterin-mediated protection are still discussed, it was aim of this study to investigate if the neuronal nitric oxide synthase (nNOS) is the major target of H4B using nNOS knock-out (nNOS^{-/-}) mice.

Methods: In a heterotopic PTX-model syngeneic C57BL6 mice (wild-type and nNOS^{-/-}) were used as donor-recipient pairs. Grafts were subjected to 16h cold ischemia time (CIT). Donors were either pre-treated with 50mg/kg H4B or untreated. Non-transplanted animals of both genotypes served as controls. Following 4h reperfusion, graft microcirculation was analyzed by confocal intravital fluorescence microscopy. Parenchymal damage and peroxynitrite-formation were histopathologically and immunohistochemically assessed, respectively. H4B levels were determined by HPLC. Finally, all groups were tested for recipient survival

Results: While prolonged CIT significantly reduced microcirculation in wild-type grafts, no damage was observed in untreated nNOS^{-/-} grafts. H4B-pre-treatment significantly restored capillary blood flow in wild-types ($p < 0.01$), however, no further beneficial effect was observed in nNOS^{-/-} organs. Furthermore, in nNOS^{-/-} grafts neither parenchymal damage nor nitrotyrosine formation were significantly influenced by H4B treatment. Intra-graft H4B levels were not affected in nNOS^{-/-} grafts by H4B pre-treatment ($p > 0.05$). Significantly prolonged recipient survival was only observed in animals receiving either nNOS^{-/-} pancreatic grafts or H4B pre-treated grafts, independently from donor genotype ($p < 0.01$).

Conclusion: The neuronal nitric oxide synthase isoform is responsible for excessive reactive oxygen species production and hence represents the major target of tetrahydrobiopterin treatment in this model.

LCMV-pseudotyped VSV for Oncolytic Virotherapy of Ovarian Cancer

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Background: Ovarian Cancer is one of the leading causes of death from gynecological malignancies in the western world. The prognosis of patients remains devastating since tumors are usually diagnosed at advanced stages and common therapeutic strategies such as surgery and chemotherapy reach their limit. A promising new approach is the use of vesicular stomatitis virus (VSV)-based oncolytic virotherapy as VSV is one of the most potent oncolytic viruses and there is no pre-existing immunity among the human population. However, VSV's glycoprotein-mediated inherent neurotoxicity has hindered clinical development so far.

Methods: To abrogate the VSV-inherent neurotoxicity, we pseudotyped VSV with the non-neurotropic envelope glycoprotein of the lymphocytic choriomeningitis virus (LCMV-GP), (Muik et al, J. Virol., 2011). Neurotoxicity of pseudotyped VSV(GP) was investigated upon intracranial injection in mice. Oncolytic potency was tested in ovarian cancer cell cultures and in a subcutaneous ovarian cancer xenograft mouse model.

Results: VSV(GP) exhibited a more than 10⁶-fold higher LD50 compared to VSV wildtype upon intracranial injection in mouse brain. Furthermore, effective oncolytic activity of VSV(GP) could be demonstrated in ovarian cancer monolayer and spheroid cell cultures. Accordingly, intratumoral injection of VSV(GP) into subcutaneous ovarian cancer xenografts in mice led to an overall response rate of 100% with complete tumor regression.

Conclusion: The results of our in vitro and in vivo studies demonstrate that LCMV GP-pseudotyped VSV exhibits a highly beneficial toxicity and efficacy profile. Thus, it represents an extremely promising candidate for oncolytic virotherapy of ovarian cancer.

Secretable antiviral entry inhibitory (SAVE) peptides for gene therapy of HIV infection

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Background: C peptides (e.g. T20, C46) are highly efficient inhibitors of HIV entry. Secreted from gene-modified cells, C peptides are expected to mediate a bystander protective effect on neighboring non-modified cells and suppress virus replication even if only a small fraction of cells is genetically modified.

Methods: Short peptides, such as the C peptides, are only inefficiently translated and exported by the cellular secretory machinery. To circumvent these limitations we expressed therapeutic C peptides as concatemers, which were subsequently processed into monomeric peptides by protease cleavage within the secretory pathway.

Results: Transfection or transduction of cell lines with retroviral vectors resulted in high-level expression and secretion of SAVE C peptides, which exerted a high antiviral activity in single-round infection assays with replication incompetent lentiviral particles pseudotyped with a variety of different HIV envelope glycoproteins. In mixed cell cultures SAVE peptides secreted from transduced cells produced a bystander effect and suppressed HIV-1 infection of non-modified cells.

Conclusion: The *in vivo* secretion of therapeutic C peptides from gene-modified T or B cells holds great promise as the cells would be expected to home to lymphatic tissues, which are the major sites of HIV replication. Secretion of the antiviral gene product in the lymphatic tissues is likely to lead to high and stable local concentrations and confer a substantial antiviral effect.

Induction of Circulating Endothelial Cells (CECs) and Circulating Progenitor Cells (CPCs) after Polyclonal Antithymocyte Globulin (ATG) Therapy in Liver Transplantation

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Background: Rabbit antithymocyte globulin (rATG) is widely used as induction agent in solid organ transplantation. Beside depletion of circulating lymphocytes there is a growing body of evidence suggesting that rATG may also play a pivotal role in modulating the immune system. As blood circulating endothelial cells (CECs) and circulating hematopoietic progenitor cells (CPCs) represent two minute fractions (CECs: 0.1% to 6.0% and CPCs: 0.01–0.20%) of blood mononuclear cells that are thought to play important roles in tissue vascularisation, the study of both cell types is currently suggested as surrogate markers for numerous pathologies. Especially the noninvasive endothelial evaluation as an early index of vascular injury following kidney transplantation has been already demonstrated.

Methods: We used four surface markers to identify viable CECs as CD31bright, CD34dim, CD45–, CD133– and viable CPCs as CD34bright, CD133+, CD45dim, CD31+cells in the peripheral blood of liver transplanted recipients (n=28) until day 20 post transplantation via FACS-analysis.

Results: An induction of CECs was exclusively observed for rATG-treated patients (n=17) increasing from 0.56% ± 0.98% pre transplantation to 1.83% ± 1.85% at day 1-2 post transplantation compared with control patients receiving standard immunosuppression (n=11) (p<0.04). In addition, the induction of CPCs was even more pronounced illustrating an increase in rATG treated patients from 0.20% ± 0.26% pre transplantation to 1.55% ± 1.75% at day 1-2 post transplantation (p<0.001). A significant elevation of blood CPCs is still detectable at day 5 (p=0.0379 compared with controls) and starts to decline at day 10 post transplantation.

Conclusion: In summary we illustrated that both CECs and CPCs were detectable in numbers that allows kinetic monitoring of these cell types post transplantation and that rATG treatment results in a transient induction. As clinical correlations between the concentration of these two populations and the effect of immunosuppressive regimens has been already proven, validation of these cell populations as biomarkers in the setting of solid organ transplantation remains to be determined.

Neurodevelopmental outcome following congenital cytomegalovirus infection in preterm infants with twin-to-twin transfusion syndrome

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Background: Congenital cytomegalovirus (CMV) infection is the most common cause of viral intrauterine infection in humans. Damage is caused by CMV replication in target organs of the fetus and indirectly by CMV-induced placental dysfunction. Up to 15% of asymptomatic infected infants develop long-term neurological sequelae. In symptomatic infants severe neurologic morbidity occurs in 60 to 90%. Congenital CMV infection can cause a wide range of brain anomalies including cysts, calcifications, ventriculomegaly and neuronal migration disorders.

Case presentation: We here report a case of preterm born monochorial-diamnial twins with twin-to-twin transfusion syndrome (TTTS) and congenital CMV infection. In the 27th week of gestation a Caesarean section was performed because of pathological cardiotocogram and doppler ultrasonography of the second twin (recipient). In the further clinical course both infants presented with severe, persistent thrombopenia, pathologic coagulation, elevated liver enzymes and direct hyperbilirubinemia. Congenital CMV infection was diagnosed on the fifth day of life by positive polymerase chain reaction in urine and blood. Serological testing of the mother's serum indicated positive values for CMV-specific IgM- and IgG-antibodies with an avidity index of 70. On postnatal day seven treatment with ganciclovir was commenced, but had to be stopped after two weeks due to liver toxicity and neutropenia. Both infants showed several neuropathological findings in serial ultrasonography and magnet resonance imaging, pathological aEEG with seizure activity and neurodevelopmental delay at the corrected age of 12 months. The present case demonstrates severe cerebral involvement following primary maternal CMV infection in combination with TTTS in preterm born twins. The severity of CMV disease in this case might be enhanced by circulatory changes followed by periods of hypoxia and ischemia due to TTTS. The complex etiology in combination with extreme prematurity, leading to pronounced brain pathology and consequent neurodevelopmental impairment make this case of special interest.

Conclusion: Current treatment strategies for congenital CMV infection focus on administration of replicase inhibitors. However, since studies are lacking and side effects are frequent, careful consideration is necessary. Treatment of the mother with CMV vaccine might have the potential to decrease incident cases of congenital CMV infection, but until now only a few studies have been published, with limited effects. Primary prevention of this disease by adequate education of every woman of childbearing potential and consequent diagnostic screening before and during pregnancy is necessary. Especially in high risk pregnancies, e.g. with signs of TTTS, monitoring for congenital infections might be of major importance in preventing additional sequelae.

Expanding the Spectrum of Neurological Disease Associated with Epstein-Barr Virus Activity

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Background: The purpose of this study was to delineate the spectrum of neurological diseases attributed to Epstein-Barr virus (EBV) activity.

Methods: The approach was a retrospective study on patients with EBV activity proven by a positive EBV antibody-specific index (AI) and/or cerebrospinal fluid (CSF) PCR.

Results: One hundred six children and adults (AI positive = 77, AI + PCR positive = 3, PCR positive = 26) were identified, most with reactivated infections. Twenty-eight showed typical EBV-related diseases (encephalitis, neuritis, meningitis), 19 further infections (HSV encephalitis, neuroborreliosis, HIV infection, bacterial meningitis), nine immune-mediated disorders (multiple sclerosis, optic neuritis), and 50 further diseases not typical for EBV. The highest AI values occurred in patients with encephalitis. EBV-related diseases occurred at peak ages below 18 and above 60 years of age. No relationship between disease category or AI values and viral loads was found. Additional reanalysis of 1,500 consecutive CSF EBV PCR studies revealed the highest positive rates among patients with further infections (n = 18/227, 7.9%) but lower rates among patients with typical EBV-related disorders (5/395; 1.3%), immune-mediated disorders (n = 2/174; 1.1%) and other conditions (n = 4/704; 0.6%).

Conclusions: Intrathecal EBV activity is not restricted to typical EBV-related disorders, unexpectedly frequent in further CNS infections and also present in non-inflammatory conditions. Prospective studies should assess the pathogenic role of EBV in these different diseases.

The Killer-Cell Immunoglobulin-like Receptor (KIR) Genotype Correlates with Acute Kidney Failure in the Early Post- Liver Transplantation Period

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Acute kidney injury (ARI) and acute renal failure (ARF) are major complications following liver transplantation (LT) leading up to chronic end-stage renal disease. The etiology of post-LT impairment is multi-factorial but it is suggested that e.g. during ischemia initial insults provoke morphological and functional changes within the vascular endothelium and tubular epithelium. As it has been demonstrated, that ischemic ARF can occur in the absence of classical T cell function and that Natural Killer (NK) cells can kill syngeneic tubular epithelial cell (TEC) in vitro, we aimed to elucidate the role of NK cells and their receptors in the context of early post-liver transplant ARI and ARF more precisely. For instance, patients with impaired kidney function (serum creatinine levels >1.2 mg/dl, n=13) illustrated heightened peripheral NK cell frequencies prior LT compared with patients showing stable renal function (n=9) (17.22% ± 10.56 versus 12.98% ± 9.09). We further retrospectively tested 89 liver transplant recipients for their killer-cell immunoglobulin-like receptor (KIR) genotype and the risk of ARI and ARF. During the first week post liver-transplantation ARI occurred in 12% and ARF in 22% of the patients, respectively. ARI was a significant risk factor for acute rejection (p=0.0009) and ARF led to elevated serum creatinine levels (>1.2 mg/dl) at the time of hospital discharge (p=0.008). Interestingly, significantly less patients having a homozygous KIR haplotype A/A (characterized by the presence of only one activating KIR gene) displayed a stable early postoperative kidney function, compared to patients with a KIR haplotype B/x (more than one activating receptor) (p=0.025, odds ratio 2.3, CI=1.3-3.9). Moreover, the absence of KIR2DL2/DS2 genes significantly influenced the risk of acute renal failure (p=0.05). A multivariate regression model of both clinical and genomic risk factors for acute kidney injury/failure confirmed a link between the KIR haplotype A/A and post-LT acute renal failure (p=0.04). In summary, we observed a higher percentage of NK cells prior to LT in patients with impaired renal function and identified the KIR haplotype A/A as an independent genetic risk factor for ARF within the first postoperative week. Our data therefore provide new aspects of an innate immune response within the setting of post-LT kidney injury and failure.

Congenital Varicella Syndrome in a Very Low Birthweight Preterm Infant

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Background: Congenital varicella syndrome (CVS) is a potential consequence of primary varicella zoster virus (VZV) infection contracted early in pregnancy. According to current knowledge, maternal herpes zoster does not harm the unborn child. In Western European countries up to 95% of women of childbearing age are seropositive for VZV-specific IgG antibodies. The incidence of primary VZV infection is 0.7–3/1000 pregnancies. The risk for CVS correlates strongly with the time of maternal disease, being highest in early pregnancy. Foetal infection during the first two trimesters is followed by either spontaneous abortion or CVS, which is expected in 12% of the infected fetuses. CVS typically manifests with brain abnormalities, eye pathologies and segmentally distributed cicatricial skin lesions and limb deformities. Based on the segmental distribution of the symptoms, it has been postulated that the clinical changes are not a direct result of intrauterine chickenpox, but that they originate from subsequent, in utero, zoster-like VZV reactivation.

Case report: We report on a male preterm infant, born at 30 weeks of gestation, who developed pustular skin lesions at the age of 4 weeks. The mother had suffered from chickenpox at 14 weeks of gestation. Apart from skin manifestations, critical bronchopulmonary dysplasia made the infant conspicuous. In keeping with the current recommendations the diagnosis of CVS was based on I. positive history of maternal varicella in the 15th week of gestation, II. typical CVS stigmata (postnatal zoster-like VZV reactivation, chorioretinitis, gastrooesophageal reflux, dysphagia) and III. proof of intrauterine VZV infection by detection of the VZV genome in diverse body material (blood, respiratory secretion, intestinal wall, skin lesions). At age 10 weeks he presented with extensive intestinal wall perforation, considered to be related to CVS, which finally led to death.

Conclusion: This case shows for the first time the clinical course of CVS in a preterm infant. Premature birth allowed to observe repeatedly developing skin lesions, that possibly correlate with VZV reactivations which, in term infants, run their course in utero.

To date, no standard treatment recommendation exists. On the basis of individual case reports it has been suggested that administration of acyclovir anticipates the progression of eye disease and neurological symptoms. If a pregnant, seronegative woman with seronegative immune status or uncertain VZV history has been exposed to the virus, postexposure prophylaxis with varicella zoster immunoglobulin (VZIG) is recommended. VZIG has been shown to mitigate maternal chickenpox symptoms. While its administration has been seen to not be able to avert the occurrence of CVS, it is presumed to reduce the rate of infected fetuses. The deleterious consequences of maternal varicella during pregnancy underline the necessity of comprehensive VZV vaccination for seronegative women of childbearing age.

HPV-basiertes Primärscreening in der Zervixkarzinomfrüherkennung in Deutschland: Eine entscheidungsanalytische Kosteneffektivitätsanalyse im Rahmen von HTA

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Hintergrund: Der HPV-Test (HPV = Humanes Papillomavirus) erzielte in Studien eine höhere Sensitivität jedoch geringere Spezifität als die aktuell zur Zervixkarzinomfrüherkennung eingesetzte Zytologie. Ziel dieses vom DIMDI in Auftrag gegebenen HTA ist eine systematische Evaluation der Langzeiteffektivität und Kosteneffektivität des HPV-basierten Zervixkarzinom-Primärscreenings in Deutschland.

Methoden: Es wurde ein Markov-Modell für den natürlichen Verlauf der Zervixkarzinomentwicklung für den Kontext des deutschen Gesundheitssystems entwickelt und validiert. Screeningstrategien mit Zytologie allein, HPV-Test allein, HPV-Test in Kombination mit Zytologie oder mit zytologischer Triage von HPV-positiven Frauen wurden mit unterschiedlichen Screeningintervallen untersucht. In das Modell gingen deutsche epidemiologische, klinische und ökonomische Daten sowie Daten zur Testgüte aus internationalen Metaanalysen ein. Zielparameter der Analysen waren die Reduktion von Zervixkrebs-/mortalität, Restlebenserwartung und das diskontierte inkrementelle Kosteneffektivitätsverhältnis (IKEV). Die Analyse erfolgte aus Kostenträgerperspektive mit jährlicher Diskontrate von 3%.

Ergebnisse: HPV-basiertes Screening war effektiver als die Zytologie (71%-97% versus 53%-80% Zervixkrebsreduktion, je nach Screeningintervall). Die IKEV lagen zwischen 2.600 Euro/Lebensjahr (LJ) (Zytologie allein ab 20J, 5-J-Intervall) und 155.500 Euro/LJ (HPV-Screening ab 30J, Zytologie 20J-29J, 1-J-Intervall). Jährliche Zytologie, wie in Deutschland empfohlen, wurde von HPV-Screening dominiert. HPV-Screening ab 30J (Zytologie 20-29J) alle 2J war kostengünstiger und gleich effektiv. Die Erhöhung des Alters für den Screeningbeginn auf 25J hatte keinen relevanten Effektivitätsverlust zur Folge, reduzierte aber den Ressourcenverbrauch.

Schlussfolgerungen: Basierend auf diesen Modellergebnissen ist das HPV-basierte Zervixkrebscreening effektiver als die Zytologie und bei Screeningintervallen von zwei oder mehr Jahren als kosteneffektiv zu bewerten. Für den deutschen Kontext könnte ein HPV-Screening ab dem 30.LJ und Zytologie im Alter von 25-29J jeweils im 2-J-Intervall eine optimale Strategie sein. Längere Screeningintervalle könnten für Frauen ohne erhöhtes Risiko und regelmäßiger Screeningteilnahme bzw. im Fall einer Abnahme der HPV-Inzidenz in der Population um mehr als 70% sinnvoll sein.

Chronic Hepatitis C infection (Typ 1b) associated with Nephrotic Syndrome in a 14 year old boy

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Background: Chronic Hepatitis C infection is known to be related with extra hepatic manifestations, including the kidney (38%). Membranoproliferative Glomerulonephritis or Membranous Glomerulonephritis with or without cryoglobulinemia is such a known manifestation of renal involvement. In the pathogenesis immune complexes are playing an important role. The immune complexes deposit in the kidney, leading to renal injury. Because of the permanent viral replications and development of new immunocomplexes the prognosis without therapy is worse, leading to impairment of the renal function, in up to 50% in adult patients. A combination therapy PegIFN α and Ribavirin is the first choice for patients with chronic hepatitis C infections. Whether responders to this combination therapy will also benefit with respect to renal improvement is controversial. Since extrahepatic manifestations are rarely seen in children with chronic hepatitis C infection, it is unknown whether antiviral therapy has an efficacy of the improvement of extrahepatic manifestations of chronic hepatitis C infection.

Case report: In April 2010 the 14 year old boy was assigned to the Department of paediatrics because of oedema of the lids and the lower legs. The patient's history showed a viral infection of hepatitis C probably transmitted by a single administration of human albumin in Croatia 2002. Chronic hepatitis C infection (genotype 1b) was already diagnosed in 2006. Routine laboratory tests confirmed a diagnosis of nephrotic syndrome with hyperlipidemia, hyopalbuminemia and gross proteinuria. He was successfully treated with prednisolon 60mg/m²/day and tapered accordingly. In September 2010 he experienced his first relapse. Steady proteinuria and increasing load of viral DNA (20.000.000IU/ml) lead to the final decision to introduce antiviral treatment with pegIFN α (1,5 μ g/kg/week s.c.) Ribavirin (1000mg). After 12 weeks of antiviral therapy neither Hepatitis C RNA nor proteinuria was detectable.

Conclusion: Further follow up of this patient will show whether sustained viral response will be achieved and paralleled by disappearance of the hepatitis C related glomerulonephritis.

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