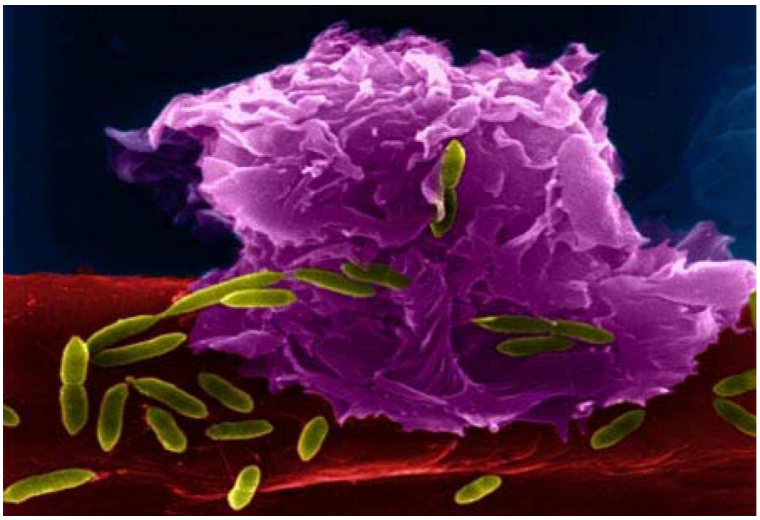
Comprehensive Center for Infection, Immunity and Transplantation

Infektiologie-Immunologie-Transplantation

3rd CIIT SCIENCE DAY

June 28th, 2012

Abstract book



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WELCOME

In 2009, the “Comprehensive Center for Infection, Immunity, and Transplantation (CIIT; please also visit: http://www.i-med.ac.at/ciit/) was established at Innsbruck Medical University (IMU). 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 IMU.

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. On the 1st Science Day in 2010, 85 posters from 46 different research teams, and on the 2nd Science Day in 2011, 59 abstracts from 34 different research teams were presented. Due to the increasing number of similar days (e.g. organised by the two doctoral programms MCBO and SPIN, this year’s Science Day is more focussed on the core of CIIT with 44 posters on infection, immunity and transplantation with only minor contributions from molecular biology and oncology or neurology. Nevertheless this smaller but fine selection will guarantee a fruitful scientific exchange.

In addition, we are very happy that Prof. Dr. Dirk Busch from the Institute of Medical Microbiology, Immunology and Hygiene of the Technical University of Munich (TU München) has agreed to deliver the keynote lecture at this 3rd Science Day and will speak about

"Mechanisms of protection towards intracellular pathogens and its implications for immunotherapy".

Finally, I would like to thank all the people who were involved in the organization of this 2nd Science Day, specifically Thomas Sonnweber, Rolando Colonia and especially Susanne Rofner, 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.

We wish all participants and guests a stimulating and interesting meeting and a fruitful scientific exchange.

Reinhard Würzner for the CIIT speakers team

Programme

VENUE: CCB (Innrain 80/82), Innsbruck

***Foyer:* Moderated Poster sessions**

13.00-13.15	Welcome & Introducing words: Reinhard Würzner
13.15-14.45	**Poster session A** (Aging, Dendritic cells, Gene Therapy)
	Poster session B (Fungal and Viral infections, Miscellaneous)
14.45-15.15	Coffee Break
15.15-16:45	**Poster session C** (T-lymphocytes, Apoptosis, Transplantation)
	Poster session D (Innate Immunity and Infection)
16.45-17.15	Coffee Break

Seminar room M01.470/M01.490), 1st floor

17.15-18.00	Greetings from IMU and introduction of the Key Note Lecturer
17:30-18.30	**Keynote Lecture:** Prof. Dr. med. Prof. Dr. Dirk Busch, Institute of Medical Microbiology, Immunology and Hygiene Technical University of Munich (TU München): **"Mechanisms of protection towards intracellular pathogens and its implications for immunotherapy"**
18.30-20.00	Discussions with light buffet and drinks

Moderated poster sessions

	Topic	Poster first authors	Moderator
13.15-14.45 Poster Sessions A & B	Aging, Dendritic cells, Gene therapy	Weinberger B, Pritz T, Arnold CR, Dubrac S, Loeffler-Ragg J, Irsara C, Scheffler J, Banki Z, Posch W, Tober R, Egerer L	Nikolaus Romani
	Fungal and Viral infections, Miscellaneous	Mauer E, Jukic E, Jank M, Blatzer M, Schafferer L, Beckmann N, Kleines M, Wegleiter K, Berktold M, Tymoszuk P, Brunner J	Reinhard Würzner
15.15-16.45 Poster Sessions C & D	T-lymphocytes, Apoptosis, Transplantation	Martinz V, Jakic B, Wiegers J, Tischner D, Wöss C, Haller M, Oberhuber R, Ashraf MI, Ritschl P, Maier H	Katja Kotsch
	Innate immunity & infection	Toto A, Posch B, Rambach G, Sonnweber T, Bellmann-Weiler R, Schroll A, Nairz M, Mitter-stiller AM, Ejaz A, Ehrlenbach S, Poolpol K, Eitzinger C	Dorothee Orth-Höller

Index

B	**B6 The impact of ornithine and arginine biosynthesis on siderophore production of *Aspergillus fumigatus***	Beckmann N
	B7 Seroprevalence of Hepatitis E virus-specific antibodies in Austria	Kleines M
	B8 Congenital CMV infection in preterm twins – Cytomegalovirus-associated enterocolitis in an extremely low birth weight preterm infant	Wegleiter K
	B9 Human plasmatic coagulation is significantly impaired by EHEC-derived Shiga toxin 2	Berktold M
	B10 The role of Stat1 in differentiation of tumor-associated macrophages in MMTV-neu mammary tumor-bearing mice	Tymoszuk P
	B11 Biomarker of inflammation in juvenile idiopathic Arthritis (JIA)	Brunner J
T-lymphocytes, Apoptosis, Transplantation	**C1 Alterations in the regulatory T cell population in atopic-dermatitis-like cutaneous inflammation in mice**	Martinz V
	C2 Endurance exercise lowers aortal plaque formation in young atherosclerosis-prone mice as a result of increased T regulatory (Treg) cells	Jakic B
	C3 Mutual antagonism of TGF-β and IL-2 in cell survival and lineage commitment of iTreg cells	Wiegers J
	C4 Defective cell death signalling along the Bcl-2 regulated apoptosis pathway compromises Treg cell development and limits their functionality in mice	Tischner D
C	**C5 Investigating the Role of BH3-only Proteins in B Cell Development**	Wöss C
	C6 Identification of a novel PKCβ phosphorylation site in $p66^{SHC}$ regulating ROS production and cell death in response to oxidative stress	Haller M
	C7 Donor age-dependent impact of $CD11c^{+}$ dendritic cells on allograft rejection	Oberhuber R
	C8 Dono p38MAPK inhibition prevents impairment of kidney function resulting from ischemia/reperfusion injury (IRI)	Ashraf MI
	C9 Characterisation of cellular infiltrates in a new established brain death model in mice	Ritschl P
	C10 Lipocalin-2 as mediator of chemokine expression and granulocyte infiltration in a murine heart transplantation model	Maier H

Innate immunity and infection	**D1 Complement activation in invasive fungal infections is triggered by different mechanisms**	Toto A
	D2 Activation and opsonisation of platelets studied in a mouse model of invasive aspergillosis	Posch B
	D3 Aspergillus species activate platelets by their cell wall polysaccharides	Rambach G
	D4 Hypoxia induced down-regulation of hepcidin is mediated by platelet derived growth factor BB	Sonnweber T
	D5 The role of Neutrophil Gelatinase-Associated Lipocalin (NGAL) in iron homeostasis of *Chlamydia pneumoniae* infected macrophages	Bellmann-Weiler R
D	**D6 Lipocalin-2 is an endogenous inhibitor of inflammation in murine nephrotoxic serum nephritis**	Schroll A
	D7 Nitric oxide-dependent regulation of ferroportin-1 controls macrophage iron homeostasis and immune function in *Salmonella* infection	Nairz M
	D8 The role of HO-1 and HIF in regulating iron homeostasis and innate immune response to *Salmonella* infection	Mitterstiller AM
	D9 Protection of Hepatitis C virus from complement mediated lysis by specific acquisition of functional CD59 but not CD46 or CD55	Ejaz A
	D10 Reduction of complement regulator CD59 expression on human kidney cells by Shiga toxin 2	Ehrlenbach S
	D11 Shiga toxin binds to complement regulatory proteins of the factor H protein family	Poolpol K
	D12 N-chlorotaurine, a long-lived endogenous oxidant, inactivates Shiga toxin 2 of enterohemorrhagic Escherichia coli	Eitzinger C

Poster Session A

13.15-14.45

Aging, Dendritic cells, Gene therapy

Posterwalk by Nikolaus Romani

A1

Memory immune response to booster vaccination in old age depends on adequate priming earlier in life

Birgit Weinberger[1], Christoph Neuner[2], Michael Schirmer[3], Rafaella Mateucci Gothe[4], Beatrix Grubeck-Loebenstein[1]

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Background: Regular booster vaccinations against tetanus and diphtheria are recommended for adults and for the elderly in many European countries. With increasing age vaccine-induced immune responses are generally weaker. Particularly in the elderly, antibody concentrations decline over time. We therefore investigated immune responses to consecutive booster vaccinations against tetanus and diphtheria in older adults.

Methods: A cohort of 87 elderly persons age (≥ 60 years) received two consecutive booster vaccinations against tetanus and diphtheria and antibody concentrations were measured before and 4 weeks after the vaccinations. After the second boost tetanus- and diphtheria-specific circulating plasmablasts were determined. For the first booster shot time since the last vaccination was approximately 10 years. The second booster vaccination was delivered 5 years later in accordance with Austrian vaccination recommendations.

Results: Prevaccination antibody concentrations were higher prior to the second boost reflecting the shorter time intervals between the vaccinations. As a consequence the proportion of persons without protective antibody concentrations (0.1 IU/ml) against diphtheria was lower prior to the second compared to the first boost. Four weeks after vaccination all participants had protective antibody concentrations against tetanus, but 6% of the cohort still had no protective antibodies against diphtheria after the second booster vaccination compared to 11% after the first boost.

Individual antibody responses were very similar after the first and the second boost, but responses against tetanus and diphtheria were only weakly linked. Antibody responses were independent of gender, health status, CMV-status and inflammatory status. However, there was a strong correlation between antibody responses and the size of the memory B cell pool as indicated by the number of specific circulating IgG-plasmablasts 7 days after the booster vaccination.

Conclusion: Our results show that elderly individuals respond similar to consecutive booster vaccinations and that vaccination history and primary responses are determining factors for the quality of booster responses.

A2

Polyfunctional $CD8^+CD28^-$ T cells are preserved in human bone marrow during aging

T. Pritz[1], K. Landgraf-Rauf[1], D. Herndler-Brandstetter[1], F. Kloss[2], R. Gassner[2], R. Rauf[3], M. Krismer[3], B. Grubeck-Loebenstein[1]

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Background: $CD8^+CD28^-$ terminally differentiated T cells are enriched in the human bone marrow (BM) and are in close contact with IL-15 producing cells, favoring the survival and expansion of effector-memory T cells. Whether these highly differentiated T cells can compensate the loss of other T cell types or whether they occupy space normally reserved for $CD4^+$ T cells or plasma cells is not known. Therefore the goal of the present study is to further explore the phenotype and function of bone marrow $CD8^+CD28^-$ T cells from elderly persons.

Methods: Bone marrow mononuclear cells (BMMC) were isolated from the iliac crest or femur of healthy donors by collagenase digestion and Ficoll gradient centrifugation. For intracellular cytokine staining BMMC and peripheral blood mononuclear cells (PBMC) of the same donor were stimulated with PMA/Ionomycin and analyzed by flow cytometry. Antigenspecific $CD8^+$ T cells were analysed by pentamer technique. The composition of the T cell repertoire in different T cell subsets was investigated by CDR3 spectratyping.

Results: $CD8^+CD28^-$ T cells accumulate in the human BM. Compared to PB bone marrow derived T cells displayed a higher activation state. Using cDNA microarray analysis it was additionally demonstrated that the expression of a set of chemokine receptors, co-stimulatory molecules and effector molecules was characterized for BM $CD8^+CD28^-$ T cells. Following stimulation BM derived $CD8^+CD28^-$ T cells produced IFNγ and TNFα, in larger amounts than effector T cells in the PB. A relatively large proportion of BM $CD8^+CD28^-$ were specific for the CMV pp65 NLV peptide, the dominant clones being Vβ8 and Vβ13.

Conclusion: These findings imply that $CD8^+CD28^-$ T cells from BM are functional. They have a proinflammatory phenotype and are cytotoxic. Their frequent specificity for CMV indicates that the BM represents a line of defense against CMV. This may be of particular importance during CMV reactivations, which occur frequently in old age.

A3

mTOR activity and autophagy in CD8$^+$ T cells of early and late differentiation stages

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Background: One of the most prominent biological indicators of aging in the human immune system is the accumulation of highly differentiated CD8$^+$CD28$^-$ T cells. These cells are believed to contribute to age-related diseases due to their high proinflammatory activity. The conditions under which these cells survive or die are therefore a matter of interest. mTOR is a central regulator of metabolism and aging and an important controller of autophagy, a catabolic survival process. To define the role of mTOR and autophagy in CD8$^+$ T cells, we analyzed mTOR signaling and autophagy in CD8$^+$CD28$^+$ and CD8$^+$CD28$^-$ T cells.

Methods: PBMCs were obtained from healthy donors and CD8$^+$CD28$^+$ and CD8$^+$CD28$^-$ T cells were separated using the MACS technology. mTOR signaling pathways as well as autophagy were analyzed by Western Blotting. Cells were either left untreated or stimulated via the T cell receptor or with the homeostatic cytokine IL-15. Rapamycin was used to induce autophagy.

Results: Following antigenic stimulation, mTOR activity was significantly lower in CD8$^+$CD28$^-$ T cells as compared to their CD8$^+$CD28$^+$ counterparts. In contrast, upon homeostatic stimulation with the cytokine IL-15 mTOR activity was higher in the CD28$^-$ subset. Within the CD8$^+$CD28$^+$ T cells, the naïve T cells respond better to antigenic stimulation than memory T cells. Following antigenic stimulation CD8$^+$ T cells show an increase in autophagy, more pronounced in CD8$^+$CD28$^+$ T cells. Upon mTOR inhibition with rapamycin we found a further increase of autophagy in CD8$^+$CD28$^+$ but not in CD8$^+$CD28$^-$ T cells.

Conclusion: We demonstrate that: 1.) CD8$^+$ T cells lose their ability to activate mTOR upon antigenic stimulation along with differentiation. In contrast, the capability of CD8$^+$ T cells to activate mTOR in response to the homeostatic cytokine IL-15 increases with differentiation. We therefore propose a model of mTOR activity in CD8$^+$ T cells as a function of stimulation and differentiation. 2.) The mTOR inhibitor rapamycin is not able to induce autophagy in activated CD8$^+$CD28$^-$ T cells, suggesting a defect of this subset in the mTOR-autophagy axis.

A4

Pregnane X Receptor (PXR) modulates CCR7 and Langerhans cell migration via TGF-β1

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Background: The pregnane X receptor (PXR) is a ligand-activated transcription factor regulating genes central to drug and hormone metabolism in the liver. We have recently shown that PXR is up-regulated in activated T-lymphocytes and modulates the T-lymphocyte function thus linking directly PXR activity to the adaptive immune response (*Dubrac et al., J Immunol, 2010*). We here show that PXR is expressed in different dendritic cell (DC) subsets and controls Langerhans cells (LC) migration through CCR7 and TGF-ß1.

Methods: To investigate the role of PXR in DC, we used various methods in cell and molecular biology such as epidermal cell cultures, western blots, quantitative PCR, flow cytometry, immunohistochemistry followed by confocal microscopy.

Results: We here show that PXR is expressed in different subsets of mouse and human immature DC especially LC and is down-regulated in mature DC. PXR ligands down-regulate CCR7 expression at the cell surface of mouse LC without affecting expression of other maturation markers such as CD40, CD86 and CXCR4 *in vitro*. In contrast, PXR antagonism upregulates expression of CCR7 by LC. Similarly, overexpression of PXR in mouse decreases expression of CCR7 at the cell surface of LC, mimicking effects of pharmacological activation of PXR. *In vivo*, PXR deficiency increases while transgenic expression of PXR decreases LC migration. Interestingly, Langerin$^+$ cells lose PXR while migrating into skin tumors and PXR expression is lowered in intra-tumoral CCR7$^+$ cells in mice. Furthermore, PXR activation increases amounts of TGF-ß1 and SOCS-1 in epidermal cells while PXR antagonism has opposite effects. Time course of TGF-ß1 and SOCS-1 expression shows an early upregulation of TGF-ß1 (T2 hrs) and its well-known target gene smad 3 (T4 hrs).

Conclusion: The present study reveals a novel role of PXR in the adaptive immune system by affecting expression of CCR7 by DC. Thus synthetic PXR agonists bear novel possibilities for immunosuppressive strategies and tolerance induction. Selective PXR modulators specifically acting on DC could become reasonable agents for novel immunosuppressive strategies for treatment of inflammatory and autoimmune diseases and prevention of allograft rejection. Similarly, in cancer, PXR antagonism might be relevant to promote DC migration and tumor-antigen presentation to lymphocytes and thus promote anti-tumor response.

A5

Role of the endothelin system in adult pulmonary Langerhans cell histiocytosis and migration of murine Langerhans cells

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Endothelin (ET) receptor blockers have been administered in patients with pulmonary Langerhans´ cell histiocytosis (PLCH) and concomitant pulmonary hypertension. The effects of these drugs on key cells of PLCH have yet to be explored. Our aim was to analyse the expression of ET receptor A and B in PLCH and investigate their functional significance in vitro. ETAR and ETBR expression was studied in 25 formalin/paraffin-embedded PLCH biopsies. For in vitro analysis, the murine LC-like cell line XS52 and freshly prepared murine LCs were used. Target expression was determined by RT-PCR, cell viability was analysed by trypan blue exclusion test and colorimetric assay, ET-1 expression by enzyme-linked immunosorbent assays and cell migration assays were performed in 48-well Boyden chambers for XS52 cells and by murine epidermal explant cultures.

The immunohistochemistry on the biopsies revealed the expression of both, ETAR and ETBR in PLCH. In vitro, the expression of the ET system was proven in murine LCs (ETAR+, ETBR+) and in XS52 cells (ETAR+). Furthermore, treating XS52 cells with the dual ET receptor blocker bosentan revealed impaired cell viability. Regarding cell migration bosentan and the selective ETAR antagonist BQ123 were capable of inhibiting ET-1 dependent migration of XS52 cells. The number of migratory LCs from murine epidermal skin explants after 48 hours of co-incubation with ET-1 was comparable to the one after CCL21 stimulation. Treatment with the dual ET receptor antagonist bosentan reduced the chemotactic potency of ET-1 in LCs. To conclude, this study proved not only the unique expression patterns of the endothelin system in PLCH and in murine LCs but also revealed the antiproliferative and antimigratory properties of ET receptor blockers in vitro apart from showing for the first time the involvement of ET-1 in Langerhans cell migration.

A6

Dog lipocalin allergen Can f 1 and the homologues human tear lipocalin evoke diverse effects in human monocyte derived dendritic cells

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Background: Several of the major respiratory allergens are lipocalins, which comprise a large group of small, mainly secretoric proteins present in all vertebrates. However, the mechanism of allergy induction by lipocalins is still enigmatic, because allergenic lipocalins do not differ significantly from non-allergenic lipocalins in amino acid sequence and tertiary structure. In this project we investigated whether the dog major allergen Can f 1 and the homologous non-allergenic tear lipocalin (Lcn1) might induce different effects in human monocyte derived dendritic cells.

Dendritic cells act at the interface between innate and acquired immunity to initiate immune responses. In this induction phase dendritic cells play a major role by providing specific stimuli for the differentiation of different T cell subsets, depending on the antigen-dendritic cell crosstalk. T helper (TH) lymphocytes can differentiate into at least four subpopulations of effector T cells, one of them are the TH2 effector cells which are crucial for the clearance of parasites and responsible for all forms of allergic inflammatory immune responses. A prerequisite for their induction is the absence of IL-12.

Methods: Human monocyte derived dendritic cells were incubated with recombinant dog allergen Can f 1 and the homologous non-allergenic human tear lipocalin and the uptake and intracellular travelling of these two proteins was followed by immunoflurorescence microscopy. In addition, production of IL-12, as a key indicator for the type of immune response induced, was measured.

Results: We found both lipocalins to be internalized by immature dendritic cells and targeted into granular structures, but the intracellular localizations of Can f 1 and Lcn1 overlap only partially (Fig. 1). Furthermore, we found that Lcn1 but not Can f 1 induces IL-12 production in immature and mature dendritic cells (Fig. 2).

Conclusion: Our results indicate differential effects of allergenic lipocalins and endogenous non-allergenic lipocalins on dendritic cells, which might be due to differential intracellular processing and targeting of the proteins. However, additional experiments are necessary to gain further insight in the mechanisms underlying the differential effects of lipocalins on dendritic cells.

A7

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 immunity. Their major task is the uptake and processing of pathogens and subsequent presentation of antigens. 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 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 activated dendritic cells in skin and liver. 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 dendritic cell lineage and an increase of the Flt3-ligand serum levels, a cytokine crucial for DC differentiation, was observed. Additionally, an accumulation of the Flt3 receptor on splenic dendritic cells, due to a rerouting defect, was observed. Similar observations were made in p14 depleted keratinocytes where the degradation of the EGF receptor was severely disturbed (Teis D. et al., 2006, JCB). This receptor accumulation and the enhanced availability of its ligand resulted in an increased downstream signaling of Flt3, a pathway crucial for dendritic cell differentiation (Sathaliyawala T. et al., 2010, Immunity). In addition we observed *in vitro* a hyperproliferation in bone marrow derived dendritic cell cultures under the influence of Flt3-ligand which phenocopies the dendritc cell expansion observed in the p14 knock out mouse model.

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

A8

Improvement of dendritic cell-based vaccines by targeting antigen to CD11c

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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: *In vitro* CD11c-specific scFv selectively targeted viral antigens to DCs and thereby significantly improved the activation of virus-specific T cells. In vaccination experiments DCs loaded with viral Ag targeted to CD11c provided improved rejection of FV-derived tumors and efficiently primed virus-specific CTL responses after virus challenge.
Conclusions: 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.

A9

Antibodies attenuate the capacity of dendritic cells to stimulate HIV-specific CTLs

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Background: Control of HIV is suggested to depend on potent effector functions of the virus-specific $CD8_+$ T cell response. Antigen opsonization can modulate the capture of antigen, its presentation and the priming of specific $CD8_+$ T cell responses. We have previously shown, that opsonization of retroviruses acts as endogenous adjuvant for DC-mediated induction of specific CTLs. However, in some HIV-positive individuals, high levels of antibodies and low levels of complement (C) fragments coat the HIV surface.

Methods: Therefore, we analyzed the impact of IgG-opsonization on the antigen-presenting capacity of DC by $CD8_+$ T cell proliferation assays following repeated prime-boosting, by measuring the antiviral activity against HIV-infected autologous $CD4_+$ T cells and by IFN-γ secretion from HIV-specific CTL clones.

Findings: We find that DC exposed to IgG-opsonized HIV significantly decreased the HIV-specific $CD8_+$ T cell response compared to the earlier described efficient $CD8_+$ T cell activation induced by DC loaded with complement-opsonized HIV. DC exposed to HIV bearing high surface IgG levels, following incubation in plasma from HIV-infected individuals, acted as weak stimulators for HIV-specific CTL clones. In contrast, HIV opsonized with plasma from patients exhibiting high C and low IgG deposition on the viral surface favored significantly higher activation of HIV-specific $CD8_+$ T cell clones.

Interpretation: Our *ex vivo* and *in vitro* observations provide the first evidence that IgG-opsonization of HIV is associated with a decreased CTL-stimulatory capacity of DC.

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A10

The Viral Vector Vaccine VSV-GP boosts immune response upon repeated applications

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[3]*Contributed equally*

Background: Vesicular stomatitis virus (VSV) is a potent candidate vaccine vector for various viral diseases. The biggest limitation of VSV, however, is its neurotoxicity, which limits application in humans. The second drawback is that VSV induces neutralizing antibodies rapidly and is thus ineffective as a vaccine vector upon repeated applications. Our group has recently shown that VSV pseudotyped with the glycoprotein (GP) of the lymphocytic choriomeningitis virus (LCMV), VSV-GP, is not neurotoxic. The aim of this project was to evaluate the potential of VSV-GP as a vaccine vector.
Methods: We used Ovalbumin (OVA) as a model antigen and analyzed immunogenicity of GP-pseudotyped and wildtype VSV containing OVA (VSV-GP-OVA and VSV-OVA) *in vitro* and *in vivo* in mouse models.
Results: We showed that both vectors infected murine bone marrow-derived dendritic cells (bmDCs) *in vitro*. These bmDCs were able to activate OVA specific $CD8^+$ and $CD4^+$ T cells. Immunization experiments in mice revealed that both VSV-OVA and VSV-GP-OVA induced functional OVA-specific cytotoxic T cells (CTLs) after a single immunization. In addition, with both viruses, mice generated antibodies against OVA. However, boosting with the same virus was only possible for the GP-pseudotyped virus but not for wild type VSV. The efficacy of repeated immunization with VSV-OVA was most likely limited by high levels of neutralizing antibodies, which we detected after the first immunization. In contrast, no neutralizing antibodies against VSV-GP were induced even after boosting.
Conclusion: Taken together, we showed that the non-neurotoxic VSV-GP is able to induce specific T cell and B cell responses against the model antigen OVA to the same level as the wild type VSV vector. However, in contrast to wild type VSV, VSV-GP-OVA boosted the immune response upon repeated applications. Thus, VSV-GP is a promising novel vaccine vector.

A11

Membrane-anchored and secreted C peptides for gene therapy of HIV infection

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Background: C peptides derived from the HIV-1 envelope glycoprotein gp41 (e.g. T-20, C46) are highly efficient inhibitors of virus entry. Here, we analyzed a membrane-anchored (maC46) and a secreted (sC46) variant of the peptide C46 for gene therapy of HIV infection *in vitro* and in mouse and macaque models.

Methods: maC46 or sC46 peptides were expressed from retroviral vectors in B and T cell lines as well as in primary human T cells. Cultures were analyzed for peptide expression and antiviral effect. For *in vivo* testing in mice, human $CD4^+$ T cells were transduced *ex vivo* with the vectors and transplanted into NOD/SCID/$\gamma^{-/-}$ mice. Upon engraftment of the gene-modified cells, mice were infected with HIV-1 and T-cell counts were monitored. For testing of maC46 in the SHIV macaque model $CD34^+$ hematopoietic stem cells were isolated from 4 pig-tailed macaques and transduced *ex vivo* with a lentiviral vector encoding maC46, GFP and a drug-selectable marker or with a control vector. Transduced cells were re-infused into the respective macaques prior to *in vivo* selection. Animals were challenged with SHIV89.6P and clinical parameters were monitored.

Results: maC46 and sC46 peptides were expressed in lymphoid cells and exerted high antiviral activity against a variety of HIV-1 strains. In mixed cell cultures peptides secreted from transduced cells produced a bystander effect and suppressed HIV-1 infection of non-modified cells. In the mouse model, efficacy of sC46 was low, while we observed a substantial increase of $maC46^+$ $CD4^+$ T cells in blood and spleen, apparently due to the selective pressure of ongoing HIV-1 infection. In the macaque model a SHIV-mediated *in vivo* selection of $maC46^+$ cells to >90% of total $CD4^+$ T cells was observed during the acute phase of disease. $CD4^+$ T cell counts made a rapid recovery, plasma viremia decreased significantly following the acute phase and significant SHIV-specific immune-responses were measured.

Conclusion: The clear accumulation of gene-modified cells in (S)HIV-infected animals indicates the outstanding antiviral efficacy of maC46. Long-term control of plasma viremia and near complete recovery of $CD4^+$ T cells in the macaque model demonstrate the high potential of a stem cell-based gene therapy of HIV-1 infection.

Poster Session B

13.15-14.45

Fungal and Viral infections, Miscellaneous

Posterwalk by Reinhard Würzner

B1

Influence of hypoxic conditions on antifungal susceptibility and biomarker release of *Aspergillus* spp. infection

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Background: Invasive aspergillosis (IA) is a major life-threatening disease in immunocompromised patients, with mortality rates up to 90%. The most common species causing aspergillosis is *Aspergillus (A.) fumigatus*, followed by *A. flavus* and *A. terreus*. During infection, fungal pathogens must adapt to various microenvironmental stresses, including hypoxia as well as high CO_2 levels. Such oxystress conditions are usually not taken into account in current models of infection, the assessment of antifungal sensitivities or the improvement of diagnosis.

Methods: We compared the *in vitro* activity of amphotericin B (amB), different azoles and echinocandins in hypoxic conditions (1% O_2, 5% CO_2) to their activity in normoxia against isolates of *A. fumigatus* (n=25) and *A. terreus* (n=16) applying Etests. For evaluation of biomarker release, the amount of β-1,3 glucan (BG) and galactomannan (GM) in *A. fumigatus* and *A. terreus* supernatants was determined by commercially available detection kits (Platelia/Fungitell).

Results: On surface cultures, we found a reduction of the minimal inhibitory concentration (MIC) for amB for both species in hypoxic conditions, resulting in lower cutoff values. Most interestingly, *A. terreus* strains, that exhibit instrinsic resistance to amB in normoxia, were shown to be susceptible to amB in hypoxia. A significant reduction in the MIC for all tested azoles was demonstrated for *A. terreus* isolates, while for *A. fumigatus* isolates differences were less pronounced. For echinocandins, no change in the MEC (minimal effective concentration) was detected between hypoxia and normoxia for all *aspergilli.* Notably, for none of the strains tested, MIC/MEC values increased in hypoxic conditions. Furthermore, the release of GM and BG was altered in hypoxia. *A. fumigatus* showed increased BG and GM release, while for *A. terreus* a decreased GM and BG release was detected.

Conclusion: Hypoxia influences *in vitro* antifungal susceptibility of *A. fumigatus* and *A. terreus* isolates, resulting in significantly lower MIC values for antifungal drugs targeting ergosterol or its biosynthesis. Our results indicate that re-assessment of antifungal susceptibility in conditions mimicking the human host might help to establish clinical breakpoints for new antifungal drugs.

B2

New insight into the mode of Amphotericin B resistance in *Aspergillus terreus*

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Background: Amphotericin B (AMB) acts fungicidal by an increase in membrane permeation and release of oxidative stressors. In general, AMB resistance in moulds is rare and detailed factors of AMB resistance in *Aspergillus terreus* (*A. terreus*) are not known.

Methods: Based on the mode of AMB mechanism we compared AMB resistant *A. terreus* (ATR) with susceptible *A. terreus* (ATS) in response to AMB. A murine model of disseminated aspergillosis was applied to analyze correlation of in vitro minimum inhibitory concentration (MIC) and in vivo outcome. To explore in vitro data the role of fungal ergosterol, AMB uptake, intracellular efflux (e. g. potassium) and the presence of prooxidant effects causing oxidative intracellular damages were analyzed in detail.

Results: A murine model of disseminated aspergillosis showed that ATS infection was more lethal when compared to ATR. While AMB treatment improved outcome of mice infected with ATS. In vitro data display ergosterol content and efflux of intracellular compounds (ions, amino acids and proteins) not playing a major role in AMB resistance. Whereas catalase activity was significant (P =0.01) higher in ATR compared to ATS. Lipid peroxidation level and oxidative Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) inactivation at several time points were significant higher in ATS.

Conclusion: Oxidative effects and damages in ATS suggest that AMB exerts its antifungal activity through formation of free radical intermediates, which causes fatal cellular injury. ATR is able to scavenge reactive-oxidant species better than ATS. Induction of membrane permeability seems to play an inferior role in AMB resistance in *A. terreus*.

B3

Aspergillus terreus – discovering signs of sexual reproduction

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Background: Similarly to the supposed strictly asexually distributing fungus *Aspergillus terreus, Aspergillus fumigatus* was thought to reproduce exclusively via asexually formed conidia, but in 2009 O'Gorman *et al.* revealed a cleistothecium formation (= fruiting body) and a sexual cycle. In analogy to *A. fumigatus* we tried to discover makers or signs that implement a sexual stage in *A. terreus*. To this aim we investigated the mating type loci of *A. terreus*.

Methods: Based on the whole genome available from *A. fumigatus* and its described mating type loci, we designed by genome comparison with *A. terreus* mating type primers for MAT1-1. Then we established a real-time PCR-based method to screen different *A. terreus* isolates. We grouped the isolates based on positive or negative MAT1-1-PCR results. We suspected double checked PCR-negative strains carrying the MAT1-2 locus. PCR-positive isolates represent the plus strains carrying the MAT1-1 locus.

In the second phase we tried to introduce fruiting body formation by crossing strains with the opposite mating type. Strains were crossed on oatmeal agar medium by four diagonal inoculations. Inoculated and sealed plates were incubated at different light and temperature conditions for several months.

Results: Our PCR-based mating type tests resulted in an almost 50:50 distribution of isolates carrying MAT1-1 or MAT1-2. This leads us to the conclusion that *A. terreus* is a heterothallic fungus with two opposite mating types. For sex to occur, hyphal contact, cell fusion followed by fusion of the nuclei need to take place between two opposite mating types. The suspected fruiting body would be a cleistothecium carrying ascospores. To date, after almost five months of incubation, no cleistothecia formation have been observed, this might be due to improper incubation conditions.

Conclusion: Although no sexual state of *A. terreus* was found so far, our results provide well-founded evidence for the heterothallic nature of *A. terreus* and a likely sexual stage. The presence of a sexual cycle would help understanding the distribution of virulence factors and antifungal resistances.

B4

A new subfamily of ABC transporters mediates excretion of extracellular siderophores in *Aspergillus fumigatus*

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Background: Siderophore biosynthesis is essential for virulence of various animal- and plant-pathogenic fungi including the opportunistic human pathogen *Aspergillus fumigatus*. *A. fumigatus* excretes the siderophores fusarinine C (FsC) and its acetylated derivative triacetylfusarinine C (TAFC).

Methods: Previously, three B-type ABC transporters termed AbcB, AbcC and AbcD were shown to be transcriptionally repressed by iron via the GATA transcription factor SreA. AbcB and AbcC, are encoded by genes located within gene clusters encoding siderophore-metabolic proteins.

Results: Here we show that these ABC tranporters are involved in siderophore excretion. Inactivation of AbcB blocked excretion of FsC, increased intracellular accumulation of the FsC degradation product anhydromevalonyl-hydroxyornithine (AMHO) and increased excretion of TAFC. Deletion of AbcC decreased TAFC excretion but increased excretion of FsC. Inactivation of AbcD reduced siderophore excretion only in an AbcC-deficient background, implying partially redundant activities of these ABC-transporters. Consistently, deficiency in these ABC transporters impaired the growth rate during iron depleted but not iron-replete conditions.

Conclusion: Interestingly, a triple mutant lacking all three ABC transporters and siderophore excretion in young cultures displayed partial siderophore excretion in old cultures, which indicates alternative siderophore excretion bypasses. Enhanced green fluorescent protein (GFP)-tagging localized AbcB in the plasma membrane. Phylogenetic analysis suggested that AbcB, AbcC and AbcD constitute a new subfamily of the ABC transporter superfamily with homologs in siderophore-producing but not siderophore-lacking fungal species, indicating involvement of all members in siderophore metabolism.

B5

The CCAAT-Binding-Complex mediates Iron Regulation in *Aspergillus fumigatus*

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Background: Iron is essential for a wide range of cellular processes but its excess is toxic. Therefore, microorganisms evolved fine-tuned mechanisms for uptake and storage of iron, to sustain iron homeostasis. In the opportunistic fungal pathogen *Aspergillus fumigatus,* the bZIP-type transcription factor HapX mediates adaption to iron starvation by activating siderophore biosynthesis and repressing iron-dependent pathways. HapX-deficiency attenuates the virulence of *A. fumigatus* underlining the importance of adaptation to iron starvation in pathogenicity. The HapX N-terminal amino acid sequence predicts interaction with the DNA-binding, heterotrimeric CCAAT-binding complex (CBC), which is conserved in all eukaryotes and believed to co-regulate up to 30% of all genes.

Methods: Here, we characterized the role of the CBC in iron regulation of *A. fumigatus* by analysis of the phenotypic consequences of genetic inactivation of the CBC subunit HapC.

Results: HapC-deficiency was deleterious during both iron starvation as well as iron sufficiency, demonstrating iron-independent regulatory functions of the CBC. In contrast, HapX is important during iron starvation only. As shown previously for HapX-deficiency, HapC-deficiency derepressed genes involved in iron-consuming pathways during iron starvation but decreased siderophore metabolism at transcriptional and metabolic levels. Inhibition of reductive iron assimilation by ferrous iron chelation blocked colony formation of both HapC-deficient and HapX-deficient conidia. Moreover, inactivation of HapC was epistatic to HapX-deficiency.

Conclusion: Taken together, these data indicate that the CBC mediates both the activating and the repressing functions of the iron-regulatory transcription factor HapX. The central role of the CBC in environmental adaptation is underlined by HapC-deficiency rendering *A. fumigatus* avirulent in a murine model of aspergillosis.

B6

The impact of ornithine and arginine biosynthesis on siderophore production of *Aspergillus fumigatus*

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The opportunistic fungal pathogen *Aspergillus fumigatus* produces extracellular siderophores for iron uptake and intracellular siderophores for storage and distribution of iron. Moreover, *A. fumigatus* employs a second high-affinity iron acquisition system, reductive iron assimilation (RIA). Siderophore biosynthesis but not RIA is essential for virulence. The main precursor of siderophores, ornithine, can be produced from glutamate in the mitochondria or cytosolic hydrolysis of ornithine-derived arginine. Here, the impact of inactivation of mitochondrial ornithine biosynthesis (*ΔargEF* mutant lacking N-acetylglutamate kinase/ N-acetylglutamylphosphate reductase) and cytosolic arginine biosynthesis (*ΔargB* mutant lacking ornithine transcarbamoyl transferase) on siderophore production was studied. *ΔargEF* and *ΔargB* are arginine auxotrophic. Growth of *ΔargEF* but not *ΔargB* is partially rescued by ornithine supplementation. Blocking RIA by ferrous iron chelation inhibited growth of *ΔargEF* but not *ΔargB.* Siderophore production of *ΔargEF* decreased while that of *ΔargB* increased with declining arginine availability. Taken together, these data indicate that the siderophore system is mainly fueled by mitochondrial rather than cytosolic ornithine production and that mitochondrial ornithine biosynthesis is feedback inhibited by arginine. In agreement with the defect in siderophore biosynthesis, *ΔargEF* displayed a dramatically reduced cellular ornithine content. In contrast, the arginine and polyamine contents were wild type-like, indicating prioritization of the later two biosynthetic pathways over siderophore production. Consistent with cellular balancing of siderophore biosynthesis and arginine metabolism, arginine was recently identified to allosterically activate the ornithine monooxygenase SidA and consequently SB-mediated ornithine consumption.

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B7

Seroprevalence of Hepatitis E virus-specific antibodies in Austria

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Background: Hepatitis E virus (HEV) infection is a major cause of acute viral hepatitis worldwide. In developing countries, it is mainly associated with fecally contaminated drinking water although parenteral and perinatal routes have been described. HEV infections in industrialized countries have been thought to be strictly associated with travel to regions of endemicity. However, locally acquired cases of HEV infection are increasingly being observed in Europe, recently. The specific sources and route of transmission of these autochthonous infections remain largely unknown. Previous studies showed that HEV is endemic in e.g. Germany and likely exists as a food borne zoonosis. In developed countries most autochthonous HEV-infections are reported in middle aged and elderly men. However, we reported recently on a 6 month old child with HEV-infection. In immuno-compromised patients chronic HEV infections including symptomatic reactivation have been observed. The prevalence of HEV in Austria is unknown.

Methods: The objective of this study was the collection of seroprevalence data on HEV in Austria. Five cohorts with 150 individuals each have been integrated into the study: blood donors, organ transplant recipients, pregnant women, children, and men above the age of 50. The sera were tested using an IgG-specific- , an IgM-specific-, and a total antibody-specific assay. Sera were regarded IgG positive if they reacted in the total antibody assay as well as in the IgG-assay. Sera were regarded IgM positive if IgM antibodies were confirmed by a positive PCR.

Results: The seroprevalence of HEV-IgG antibodies was 2.7% in men above the age of 50, 3.3% in blood donors, 2.0% in transplant recipients, 0% in pregnant women, and 0% in children. IgM antibodies confirmed by PCR were not detectable in any group.

Conclusions: This indicates that HEV infections do occur in Austria and should be considered as a differential diagnosis in patients with otherwise unexplained hepatitis.

B8

Congenital CMV infection in preterm twins – Cytomegalovirus-associated enterocolitis in an extremely low birth weight preterm infant

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Background: Cytomegalovirus (CMV) infection is the most common congenital infection in humans. A primary CMV infection occurs in 2% of all pregnancies and remains asymptomatic in 95% of cases and is transmitted to the fetus in 40%. Especially preterm infants with a low birth weight are at high risk for serious clinical manifestations.

Results: We report on female monochorial twins with severe growth discrepancy, born with a gestational age of 31 weeks. The second twin presented with symptoms of premature birth and extremely low birth weight of 560g. Maternal antibodies were IgG positive and IgM negative for CMV. Routinely checked CMV-PCR in urine was positive. The first twin showed an asymptomatic clinical course. At the age of two months the second twin presented with thrombocytopenia, hepatosplenomegaly, elevation of transaminases with hyperbilirubinemia and severe necrotizing enterocolitis, which did not improve with antibiotic therapy and oral food deprivation. CMV, detected in blood, urine and stool, was identified as a causal pathogen. A retrospective CMV analysis in dried blood spot specimen, which was taken at the age of 48 hours, confirmed congenital CMV infection. Due to antiviral treatment with CMV gammaglobulin and ganciclovir the patient continuously improved. Discharge from hospital was possible at the age of four months. Neurological examination and otoacoustic emissions were normal in both patients.

Conclusion: This report shows a congenital CMV infection of preterm twins and illustrates the great variability of clinical symptoms correlating with birth weight. A congenital CMV infection cannot be excluded by a primarily asymptomatic course. Even if the onset of the disease is late, confirmation of congenital infection can still be performed by the analysis of the CMV DNA in the dried blood spot specimen.

B9

Human plasmatic coagulation is significantly impaired by EHEC-derived Shiga toxin 2

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Background: Infection with Shiga toxin producing *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). Consecutively the coagulation system is activated. It has also been postulated, that Stx itself acts as an activator of blood platelets. 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, on the plasmatic coagulation and on antithrombin (AT), a strong inhibitor of the plasmatic coagulation cascade.

Methods: Stx2 was incubated with platelet-rich-plasma (PRP) and aggregometry was performed to evaluate the aggregation inducing capacity of Stx2. In flow cytometry, activation of platelets was shown measuring the expression of two platelet-activation markers, CD62P (P-selectin) and CD63. For evaluation of a possible impairment of the plasmatic coagulation due to Stx2 thromboelastometry (ROTEM) analyses with platelet-poor-plasma (PPP) were performed and Coagulation Time (CT) and Clot Formation Time (CFT) were measured. ELISA was performed to evaluate a binding of antithrombin (AT) to Stx2.

Results: Neither in aggregation, nor in the expression of the investigated activation markers CD62P and CD63, a Stx2-induced effect on platelets could be observed. In ROTEM a clear reduction of both, the Coagulation Time (CT) and the Clot formation Time (CFT) in PPP was observed. However, ELISA revealed a strong binding of AT to Stx2.

Conclusion: Stx2 is not able to activate human platelets *in-vitro*. However, a strong binding of AT to Stx2 was observed. A reduction of both CT and CFT in ROTEM under influence of Stx2 points towards a significant functional correlate of the Stx2-AT binding. We hypothesize, that in HUS-associated thrombotic disorders, Stx2 has a direct and possibly significant impact on the plasmatic branch of the coagulation system.

B10

The role of Stat1 in differentiation of tumor-associated macrophages in MMTV-neu mammary tumor-bearing mice

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Background: The Tumor-Associated Macrophages (TAMs) are one of the major non-neoplastic cell populations in a variety of animal and human tumors, correlated with a bad prognosis for the patient and an increased risk of recurrence and metastasis. They were proven to create 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.

Methods: Bone marrow cultures in vito and functional assays with MACS-purified TAMs are used.

Results: The TAMs, which infiltrate both Stat1-proficient and deficient MMTV-neu tumors express the CD11b+ CD11c+ F4/80+ GR-1- surface phenotype and have broad immunosuppressive properties. Although functionally similar, the Stat-deficient TAMs are approximately 50% less abundant than their wildtype counterparts. In the presented work the techniques of in vitro cultures, in vivo experiments and functional assays with ex-vivo isolated TAMs were applied to identify the bona-fide TAM precursor population in bone marrow and bloodstream and to point out the growth factors which orchestrate their differentiation.

Conclusion: Stat1 is suggested to regulate the recruitment of inflammatory monocytes to the tumor bead and their maturation into TAMs via tumor-derived M-CSF.

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B11

Biomarker of inflammation in juvenile idiopathic Arthritis (JIA)

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Background: Juvenile idiopathic arthritis (JIA) is a relevant autoimmune disease in children. T cells, B cells, and damage-associated molecular patterns (DAMPS) are involved in the pathogenesis of the disease. Biomarkers for JIA and its subtypes are not established. Pro-inflammatory pathways activate enzyme indoleamine 2,3-dioxygenase (IDO) which enhances tryptophan (Trp) conversion to kynurenine (Kyn). Thus, in conditions of chronic immune activation reduced Trp availability and production of Kyn and its down-stream metabolites may inhibit cell proliferation. In rheumatoid arthritis (RA) Trp concentrations are lower in patients than in controls and the Kyn/Trp ratios are higher and correlate with neopterin concentrations [1-3].

Methods: In this study, Trp and Kyn metabolism was investigated in children with JIA and compared to serum neopterin concentrations. Fifty-four sera of 25 JIA patients and 10 samples of synovial fluid were examined with HPLC (Trp and Kyn) and Elisa (Neopterin, BRAHMS, Hennigsdorf, Germany). Eighteen sera from 18 children with non-inflammatory diseases were used as controls.

Results: Trp in the sera of patients was mean 57.2 + SD 19.0 µmol/L and Kyn was mean 2.40 + SD 0.81 µmol/L). Serum neopterin was 5.69 + SD 1.72 nmol/L. In the synovial fluid, neopterin was mean 10.5 + SD 7.41 nmol/L), Trp was 36.7 + SD 17.4 µmol/L and Kyn was 2.13 + SD 0.75 µmol/L). In control patients, neopterin was 6.93 + SD 3.10), Trp was 57.6 + SD 14.8) and Kyn was 2.60 + SD 1.60 µmol/L.

Conclusion: Serum Trp concentrations showed no relevant difference in JIA patients vs. controls. IDO activity reduces Trp primarily in the synovial fluid in JIA patients.

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[2] Fagerer N. et al. Expression of neopterin and chemokines in rheumatoid arthritis and cardiovascular disease. Pteridines 2011;22:7-12.

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Poster Session C

15.15-16.45

T-lymphocytes, Apoptosis, Transplantation

Posterwalk by Katja Kotsch

C1

Alterations in the regulatory T cell population in atopic-dermatitis-like cutaneous inflammation in mice

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Although the important role of abnormal immune reactivity in the multifactorial pathogenesis of atopic dermatitis (AD) is widely accepted, the complex network of the AD immune system remains still unclear. Repeated high dose of topically applied vitamin D3 (VitD3) or overexpression of thymic lymphopoietin (TSLP) under the K14 promotor result in Langerhans-cell-dependent AD-like inflammation in mouse skin, characterized by epidermal hyperplasia, hyperkeratosis and dermal inflammation. These symptoms are associated with enhanced mRNA expression of TSLP and other Th2 cytokines and chemokines in the skin and with elevated serum IgE levels. Mice with AD-like inflammation show greater numbers of skin draining lymph node (sdLN) cells and increased numbers of emigrated skin dendritic cells (DC) and regulatory T cells (T_{regs}). Proliferation of natural and induced T_{regs} is similar in VitD3 treated mice but both populations are increased as compared to vehicle-treated controls. Short-term topical application of VitD3, inducing acute AD-like inflammation, upregulate T_{reg} activation markers ICOS, CTLA-4 and GARP as well as IL-10 secretion by T_{regs}. In K14-TSLP transgenic mice, a model of chronic AD-like skin inflammation, T_{regs} induce ICOS, whereas production of IL-10 is not altered as compared to controls. Furthermore, AD-like inflammation modulates expression of costimulatory molecules on various DC subsets in sdLN. Finally, depletion of Langerin-expressing DC in Langerin-DTR (diphtheria toxin receptor) mice demonstrates that a lack in Langerin$^+$ DC promotes development of T_{regs}. While T_{regs} seem upregulated and activated in acute AD, defective regulatory function cannot be excluded in chronic AD as reported in various cohorts of AD patients.

C2

Endurance exercise lowers aortal plaque formation in young atherosclerosis-prone mice as a result of increased T regulatory (Treg) cells

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Background: Physical exercise has been shown to be effective in reducing both morbidity and mortality in patients with cardiovascular disease. In this study, we investigated if endurance training can be used to treat and possibly prevent atherosclerosis in both, wild-type (C57BL/6) and atherosclerosis-prone (ApoE$^{-/-}$) mice. Specifically, we were interested, if any beneficial effect of physical exercise may be mediated by an increase of the number of T regulatory cells (Tregs).

Methods: 14 week ("young") and 49-52 week ("aged") female wild-type and ApoE$^{-/-}$ mice were trained on a 5-week treadmill programme. The programme consisted of 1 hour of running for 5 days per week at a set speed. The running mice were compared to mice with a sedentary lifestyle. The mice were fed either normal chow diet or cholesterol-enriched ("Western") diet *ad libitum*, (n=6 in each group). The aorta was then surgically removed for Oil-Red staining to determine total plaque size. Serum cholesterol and triglycerides were measured with ELISA. **... truncated by editor (RW)!**

Results: Young and aged wild-type mice fed a Western diet on a sedentary lifestyle showed significantly increased bodyweight compared to the other wild-type groups fed normal chow diet. When comparing running to resting young ApoE$^{-/-}$ mice (independent of diet), significantly decreased plaque formation was found in the trained groups. Exercise in the aged ApoE$^{-/-}$ mice had no beneficial lowering of plaque formation. Lastly, we observed a decrease in plaque formation in the exercising aged wild-type mice, irrespective of diet. Young ApoE$^{-/-}$ mice had increased cholesterol levels compared to wild-type mice, on same lifestyle settings. Higher triglyceride levels were also observed in Western fed ApoE$^{-/-}$ mice (both in resting/running groups) compared to the respective wild-type groups. Moreover, ApoE$^{-/-}$ mice on a Western diet had higher levels of cholesterol levels compared to ApoE$^{-/-}$ mice on a normal diet. An increase of Tregs was found in lymph nodes of exercising young ApoE$^{-/-}$ and wild-type mice, irrespective of diet. In contrast, the same trend was not observed in the aged mice. However, aged exercising ApoE$^{-/-}$ and wild-type mice on a normal diet displayed an increase in lymph node Tregs.

Conclusion: Physical exercise leads to less aortal plaque formation in the young ApoE$^{-/-}$ mice but not in the aged mice, and does not seem to depend on lower lipids in serum. The decrease of atherosclerotic plaques in the exercising young ApoE$^{-/-}$ mice might be a consequence of the induction of Tregs found in lymph nodes. In contrast, the increase of Tregs in aged mice does not lead to a regression of plaque formation.

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C3

Mutual antagonism of TGF-β and IL-2 in cell survival and lineage commitment of iTreg cells

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Background: Regulatory T-cells (Treg) expressing the transcription factor Foxp3 play a key role in the maintainance of immune homeostasis and prevention of autoimmune diseases. Foxp3+Treg cells can be divided into two types: natural Treg (nTreg) cells, that develop in the thymus, and adaptive (or induced) Treg (iTreg) cells that are generated outside the thymus. iTreg cells carry high hopes for the treatment of chronic inflammatory and autoimmune diseases and can be generated and expanded in the presence of transforming growth factor beta (TGF-beta) and Interleukin-2 (IL-2). Knowledge about factors stabilizing their lineage commitment and lifespan, however, is limited.

Methods: We investigated the behaviour of iTreg cells, derived from apoptosis-defective mouse mutants, during activated cell autonomous cell death (ACAD), triggered by cytokine-deprivation, or activation induced cell death (AICD) after restimulation of the T cell receptor, and compared these responses with those of effector T cells.

Results: We observed that iTreg cells were much more sensitive to IL-2-deprivation but poorly susceptible to AICD. In fact, when apoptosis was compromised, TCR-religation resulted in methylation-independent, ERK- and, to a lesser extent, PI3K/mTOR-mediated loss of Foxp3 expression, impaired suppressive capacity and effector cytokine production. *In vivo,* iTreg cells prevented colitis induction yet rapidly lost Foxp3-GFP expression, gained ability to produce effector cytokines and mediated the induction of Th1 cells. Surprisingly, iTreg cell conversion was limited by TGF-beta-mediated transcriptional activation of proapoptotic BH3-only protein genes, triggering *Bim/Bcl2L11*-dependent apoptosis. Survival and regulatory T cell fate were only secured when both cytokines were present simultaneously.

Conclusion: Thus, the very same cytokine that drives the generation of iTreg cells can trigger their demise when IL-2 becomes limiting. Our results provide novel insights in iTreg cell biology that will assist optimization of iTreg cell based therapy.

C4

Defective cell death signalling along the Bcl-2 regulated apoptosis pathway compromises Treg cell development and limits their functionality in mice

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The Bcl-2 regulated apoptosis pathway is critical for the elimination of autoreactive lymphocytes, thereby precluding autoimmunity. T cells escaping this process can be kept in check by regulatory T (Treg) cells expressing the transcription and lineage commitment factor Foxp3. Despite the well-established role of Bcl-2 family proteins in shaping the immune system and their frequent deregulation in autoimmune pathologies, it is poorly understood how these proteins affect Treg cell development and function. Here we compared the relative expression of a panel of 40 apoptosis-associated genes in Treg vs. conventional CD4(+) T cells. Physiological significance of key-changes was validated using gene-modified mice lacking or overexpressing pro- or anti-apoptotic Bcl-2 family members. We define a key role for the Bim/Bcl-2 axis in Treg cell development, homeostasis and function but exclude a role for apoptosis induction in responder T cells as relevant suppression mechanism. Notably, only lack of the pro-apoptotic BH3-only protein Bim or Bcl-2 overexpression led to accumulation of Treg cells while loss of pro-apoptotic Bad, Bmf, Puma or Noxa had no effect. Remarkably, apoptosis resistant Treg cells showed reduced suppressive capacity in a model of T cell-driven colitis, posing a caveat for the use of such long-lived cells in possible therapeutic settings.

C5

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. There is a complete rescue when both BH3-only proteins are lacking and the surviving cells also seem to be able to mount a T-dependent immune response.

Conclusion: We conclude that BAFF acts by modulating the expression and/or function of Bim and Bmf, but the molecular basis 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.

C6

Identification of a novel PKCβ phosphorylation site in p66^{SHC} regulating ROS production and cell death in response to oxidative stress

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Background: Reactive oxygen species (ROS) produced during ischemia/reperfusion are main contributors to the deterioration of organ function following transplantation. Knock-out of p66^{SHC} in mice resulted in a 30% increase in lifespan which correlated with an increased resistance to oxidative stress. Mouse embryonic fibroblasts (MEFs) from p66$^{SHC-/-}$ mice were shown to be less sensitive towards apoptosis in response to different stress stimuli due to a decreased ROS production. It has been reported that p66^{SHC} directly produces H_2O_2 through the oxidation of cytochrome *c* at the mitochondrial electron transport chain (Giorgio et al., Cell 122, 2005). P66^{SHC} has been linked to IR-induced organ damage and thus might be a potential therapeutic target. PKCβ has been implicated in the activation of p66^{SHC} through phosphorylation of Ser36 which is also a target for several other kinases (Pinton, Science 2007).

Methods: As cellular models we used immortalized MEFs from p66$^{SHC-/-}$ or PKCβ$^{-/-}$ mice. Cells were stained for ROS using reduced MitoTracker Red, detected with standard microscopy, or DCF-DA, measured with FACS. For survival assays cells were stained with Annexin V/PI and analyzed by FACS. Hydrogen peroxide (H_2O_2) and tert-butyl hydroperoxide (t-BHP) were used as pro-oxidants. Gö6976 is a selective PKCα/β inhibitor.

Results: We could confirm the role of PKCβ in p66^{SHC}-induced ROS production using PKCβ$^{-/-}$ MEFs and different PKC inhibitors. However, Ser36 phosphorylation of p66^{SHC} was only minimally affected by lack of PKC activity. Using an *in silico* approach, one novel PKCβ phosphorylation site in p66^{SHC} was identified and confirmed in *in vitro* kinase assays using peptide substrates. Serine to alanine substitution of this PKCβ phosphorylation site resulted in a p66^{SHC} mutant, which was unable to rescue the wild type ROS phenotype when expressed in p66$^{SHC-/-}$ MEFs and provoked an increased resistance towards oxidative stress. Apoptosis induction after STS treatment or serum deprivation was unaffected. Mutation of the newly identified site also abrogated mitochondrial accumulation of p66^{SHC} in response to cellular stress. This may be explained by decreased binding of the p66^{SHC} mutants to the prolyl isomerase Pin1 which is essential for its mitochondrial translocation (Pinton, Science 2007).

Conclusion: Our work identified a novel PKCβ phosphorylation site in p66^{SHC}, which is essential for its ability to generate ROS and apoptosis following oxidative stress.

C7

Donor age-dependent impact of CD11c^{+} dendritic cells on allograft rejection

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Background: In large-scale clinical studies, the use of organs from old donors has been associated with increased frequencies of acute rejections. Herein, we investigated the impact of donor age on recipient immune responses in vitro and in vivo.

Methods and Results: Hearts from young (3 mo) or old (18 mo) C57BL/6 (B6) donor mice were grafted into young (3 mo) DBA/2 recipients. Old hearts were rejected more rapidly than young hearts and H&E staining revealed higher rejection scores. Furthermore, recipients of old allografts showed increased frequencies of alloreactive IFN-γ producing cells as well as higher percentages of CD8+ effector and CD8+ IFN-γ+ T cells. To determine whether the more potent immune response observed after transplantation of old organs is promoted by aged graft-derived leukocytes or aged parenchyma, chimeric animals were generated by transplanting bone marrow from young B6 mice into lethally irradiated old or young B6 mice. Transplantation of chimeric hearts into young DBA/2 mice resulted in comparable survival rates, rejection scores and recipient systemic immune responses. To investigate in depth the relevance of CD11c+ Dendritic cells (DCs) among graft-derived leukocytes, depletion of DCs was performed using liposomal clodronate. Following transplantation of old or young DC-depleted B6 cardiac allografts into young DBA/2 animals, survival rates, rejection scores and systemic immune responses were similar between groups. Finally, immunogenic properties of flow-sorted splenic DCs were evaluated. DCs from old B6 mice induced a higher proliferative response and were more potent in priming allogenic responder cells in vitro.

Conclusion: Taken together, these results highlight the impact of age on graft-derived Dendritic cells in their capacity to trigger the host immune response after solid organ transplantation.

C8

Dono p38MAPK inhibition prevents impairment of kidney function resulting from ischemia/reperfusion injury (IRI)

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Background: In the course of solid organ transplantation excessive production of reactive oxygen species (ROS) is a major contributor to the development of ischemia/reperfusion injury (IRI). Preventing ROS actions through the use of antioxidants so far proved unsatisfactory in the clinical setting, most likely due to the difficulty to timely and efficiently target these substances to the site of ROS production and action. 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. We recently also obtained evidence, which linked the activation of p38MAPK to mitochondrial ROS accumulation and cell death during hypoxia/reoxygenation (HR). These data suggest that mitochondrial changes preceding the onset of cell death are subject to regulation by intracellular signaling pathways. This insight could be therapeutically exploited for the prevention of IR-induced organ damage. Here we further dissected the contribution of p38 to IR- and HR-induced damage and provide first evidence for a therapeutic benefit of p38 inhibition *in vivo*.

Methods: Kidney transplantation and kidney clamping in the rat were used for the induction of IRI. HR was predominantly analyzed in HL-1 cardiomyocytes and primary MEFs. Intracellular signaling was monitored by using phosphorylation-specific antibodies. Mitochondrial ROS levels were determined by imaging of cells pre-loaded with Mitotracker Red CM-H2XROS. **... truncated by editor (RW)!**

Results: In the work presented here we provide further evidence for the involvement of p38 in causing redox stress and suggest MAPKAP-kinase 2 (MK2) as a downstream effector. Reperfusion following kidney clamping or transplantation was marked by a profound increase in the activity of p38 and the putative effector MK2 which was significantly prevented by the p38 inhibitor BIRB-796. p38 inhibition almost completely prevented functional impairment caused by IR during kidney clamping as measured by reduced serum creatinine, urea, cystatin c and NGAL levels. p38 inhibition also protected from oxidative damage and significantly reduced the percentage of apoptotic cells and the expression of TNFα and HO-1 during IR. Most importantly, a significant reduction of functional impairment was also achieved in the kidney transplant model.

Conclusion: Inhibiting p38 signaling during IR may provide a potent strategy for limiting IRI.

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C9

Characterisation of cellular infiltrates in a new established brain death model in mice

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Background: Brain death (BD) has been shown to be an independent risk factor for allograft survival as well as graft function. As the great majority of transplanted organs are still derived from deceased organ donors, we introduced a model of BD in mice to elucidate in more detail the molecular and cellular mechanisms impacting donor organ quality.

Methods: Mean arterial pressure in anesthetized mice (n=5) was measured via an intrarterial catheter in the left common carotid artery. Mice were ventilated via a tracheotomy with a tidal volume of 200µl and frequency of 150/min. For BD induction, a fogerthy catheter 3 Fr. was inserted through a bore hole in the parietal scull. The balloon was inflated over a period of 10 min until brain death occurred. BD was confirmed by cessation of spontaneous respiration and maximally dilated, fixed pupils as well as missing brainstem reflexes. BD was kept for 3 hours before organs were taken for analysis. Naive mice without any treatment served as control group (n=5).

Results: Real time RT-PCR (TaqMan©) revealed that inflammatory cytokines or adhesion molecules including IL-6, TNFα, IFNγ and P-Selektin were clearly induced in hearts and kidneys as a consequence of BD whereas no cytokine induction was detectable in livers. Moreover the percentage of CD3+CD4+ as well as CD3+CD8+ T cells was significantly increased in peripheral blood mononuclear cells and splenocytes in the BD group ($p<0.05$). In addition the amount of T regulatory (Treg) cells characterized by their CD3+CD4+CD25+FoxP3 expression was significantly elevated ($p<0.001$). BD resulted further in a significant induction of splenic CD11c+B220- conventional dendritic cells (cDCs) and although no changes in the frequency of NKp46+ Natural Killer (NK) cells was detectable, BD itself resulted in a significant induction of mean fluorescence intensity (MFI) of NKp46 expression ($p<0.001$) suggesting an activation of NK cells.

Conclusion: Donor BD is a major risk factor for long term survival of solid organs. Our data highlight the organ specific inflammation due to BD and the activation of various lymphocyte subsets. A more comprehensive immunological analysis will further uncover the importance of various cell subsets in the setting of BD and whether any donor/graft pre-treatment strategies may improve allograft survival.

C10

Lipocalin-2 as mediator of chemokine expression and granulocyte infiltration in a murine heart transplantation model

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Our previously published data demonstrated that neutrophil gelatinase-associated lipocalin (NGAL/Lcn-2) expression is associated with ischemia/reperfusion injury (IRI) following transplantation and correlates with polymorphonuclear cell infiltration. To elucidate the regulatory role of Lcn-2 during IRI we aimed to investigate the regulation of Lcn-2 expression by hypoxia as well as the effects of Lcn-2 on the expression of key chemokines, chemokine receptors, and adhesion molecules in a heterotopic murine heart transplantation model using wild-type and Lcn-$2^{-/-}$ mice. Transient transfection studies showed that neither hypoxia alone nor hypoxia and overexpression of HIF-1α or HIF-1β significantly induced the Lcn-2 promoter. Significant differences between Lcn-$2^{+/+}$ and Lcn-$2^{-/-}$ grafts were revealed in the expression of genes encoding various chemotactic proteins and in granulocyte infiltration. Our results indicate that Lcn-2 indirectly affects granulocyte infiltration in the reperfused graft by modulating the expression of chemokines and their receptors. Understanding these regulatory mechanisms will be crucial to establishing prevention strategies for IRI and consecutive allograft dysfunction following solid organ transplantation.

NOTES

Poster Session D

15.15-16.45

Innate immunity and infection

Posterwalk by Dorothee Orth-Höller

D1

Complement activation in invasive fungal infections is triggered by different mechanisms

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Background: Invasive aspergillosis (IA) has emerged as a leading cause of morbidity and mortality in immunocompromised patients; the most common inducers are *Aspergillus (A.) fumigatus* and *A. terreus.* Activation of the complement cascade, which is an important weapon of innate immunity, might considerably influence the course of IA by exerting its beneficial but also detrimental effects. For that reason we studied different mechanisms of complement activation in IA.

Methods: Conidia and hyphae of *A.fumigatus* and *A.terreus* were incubated with serum. Deposition of complement factors on conidia was monitored by FACS, opsonization on hyphae was detected by fluorescence microscopy. Human thrombocytes were incubated with a fungal culture supernatant of *A. fumigatus* in the presence of serum. Thrombocyte activation and deposition of complement factors were examined by FACS.

Results: Conidia of *A. fumigatus* and *A. terreus* were able to trigger complement activation with subsequent deposition of complement factors on the conidial surface. The isolate ATS of *A. terreus,* which differed from other *A. terreus* isolates by an unusual sensitivity towards the antimycotic drug amphotericin B (AmB), was even better recognized by the complement system than its AmB-resistant counterpart (ATR). Similarly, the hyphae of the ATS isolate triggered more deposition of complement factors on the hyphal surface than ATR hyphae.

As a second mechanism for complement stimulation in IA, the fungus-induced alteration of host cells and subsequent recognition by the complement system as “altered-self” was investigated. Fungal supernatant activated human thrombocytes, as shown by degranulation; furthermore, the platelet membrane was modified with increased exposure of phosphatidylerine and binding of Annexin V. As a consequence strong opsonization of the thrombocytic surface with complement factors C3, C1q, C5, C7, and TCC could be detected on the thrombocytes.

Conclusion: Complement activation can take place in IA by direct contact with fungal conidia and hyphae, but also by fungus-induced alteration of host cells such as platelets. The fact that different mechanisms can trigger the complement cascade underlines the importance of this innate immune system. Putative consequences include support of phygocytosis and thus prevention of fungal dissemination. However opsonisation of activated platelets might also contribute to thrombosis and thrombocytopenia.

D2

Activation and opsonisation of platelets studied in a mouse model of invasive aspergillosis

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Background: Invasive aspergillosis is associated with a high lethality among immunocompromised patients despite improved antifungal drugs. Recent results indicated that platelets and the complement system are important innate immune weapons to recognize and combat fungal pathogens. We studied activation and complement opsonisation of platelets in a mouse model of inhalative or intravenous aspergillosis with *Aspergillus terreus*. The influence of echinocandins on these parameters was also investigated.

Methods: Balb/c mice were immunosuppressed with cortison acetate or cyclophosphamid, followed by infection with *A. terreus* conidia intravenously or by inhalation, respectively. Three groups of mice were treated every day with caspofungin, anidulafungin and micafungin. The clinical status of all animals was determined daily. Blood samples were taken at different time points, and the activation marker CD62P as well as deposition with the complement factor C3 on the surface of the platelets were measured by FACS.

Results: When mice were infected intravenously with *A. terreus* platelet activation was higher than in uninfected control mice, as shown by increased presence of CD62P on the platelet surface. Similarly, enhanced opsonisation of platelets with complement factor C3 could be detected in infected compared to non-infected animals. In contrast, no increased platelet activation nor opsonisation were detected in mice infected with *A. terreus* by inhalation. Treatment of the mice with caspofungin or anidulafungin induced enhanced opsonisation of platelets with C3 at day 3, compared to control animals; no difference was detected at later time points.

Conclusion: The mouse models for intravenous versus inhalative infection with *A. terreus* differed significantly in the parameters for platelet activation and opsonisation with complement factors. The platelets were modified in intravenously infected animals and subsequently recognized by the complement system as “altered-self”. In contrast, no platelet modifications could be detected in animals infected by inhalation. Antifungal therapy can influence the process of platelet activation and opsonisation.

D3

Aspergillus species activate platelets by their cell wall polysaccharides

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Background: Invasive aspergillosis (IA) is an opportunistic disease that mainly affects immunocompromised patients. *Aspergillus (A.) fumigatus* and *A. terreus* are the most common pathogenic species causing IA. Their interactions with platelets, that had recently been recognized to belong to the innate immunity and harbour antimicrobial functions, might considerably influence the pathogenesis of IA and were therefore studied in detail.

Methods: Thrombocytes were incubated with conidia of different *A. fumigatus* and *A. terreus* isolates. Furthermore, cell wall components were separated from protoplasts by enzymatic digestion and the whole fraction was added to platelets. In comparison, single purified cell wall compounds were given to the cells. Thrombocyte activation was monitored in all experiments by FACS analysis, using CD62P as marker.

Results: Conidia of *A. fumigatus* and *A. terreus* both induced significant activation of platelets; the intensity of thrombocyte stimulation varied considerably between conidia of different isolates. The fraction containing all the cell wall components was also capable of inducing platelet degranulation. This reaction could not be mimicked by purified 1,3-β-glucan, nor by galactomannan or chitin. In contrast, the polysaccharide galactosaminogalactan, which had been shown to cover the fungal surface, harboured the capacity to activate the thrombocytes in a dose-dependent manner.

Conclusion: Direct contact of the conidial surface with the platelets resulted in platelet activation and degranulation. A similar process can be hypothesized to take place after angioinvasion in the course of invasive aspergillosis. Galactosaminogalactan could be identified as active compound to trigger or at least to participate in this effect. Putative consequences of platelet activation might be a better antimicrobial function of the thrombocytes, but also occurrence of thrombosis.

D4

Hypoxia induced down-regulation of hepcidin is mediated by platelet derived growth factor BB

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Background: Hypoxia affects body iron homeostasis, which may impact on hematopoietic response as well as immunosurveillance during oxygen deprivation. However, underlying mechanisms of the adaption of iron homeostasis to hypoxic challenge are incompletely understood.

Methods: Using a standardized hypoxia chamber, 23 healthy volunteers were subjected to exercise under hypoxic conditions, equivalent to an altitude of 5600 meters, for 6 hours. Consecutive in *vivo* experiments were performed with a standardized hypoxia chamber adjusted for mice, respectively.

Results: Exposure of subjects to hypoxia resulted in a significant decrease of serum levels of the master regulator of iron homeostasis hepcidin and elevated concentrations of platelet derived growth factor(PDGF)-BB. Using correlation analysis, we identified PDGF-BB to be associated with hypoxia mediated hepcidin depression in humans. In mice, the hypoxia mediated down-regulation of hepatic hepcidin mRNA expression was paralleled by elevated serum PDGF-BB protein concentrations and higher serum iron levels as compared to normoxically housed control mice. PDGF-BB treatment of HepG2 cells and C57BL/6 mice resulted in suppression of both, steady state and BMP6 inducible hepcidin expression. Mechanistically, PDGF-BB appeared to inhibit hepcidin transcription by down-regulating the protein expression of the transcription factors CREB and CREB-H, because the latter event could be reversed by pharmacological blockade of PDGF-BB receptor signaling.

Conclusion: Hypoxia decreases hepatic hepcidin expression by a novel regulatory pathway exerted via PDGF-BB, leading to increased availability of circulating iron, which can be used for erythropoiesis but may also impact on immunosurveillance.

D5

The role of Neutrophil Gelatinase-Associated Lipocalin (NGAL) in iron homeostasis of *Chlamydia pneumoniae* infected macrophages

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Background: Neutrophil gelatinase-asscociated lipocalin (NGAL/ Lipocalin-2/Lcn-2) or 24p3 is a 25 kDA protein of the lipocalin superfamily produced by different cell types including immune cells. Lcn-2 plays a direct role in iron transport into the host cell which is important for intracellular pathogens needing sufficient iron supply for proliferation and pathogenicity. The cytokine Interleukin-10 (IL-10) is an important regulator of cytokine production, mainly playing an anti-inflammatory role in the immune system modulating iron availability. In the present study, we investigate the role of Lcn-2, iron and IL-10 in primary macrophages infected with *Chlamydia pneumoniae*.

Methods: Primary peritoneal macrophages were obtained from wild type and Lcn-2-/- mice. Cells were infected with *Chlamydia pneumoniae* at a MOI of 10 and stimulated with iron, anti-interleukin-10 or both, or left untreated. For determination of infection cells were harvested after 12 hours following DNA preparation for *C. pneumoniae* RT-PCR. RNA and proteins for the analysis of cytokine and proteins of iron metabolism were determined iafter 12 and 24 hours respectively.

Results: Chlamydial infection was more pronounced in Lcn-2-/- mouse peritoneal macrophages compared to Lcn-2+/+ cells. Moreover a significant increased infection was found after stimulation with iron and anti-IL10 and this effect was even more pronounced with combined stimulation of Lcn-2 -/- macrophages. Intracellular H-Ferritin revealed an increase after stimulation with iron, whereas chlamydial infection did not change the H-Ferritin levels in Lcn-2+/+ macrophages. On the other hand Lcn-2-/- cells showed a decrease of H-Ferritin after *C. pneumoniae* infection which was only reversed by addition of iron. Whereas transferrinreceptor was downregulated in both cell lines, DMT-1 and the iron exporter ferroportin revealed divergent regulations.

Conclusion: Lipocalin-2 and interleukin-10 are important modulators of iron homeostasis in macrophages infected with *C. pneumoniae* thus influencing the course of infection. Enhanced expression of IL-10 in Lcn-2-/- macrophages may be a protective mechanism to limit iron bioavailability for the intracellular pathogen in the absence of Lcn-2.

D6

Lipocalin-2 is an endogenous inhibitor of inflammation in murine nephrotoxic serum nephritis

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Background: Lipocalin-2 (Lcn-2) has been described to be involved in divergent processes such as acute kidney injury or bacterial host defence. Our study was designed to evaluate the functional role of Lcn-2 in nephrotoxic serum nephritis (NTS).

Methods/Results: Mice subjected to NTS expressed increased Lcn-2 in proximal tubular epithelial cells and cells of the innate immune system. Lcn-2 knock-out mice exhibited more glomerular damage with increased proteinuria and interstitial mononuclear leukocytic infiltrates compared to wild-type mice. By inducing NTS in lethally radiated LCN2 knock out mice reconstituted with WT bone marrow , we found Lcn-2 expressed in circulating innate immune cells to protect from NTS. In contrast, the lack of Lcn-2 in these cells led to decreased rates of apoptosis but increased necrosis and formation of high-mobility group box 1 (HMGB-1) in the kidney. Via TLR-2 signalling HMGB-1 increased the transcription rate of pro-inflammatory cytokines in tubular epithelial cells and macrophages resulting in increased disease activity. In parallel, Lcn-2 was also found to be increasingly transcribed by TLR-2 signalling.

Conclusion: Thus, Lcn-2 expressed in innate immune cells is protective in NTS by inducing concerted apoptosis and inhibiting the formation of HMGB-1 thereby limiting cytokine production via TLR-2 signalling. In parallel, TLR-2 dependent transcription of Lcn-2 is an endogenous inhibitor of inflammation in NTS.

D7

Nitric oxide-dependent regulation of ferroportin-1 controls macrophage iron homeostasis and immune function in *Salmonella* infection

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Background: The transcriptional expression of nitric oxide (NO) synthase-2 (Nos2) is controlled by iron, while NO affects the binding activity of iron regulatory proteins and thus cellular iron homeostasis. This interplay links the maintenance of iron homeostasis to the optimal formation of NO for host defence.

Methods: We examined the reciprocal interactions between NO production and iron homeostasis in wild-type and *Nos2*$^{-/-}$ macrophages and mice both under steady-state conditions and in response to infection with *Salmonella enterica* serovar Typhimurium using quantitative RT-PCR, Western blotting and Luciferase reporter assays.

Results: We found that NO induces the transcription of ferroportin-1 (Fpn1), the major cellular iron exporter. *Nos2*$^{-/-}$ macrophages displayed increased iron content due to reduced Fpn1 expression. At the systemic level, *Nos2* disruption led to a significant accumulation of iron in the spleen due to iron deposition in *Nos2*$^{-/-}$ macrophages. Mechanistically, lack of NO formation resulted in impaired nuclear factor erythroid 2-related factor-2 (Nrf2) expression, while pharmacological NO donors enhanced the binding activity of Nrf2 in peritoneal macrophages, subsequently leading to increased Fpn1 transcription and cellular iron egress. In addition, macrophages from *Nos2*$^{-/-}$ mice showed reduced expression of pro-inflammatory cytokines TNF-α, IL-12 and IFN-γ upon infection with the intracellular pathogen *Salmonella* Typhimurium, while administration of the iron chelator deferasirox restored cytokine production in these cells, as did over-expression of Nrf2.

Conclusion: Our results demonstrate that diminished NO formation results in increased iron accumulation in macrophages, which is attributable to reduced Nrf2 activity and down-regulation of Fpn1 transcription. The accumulation of iron in *Nos2*$^{-/-}$ macrophages reduces the expression of M1-type innate host response mechanisms and impairs anti-bacterial host defences, suggesting that part of the protective effect of NO against infection with intracellular pathogens is due the radical's ability to modulate macrophage iron homeostasis.

D8

The role of HO-1 and HIF in regulating iron homeostasis and innate immune response to *Salmonella* infection

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Background: Macrophages play an essential role in innate immune regulation which also takes place in hypoxic microenvironments of infected tissues. 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 (Hif1α) and 2 (Hif2 α) have been found to regulate macrophage innate immune responses. Similar to Hif, heme oxygenase-1 (HO-1), the enzyme cleaving heme to ferric iron, billiverdin and carbon monoxide, is involved in the regulation of stress response, iron homeostasis and host pathogen interactions. The protective properties of HO-1, Hif1α and more recently Hif2α have been studied in models of inflammation, however the underlying molecular mechanisms or mode remain largely unknown.

Methods/Results: Using RAW264.7 murine macrophages and siRNA knock down of target genes, we found that the absence of both genes (hif1α and hmox) lead to regulation of the iron exporter ferroportin-1 (Fpn1) mRNA and protein expression. To study the role of Hif1α, Hif2α and HO-1 in a macrophage infection model using *Salmonella enterica* serovar Thyphimurium a more reliable technology to study host pathogen interactions was adapted for RAW264.7 cells. The lenti-virus based (tetracycline inducible) shRNA expression system results in stable and reproducible gene knock down in infected macrophages. In RAW264.7 cells, the deficiency of the three stress response genes shows significantly increased killing of Salmonella compared to the control cells.

Conclusion: Our data suggest a role for HO-1, Hif1α and Hif2α in the regulation of iron homeostasis in macrophages and strengthens anti- bacterial immune effector pathways.

D9

Protection of Hepatitis C virus from complement mediated lysis by specific acquisition of functional CD59 but not CD46 or CD55

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Background: Viruses of different families encode for regulators of the complement system (RCAs) or acquire such RCAs from the host to get protection against complement-mediated lysis (CML). As hepatitis C virus (HCV) shares no genetic similarity to any known RCA and is detectable at high titers in sera of infected individuals, we investigated whether HCV has adapted host-derived RCAs to resist CML.

Methods: To investigate the presence of RCAs on the surface of HCV we performed virus capture assay using specific antibodies and captured virus was estimated using quantitative PCR. Confirmation was made using western blot. Functionality of the RCA on virus was analysed by using blocking antibodies and Huh7.5/HCV-JC1_luciferase reporting system.

Results: Here we report that HCV selectively incorporates CD59 while neither CD55, nor CD46 are associated with the virus. The presence of CD59 was shown by capture assays using patient- and cell culture-derived HCV isolates. Association of CD59 with HCV was further confirmed by Western blot analysis using purified viral supernatants from infected Huh 7.5 cells. HCV captured by antibodies specific for CD59 remained infectious for Huh 7.5 cells. In addition, blocking of CD59 in the presence of active complement reduced the titer of HCV most likely due to CML. HCV produced in CD59 knock-down cells were more significantly susceptible to CML compared to wild type virus, but neither replication, assembly nor infectivity of the virus seemed to be impaired in the absence of CD59.

Conclusion: Our data provide evidence that CD59 is selectively incorporated into the membrane of HCV as, (i) the virus capture assay was selective for this RCA and does not provide any signal above background for CD46 or CD55 independent on the viral genotype; (ii) HCV retained by the virus capture assay promoted infection of susceptible Huh7.5.1 cells; (iii) blocking of CD59 in the presence of active complement reduced the viral titer most likely due to complement mediated lysis.The acquisition of CD59 seems to be selective as neither CD55 nor CD46 were detected in cell-culture isolates or patient-derived HCV. In summary our data indicate that HCV incorporates selectively CD59 in its envelope to gain resistance to CML in serum of infected individuals.

D10

Reduction of complement regulator CD59 expression on human kidney cells by Shiga toxin 2

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Background: Enterohemorrhagic *Escherichia coli* (EHEC) virulence factor Shiga toxin 2 (Stx2) is the main cause for the hemolytic uremic syndrome (HUS) which is characterized by the clinical triad of hemolytic anemia, thrombocytopenia and acute renal failure. In addition to the EHEC-induced HUS there are inherited atypical forms of HUS (aHUS), caused by mutations in regulators of the complement system, such as the soluble factor H or the cell-surface associated CD46 (MCP). Both CD46 and CD55 play a role in the deactivation of the C3- and C5 convertases, whereas CD59 modulates membrane attack assembly.

The aim of the present study was to determine the effects of Stx2 on the membrane bound complement regulators CD46, CD55 and CD59 in two different human renal cell lines, human kidney glomerular endothelial cells (GEnC) and human kidney proximal tubular epithelial cells (HK-2).

Methods: Both cell lines were incubated with 20 pg/µl or 200 pg/µl Stx2 at 37℃ for 4 h and the expression of CD46, CD55 and CD59 on the cell surface was assessed by flow cytometry. A number of 106 cells of every incubation group (Stx2 and control) was frozen down for subsequent analyses by RT-qPCR. β-actin was used as housekeeping gene for relative quantification of qPCR values of the target gene. Primers for CD59 and β-actin were used as described by Pattyn et al. (Nucleic cids Res. 31, 2003) and Kinderlerer et al. (Arthritis Res. Ther. 8, 2006), respectively.

Results: No influence of Stx2 on CD46 and CD55 expression on HK-2 cells was found. On GEnC cells a slight, but not significant decrease of CD46 and CD55 was detected. However a significant reduction of CD59 was shown for this cell line (20 and 200 pg/µl) whereas on HK-2 cells the significant reduction of CD59 became significant only after incubation with 200 pg/µl.

The mRNA of CD59 was significantly reduced in HK-2 cells after incubation with 200 pg/µl Stx2 and highly significantly reduced by half in GEnC cells after incubation with only 20 pg/µl Stx2 compared to the PBS control.

Conclusion: Together with our previous findings, showing that Stx2 activates the complement system, we draw the conclusion that Stx2 additionally reduces the complement regulator CD59 on the cell surface of human renal cell lines which implies that the cells become more vulnerable against complement attack.

D11

Shiga toxin binds to complement regulatory proteins of the factor H protein family

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Background: Shiga toxin (Stx) is the most important virulent factor of enterohemorrhagic *E. coli* (EHEC) which initially usually cause gastrointestinal infection. Approximately 10% of cases develop to hemolytic uremic syndrome (HUS). There are limited data on the pathogenesis to develop HUS. However, a role of complement in HUS has been reported recently: Stx2 activates complement via the alternative pathway and binds to regulatory protein factor H (FH). In this situation, the effect of complement activation can enhance the destruction of the kidney. Complement factor H-related protein 1 (FHR1) and factor H-like protein 1 (FHL1) are proteins of the FH protein family that show a structure and sequence relationship to FH. Furthermore they also act as regulators of the alternative pathway. Here, we determine whether Stx2 binds to complement regulatory proteins FH, FHR1, and FHL1 and characterize the impact of that binding.

Methods: By use of ELISA, Stx2 was immobilized onto microtiter plates. After blocking, FH or FHR1 or FHL1 were applied and anti-FH was used for detection. Cofactor activity assay of FHL1 bound to Stx2 was determined by measuring the factor I-mediated degradation of C3b and was analyzed by SDS-PAGE and Western blot.

Results: Stx2 binds to FH (as published) but also to FHR1 and FHL1. FHR1 dose-dependently competed with FH for Stx2 binding. The FHR1 binding site for Stx2 was located to the C-terminal short consensus repeats (SCRs) 3-5, resembling SCRs 18-20 of FH. Both allotypes of FHR1 (FHR1*A and FHR1*B) bind to Stx2. FHL1 retains its function (cofactor activity) for factor I-mediated C3b inactivation when bound to Stx2. Investigating of binding subunit of Stx, showed that Stx beta subunit binds to FH and FHR1.

Conclusion: The binding properties of Stx2 to FH, FHR1, and FHL1, indicate multiple interactions of Stx with the complement system. Our study demonstrates that Stx2 may affect the regulatory function of FH and shares the same region with FHR1 for binding to Stx2. This competition may result in a decreased regulatory function of FH. We suggest that FH, FHR1, and FHL1 represent key immune proteins for pathogenesis of Stx-related HUS.

D12

N-chlorotaurine, a long-lived endogenous oxidant, inactivates Shiga toxin 2 of enterohemorrhagic Escherichia coli

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Background: N-chlorotaurine (NCT), a main representative of the long-lived oxidants produced by stimulated human granulocytes and monocytes, is known to exert broad-spectrum antimicrobial activity. Loss of virulence of pathogens has been shown to be one of the first events that occur during their inactivation by NCT. The aim of this study was to investigate the impact of NCT on a virulence factor from the molecular process to the functional consequences. Shiga toxin 2 (Stx2) was selected as a model since it is an important toxin secreted by enterohemorrhagic Escherichia coli (EHEC).
Methods: Stx production in relation to bacterial growth in the presence of NCT was tested by quantitative colony counts and Stx2 ELISA. NCT-treated supernatant of EHEC was incubated with Vero cells, and the toxin function was evaluated by cytopathic effect and by cell viability assays. Purified and fluorochrome labeled Stx2 was incubated with NCT and added to human glomerular endothelial cells (GEnC). Attachment and penetration of Stx2 into the cells was monitored by confocal microscopy. In addition, surface-binding was investigated by FACS analysis. SDS-PAGE was performed with purified NCT-treated Stx2. Shifted bands were subjected to mass spectrometry.
Results: Bacterial growth and Stx2 production were both inhibited at a threshold of 2 mM NCT. Vero cell assays proved that 5.5 mM NCT impaired the function of Stx. Confocal microscopy and FACS analyses showed that the binding of Stx2 to two different human renal cell lines was inhibited, and no NCT-treated Stx2 entered the cytosol. Mass spectrometry clearly displayed oxidation of thio groups and aromatic amino acids.
Conclusions: NCT oxidizes and inactivates Stx2. Therefore, long-lived oxidants may act as powerful tools of innate immunity against soluble virulence factors of pathogens. Moreover, NCT and novel analogs are in development as topical antiinfectives. Inactivation of virulence factors may contribute to therapeutic success.

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