BRIDGE GAPS
CROSS ROADS

'All together now'

CCHD IAI ICA MCCA

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Experience innovative perspectives. Vienna 2016.

Program and Abstract Book

Joint Symposium of 4 PhD Programs
February 1st - 3rd, 2016
Hörsaalzentrum AKH - Ebene 7 + 8, Hörsaal 3
Medical University of Vienna
Währinger Gürtel 18-20, 1090 Vienna

FWF MEDICAL UNIVERSITY OF VIENNA vetmeduni vienna UNIVERSITÄT SALZBURG

Immunity in Cancer & Allerg
PhD program - University of Salzbur
ICA CHD MCCA

Bridge Gaps - Cross Roads

Joint PhD Symposium
February 1st - 3rd 2016

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A WARM WELCOME TO ALL OF YOU

With great pleasure we welcome all of you in Vienna to our Joint Symposium ***Bridge Gaps - Cross Roads***.
It is the first time in the history of our doctoral programs that 4 PhD programs from Vienna and Salzburg organize a joint PhD symposium. It took a lot of effort to consider everyone's interest and still generate an attractive scientific program for all of us. We are very happy that 21 renowned international scientists will present latest developments in their fields and 92 PhD students will have the opportunity to present their work in oral presentations and poster sessions.

"The truth is rarely pure and never simple."
- Oscar Wilde

This symposium stands out due to its large variety of topics, helping us to keep an open mind for „the big picture" in life sciences and not to get lost in a narrow-minded view on our research.

'All together now'

CCHD IAI ICA MCCA

We cordially thank the speakers of our PhD programs, Maria Sibilia, Stefan Böhm, Winfried Pickl, and Josef Thalhamer, who made this symposium possible and supported us with their know-how at every step of the organization. We also express our deep gratitude to all students who continuously contributed to the organization of this symposium and to our secretaries who helped out with their experience in organizing meetings. We would also like to thank our sponsors from industry for kindly supporting our symposium.
Finally, we hope that all of you will enjoy our symposium and we are looking forward to great talks, stimulating discussions, and memorable social events.

Leo Edlinger
"Inflammation and Immunity"

Jelena Gotovina and Martina Bucsaiova
"Cell Communication in Health and Disease"

Chiara Palladino and Dominika Polak
"Molecular, Cellular and Clinical Allergology"

Florian Zauner and Theresa Neuper
"Immunity in Cancer and Allergy"

The Organizing Committee

CHD

To offer state-of-the-art patient treatment, health-care providers need to act not only within the wards, but also in laboratories. At the Medical University of Vienna, which operates the Vienna General Hospital, students are educated in medicine so as to administer effective relief to patients. In addition, they are also trained in biomedical sciences in order to discover cures for diseases. Sickness arises when the coordinated communication within and between cells in multicellular organisms falls into disarray. This faulty communication applies to widespread pathologies, including neurodegeneration, atherosclerosis, and other chronic inflammatory diseases. Hence, understanding cellular communication is essential for defining pathological alterations and for developing appropriate therapeutic agents. The doctoral training course "Cell Communication in Health and Disease" (CCHD) provides students with challenging research projects ranging from basic biomedical sciences to translation into clinical application. CCHD students acquire intellectual and technical skills employable in highly divergent areas, as they are exposed to four research themes dealing with organ-independent ubiquitous regulatory systems (neurobiology, vascular biology, immunology, and inflammation research). Since 2007, 68 students began and 37 completed their PhD theses within CCHD. Current thesis projects focus on (i) Kv7 channels and purinergic receptors, amphetamine actions, medial prefrontal cortical circuits, and microglia in pain pathways (neurobiology); (ii) roles of leukocytes in vascular patency, complement inhibitors and malondialdehyde-adducts in atherosclerosis, and the role of platelets in bacterial infections (vascular biology); (iii) IgE and IgG in allergies and tumour initiation/ progression, TREM2 and innate immunity responses, T cell Lck kinase and the organization of antigen presenting cells, as well as antiviral proteins and necroptosis (immunology); and (iv) monogenic defects and mechanisms in autoinflammation and immunodeficiencies and adipose tissue inflammation in hepatitis and insulin resistance (inflammation). By gathering students working in such complementary fields, they not only acquire the scientific knowledge of their own projects, but also that of their program peers. Thereby, graduates of CCHD gain all the necessary qualifications for international careers in the biomedical sciences.
Although CCHD is in its eighth year, this is the first international symposium organized in collaboration with three other doctoral programmes. This will further intensify the multidisciplinary character of this event, and I would like to thank the students for putting together the program and for organizing the entire symposium. I look forward to illuminative seminars and lively discussions and I do hope that not only the CCHD students, but also all other participants will keep this CCHD workshop in mind as an unforgettable event.

Stefan Boehm, coordinator of CCHD

The international PhD program Inflammation and Immunity (IAI) was officially initiated in July 2007 dedicated to unify research groups at the Medical University of Vienna successfully working in this very broad biomedical research field, to foster their scientific collaboration, and to train the next generation of excellent young researchers to become experts in modern concepts and techniques of molecular biology, immunology, cell biology, allergology, infectiology and tumorbiology. In 2010 and 2013 the IAI was positively evaluated by the FWF and research groups from the Veterinary Medical University Vienna joint the faculty. Today 10 groups work in 4 different research areas: (i) Basis aspects of Immunity, (ii) Inflammatory diseases, (iii) Infectiology and Vaccinology, and (iv) Allergy and Hypersensitivity. The IAI attracts highly qualified graduate students selected from applicants from all over the world and offers students the opportunity to work on high profile scientific projects at the forefront of modern biology in research groups with great scientific competence. We provide students with a creative and excellent environment for research and educational training in translational biomedicine to prepare them for a successful career in basic and/or applied research.

Every year the IAI students organize an international workshop by inviting recognized research leaders working on a topic of their choice. This provides them with a unique networking opportunity and allows them to present their own research progress and get in close contact with the guests speakers during the social events organized by the students on these occasions. In 2014 and 2015 the IAI students started to organize these international workshops together with the CCHD PhD program to strengthen the multidisciplinary character of this event. This year we will further extend our efforts by organizing a joint international workshop together with the CCHD, MCCA and ICA PhD programs.

My special thanks go to all the students of the Organizing Committee for their impressive commitment and ability to coordinate all the different ideas and wishes. I am looking forward to a great meeting and hope that all the participants will also enjoy the outstanding presentations and lively discussions.

Maria Sibilia, coordinator of IAI

Immunity in Cancer & Allergy
ICA
PhD program - University of Salzburg

The doctoral program “Immunity in Cancer and Allergy - ICA” focuses on two pathologies of the immune system, i.e. the overwhelming allergic immune response and the inefficient immune response against certain tumors. Both diseases are a growing concern and there is an urgent medical need to elucidate the underlying mechanisms for the development of new therapies. The scientific goal of the program is unraveling the cellular and molecular immunological mechanisms and pathways and to develop rational and molecule-based strategies for the treatment of these diseases.

For this purpose, ICA selects excellent graduate students from all over the world, provides an intellectually stimulating environment, excellent instrumental and methodological infrastructure and ambitious scientific projects. Eleven research groups with international reputation guarantee high quality research and training. Furthermore, the college structure ensures that students benefit from the collective experience of the researchers. Since its start in 2008, ICA has already strongly influenced the research and teaching landscape of the biological sciences at the University of Salzburg, and moreover, had a positive impact on the scientific quality and visibility of the involved groups, and the Paris Lodron University of Salzburg.

From its beginning, ICA held joint meetings in order to expand its interactions with researchers from related scientific areas and to encourage interdisciplinary approaches. We started with regular joint ICA Symposia with other biological departments, and in 2014 we organized a first joint Symposium of the Salzburg ICA together with the Viennese PhD program MCCA. Our meeting activities will culminate in the forthcoming “quadripartite” Symposium of the doctoral colleges CCHA, IAI, ICA and MCCA.

I would like to express my thanks and appreciation to all doctoral students who contribute to the organization of this event, and I am looking forward to a great meeting with exciting lectures and lively discussions.

Josef Thalhamer, coordinator of ICA

The PhD-Program Molecular, Cellular and Clinical Allergology, MCCA, is funded by the Austrian Science Fund (FWF), the Medical University of Vienna and the Veterinary University of Vienna, Austria. The goal of the MCCA-PhD program is to select, educate and promote the best possible PhD students in the field of allergy research and to strengthen long-term perspectives of allergy research in Austria but also abroad and to develop innovative strategies for diagnosis, therapy and prevention of allergic diseases. The faculty members mentoring the individual PhD-projects have been carefully selected to cover the field of allergology starting from the disease-causing allergen molecules, the allergen-specific immune responses *in vitro* and *in vivo*, to the clinical application and should thus guarantee an educational program spanning the entire field of allergology. The activities within the PhD Program shall foster the sustained development of allergy research at the Medical University of Vienna and the Veterinary University of Vienna.
Students who decide to pursue their PhD degree within the MCCA program have the exceptional possibility of obtaining profound insights into cutting-edge clinical medicine while at the same time becoming deeply immersed into pertinent questions of molecular and cellular allergology. Depending on the track the actual PhD-student's project will be located in, aspects of molecular, cellular or clinical allergology will be the dominating theme. Compulsory training within the two complementary tracks will round-up education and training of individual students and ensure that a holistic view of the entire discipline, i.e. allergology, is obtained eventually.
The MCCA program is currently operated by 16 dedicated Faculty Members (9 female, 7 male) comprising a truly interdisciplinary mix. The MCCA faculty covers besides the most basic science aspects, i.e. molecular biology, protein chemistry, structural biology, cell biology, animal models, also a number of medical specialities such as dermatology, respiratory diseases and ENT, hematology, immunology, pathophysiology, laboratory medicine and pediatric allergies including food allergy. Despite this interdisciplinary setting, the strong dedication to allergy research is the unifying principle bringing individual researchers with such seemingly unrelated backgrounds closely together. It is their common aim to combat an important disease, i.e. IgE-associated allergy, and to develop in a concerted effort, novel strategies for diagnosis, prevention and cure.
Besides an ambitious and intensive training program, MCCA offers a personalized mentoring-environment for the individual student based on intra- and extramural scientists and members of the international scientific advisory board (ISAB) providing support, advice as well as further career-planning. Moreover, the program has committed itself to provide plenty of opportunities to the PhD-students to 'meet and mingle' (retreats, symposia, allergy club meetings, etc.) in order to provide the necessary scientific but also social framework for a fulfilling life within MCCA.
The joint international symposium together with the PhD programs CCHD, IAI and ICA represents a fantastic opportunity to exchange up-to-date scientific findings and knowledge, to discuss ongoing projects sometimes from very different perspectives and to provide a platform for possible future collaborations. My deep gratitude goes to all PhD Students, Faculty Members, Guest Speakers, companies and institutions, who made the Joint Symposium 'bridge gaps – cross roads' a reality.
I personally wish all participants a most memorable scientific meeting, lots of new ideas and insights for your ongoing and future research as well as a good dose of fun while meeting and mingling!

(MCCA-Speaker)

MCCA

February 1st 2016

8:00-8:30	Registration
8:30-9:00	Opening: **Michaela Fritz, Vice Rector for Research & Innovation, MUV** **Maria Sibilia, Stefan Böhm, Winfried Pickl, Josef Thalhamer**
9:00-9:40	**Peter Sicinski** *Identification of Cell Cycle-Regulating MicroRNAs.*
9:40-10:20	**Riccardo Dalla-Favera** *Pathogenesis of Diffuse Large B-Cell Lymphoma.*
10:20-10:40	Coffee break
10:40-11:20	**Thomas Jenuwein** *Establishment & maintenance of mammalian heterochromatin.*
10:20-11:30	Lena Müller *NCoR1 balances conventional versus innate T cell development.*
11:30-12:30	Lunch
12:30-13:10	**Sergei Grivennikov** *Molecular and cellular mechanisms of tumor-elicited inflammation in colorectal cancer.*
13:10-13:50	**Miguel Soares** *Tissue damage control in immune mediated inflammatory diseases.*
13:50-14:00	Alexander Puck *The soluble cytoplasmic tail of CD45 (ct-CD45) is an inhibitory factor in human adult serum that induces quiescent anergy in T cells.*
14:00-14:20	Coffee break
14:20-15:00	**Mohamed Bentires-Alj** *Cancer targeted therapy and tumor heterogeneity: act locally, think globally.*
15:00-15:40	**Christian Stockmann** *The hypoxic response in Natural Killer cells: Linking cytoxicity and tumor immune surveillance to angiogenesis.*
15:40-15:50	Nora Zulehner *Dau c 1, the Bet v 1-homolog in carrot, bears sensitizing activity: evidence at the T cell level.*
15:50-16:10	Coffee break
16:10-16:50	**Miriam Erlacher** *Targeting Bcl-2 proteins during hematopoietic stem cell transplantation.*
16:50-17:30	**Tim Somervaille** *Transcription factor gene derepression as an oncogenic mechanism in AML.*

February 2nd 2016

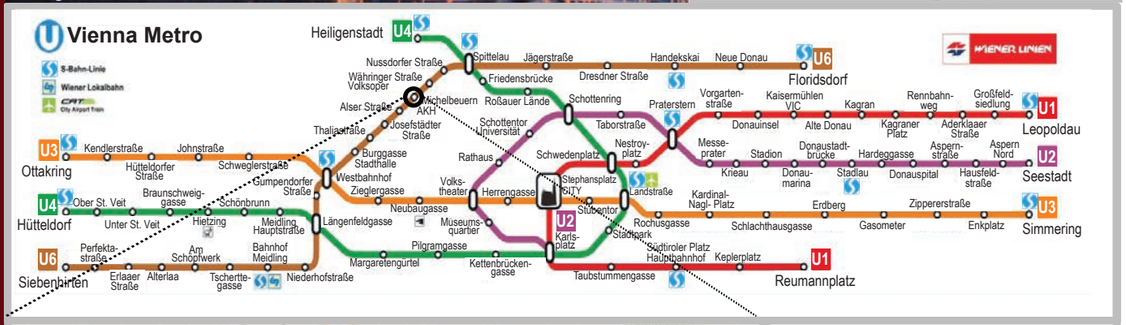
8:00-8:20	Registration + Coffee
8:20-9:00	**Marsha Wills-Karp** *Regulation of IL-33 in Allergic Asthma.*
9:00-9:10	Eva Waltl *Comparison of five different damaging models of the respiratory epithelium.*
9:10-9:20	Christian Zwicker *Cross-talk between the probiotic strain E. coli 083 and the host immune system and its consequences for the development of allergic airway inflammation.*
9:20-10:00	**Gábor Tamás** *Similarities and differences of human and rodent neocortical synapses, neurons and networks.*
10:00-10:10	Szabolcs Biró *A novel extra-dimensional attentional set-shifting task for rodents to explore prefrontal networks.*
10:10-10:35	Coffee break
10:35-11:15	**Daniel Campbell** *Novel signaling pathways that regulate effector and memory T cell development.*
11:15-11:25	Almedina Kurtaj *The immune response against the timothy grass pollen allergen Phl p 5 in non-allergic humans.*
11:25-12:05	**Steffen Massberg** *tba*
12:05-12:15	Florian Puhm *The generation of malondialdehyde-positive microvesicles.*
12:15-13:00	Lunch sponsored by CeMM
13:00-14:30	**POSTER SESSION**
14:30-15:10	**Wulf Haubensak** *Network designs for emotional behavior.*
15:10-15:20	Shreyas Bhat *Mechanism of low-efficacy substrate efflux at the human serotonin transporter.*
15:20-16:00	**Franziska Denk** *Epigenetics – could the past come back to haunt us?*
16:00-16:10	Mira Kronschläger *Impact of glial cell activation on synaptic plasticity in nociceptive pathways.*
16:10-16:30	Coffee break
16:30-17:10	**Thomas Bieber** *Pathophysiology of atopic dermatitis: An update.*
17:10-17:20	Bernhard Kratzer *Shielding of the major mugwort pollen allergen Art v 1 inside of virus-like nanoparticles makes it invisible for B-lymphocytes in vivo.*
17:20-18:00	**Teunis Geijtenbeek** *C-type lectin receptors in infection and immunity***.**
18:00-18:10	Theresa Neuper *NOD1 regulates DC functions by modulating IL-10 signaling.*

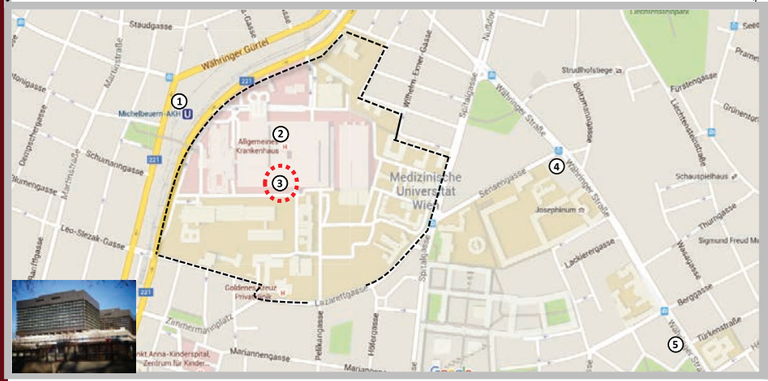
February 3rd 2016

9:00-9:30	Registration + Coffee
9:30-10:10	**Denisa Wagner** *Neutrophil extracellular traps in inflammation and thrombosis.*
10:10-10:20	Maté Kiss *Complement factor H deficiency dampens myeloid cell recruitment independent of its complement regulatory activity.*
10:20-11:00	**Marco A. Cassatella** *Advances on the biology of polymorphonuclear neutrophils.*
11:00-11:30	Coffee break
11:30-13:00	**POSTER SESSION**
13:00-13:45	Lunch
13:45-14:25	**Christoph Peters** *Lysosomal Proteases in Cancer Progression.*
14:25-14:35	Julia Gutjahr *The role of CD44 in the pathophysiology of chronic lymphocytic leukemia.*
14:35-15:15	**Brian Evavold** *Two dimensional regulated protein interactions and force awakens T cell activation.*
15:15-15:25	Sandra Rosskopf *Generation of allergen-specific T cell stimulator cells to investigate coinhibitory pathways in allergy.*
15:25-16:00	**Award and Closing ceremony**

Conference Schedule

Foto: Igor Mattoni





1. Michelbeuern – Allgemeines Krankenhaus (AKH) U-Bahn Metro Station (Line U6).
2. AKH (Vienna General Hospital).
3. **Hörsaalzentrum.**
4. Hotel Atlanta, Währinger Straße 33, 1090 Vienna.
5. Hotel Regina, Rooseveltplatz 15, 1090 Vienna.

Directions to:
Hörsaalzentrum, Ebene 7+8, Hörsaal 3, Allgemeines Krankenhaus, Währinger Gurtel 18 – 20, 1090 Vienna, Austria.

Guests arriving from the Wien Schwechat airport can reach the Hörsaalzentrum by:

1. **Taxi:** Taxis are available right outside the airport terminal. It takes 20 – 25 mins to reach the conference venue from the airport with fares ranging from 30 – 40 € (higher during night time). Contact: +43 676 7408005, email: http://www.123taxi.at/de/.

2. **City Airport Train (CAT):** CAT shuttles non-stop from the airport to Wien-Mitte Landstraße once in 30 mins (Operating time: 05:36 to 23:36). A single CAT ticket costs 12 € and a return ticket costs 19 € available in ticket vending machines within the airport. Destination time is 16 mins. From Wien-Mitte Landstraße, take U4 green metro line to Spittelau (towards Heiligenstadt). From Spittelau take U6 brown metro line to Michelbeuern AKH (towards Siebenhirten). Metro tickets can be bought through vending machines in the metro station.

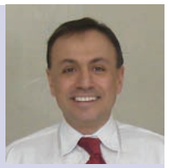
3. **Schnellbahn (S – Bahn) S7:** S – Bahn S7 trains have the same route as that of CAT trains with multiple stops running once in every 15 – 30 mins. Journey time to Wien-Mitte Landstraße is 25 mins and a single ticket costs 4,40 €. From Wien Mitte, follow the same directions as given above for the CAT. S-Bahn tickets can be bought by ÖBB vending machines or ÖBB counters within the airport.

4. **Bus:** Vienna airport bus lines to Westbahnhof can be taken from the airport. Journey time is around 40 mins and a single way ticket costs 8 € (13 € for return ticket). Get off at Wien Westbahnhof and take U6 brown metro line to Michelbeuern AKH (towards Siebenhirten).

Useful websites:
http://www.wienerlinien.at/ http://www.vor.at/ https://www.oebb.at/ Google Maps

February 1st - Plenary Talks

Identification of Cell Cycle-Regulating MicroRNAs.



Per Hydbring, Veronica Matia and Peter Sicinski
Department of Cancer Biology, Dana-Farber Cancer Institute, and Department of Genetics, Harvard Medical School, Boston, Massachusetts, USA

We performed nine genome-wide screens for microRNAs targeting all major cyclins and cyclin-dependent kinases (cyclins D1, D2, D3, E1, E2, CDK1, CDK2, CDK4 and CDK6). We identified a novel class of microRNAs that target multiple cell cycle proteins, which we called "cell cycle-regulating microRNAs". Some of these microRNAs are down regulated in human cancers. We found that ectopic expression of cell cycle-regulating microRNAs in human cancer cells blocked their proliferation, and in some cases triggered apoptotic cell death. We profiled the response of 120 human cancer cell lines from Cancer Cell Line Encyclopedia to selected microRNAs and developed biocomputational methods to predict the response based on the mutational status or gene expression profiles. Lastly, we used nanoparticles to deliver cell cycle-regulating microRNAs to tumor-bearing mice, and we showed that it slowed-down tumor growth in vivo.

Pathogenesis of Diffuse Large B-Cell Lymphoma.

Riccardo Dalla-Favera, MD
Institute for Cancer Genetics, Columbia University, New York, USA

Diffuse Large B cell Lymphoma (DLBCL) represents the most common form of B cell derived non-Hodgkin Lymphoma (B-NHL), accounting for ~30% of the de-novo diagnoses and also arising as a frequent clinical evolution of Follicular Lymphoma (FL) and Chronic Lymphocytic Leukemia (CLL), DLBCL and FL derive from B cells at different stages of germinal center (GC) development. In order to identify the genetic lesions associated with DLBCL pathogenesis, we have integrated whole-exome sequencing analysis and copy number variation analysis for a comprehensive definition of the DLBCL coding genome. The results have identified a novel set of recurrent genetic lesions, which, in turn, identify mutant genes regulating important pathways involved in GC development, including those involved implicated in chromatin remodeling, transcriptional control of apoptosis and differentiation, NF-kB activation and immune escape. Recent results have identified a pervasive mutational deregulation of a transcription factor network (MEF2B-BCL6-FOXO1) involved in the control of GC development. The normal role of these transcription factors and the consequence of their mutations in DLBCL have been investigated using GC-directed conditional transgenic mice.

Establishment & maintenance of mammalian heterochromatin.

Group Thomas Jenuwein
Department of Epigenetics, Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

Heterochromatin is important to protect genome integrity and to stabilize gene expression programs via the partitioning of repressed versus active chromatin domains. Mammalian heterochromatin is characterized by its underlying repetitive DNA sequence and epigenetic hallmarks, such as H3K9me3 methylation, DNA methylation and noncoding repeat RNAs. Despite the identification of these key components that ensure heterochromatin integrity, it is still unclear how heterochromatin is initiated and which mechanisms maintain heterochromatin structure. I will discuss novel insights on how repeat-rich DNA and RNA interact with chromatin-modifying enzymes and nucleosomes to enforce mammalian heterochromatin formation.

Molecular and cellular mechanisms of tumor-elicited inflammation in colorectal cancer.

Sergei Grivennikov
Fox Chase Cancer Center, Philadelphia, USA

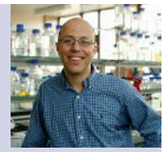
Solid tumors exhibit immune infiltrates and enhanced expression of inflammatory mediators – a phenomenon defined as 'Tumor-elicited inflammation' (TEI). In colorectal cancer (CRC), oncogenic events lead to early epithelial barrier deterioration and microbiome-driven expression of cytokines. Among them, IL-23 and IL-1 family cytokines are essential to enforce TEI and induce other pro-tumorigenic cytokines, such as IL-22 and IL-17. Analysis of IL-17A "reporter" and conditional receptor knockout mice revealed that not only conventional „Th17" cells but also innate lymphocytes (ILC3) integrate IL-23 and IL-1 driven signals in CRC and drive tumor growth/progression. Broad spectrum of commensal bacteria with nevertheless very distinct common properties was found to be associated with CRC tumors to induce TEI inflammatory reaction in a special subset of tumor-associated myeloid cells. These myeloid cells interacted with lymphoid cells in the tumor to induce production of TEI-relevant cytokines, which are essential for tumor growth and progression. Rapid colonic transformation model showed potential for short term pharmacological inhibition of IL-1 driven TEI in controlling IL-17 expression and tumor growth. Tumor elicited inflammation induction and maintenance is therefore essential for CRC growth and progression.

Tissue damage control in immune mediated inflammatory diseases.

Miguel Soares, PhD
Instituto Gulbenkian de Ciência, Oeiras, Portugal

Damage control refers to those actions that are made towards minimizing damage associated with different emergency situations. Depending on context, damage control may refer to emergency procedures dealing with the sinking of a ship or to surgery procedures dealing with severe trauma or even to a company in Marvel Comics, which repairs damaged property arising from conflicts between super heroes and villains. By extension, "tissue" damage control refers to adaptive responses that minimize the extent of tissue damage and organ dysfunction associated with the pathogenesis of immune mediated inflammatory conditions, including in infectious diseases. Presumably, tissue damage control is regulated by a restricted number of evolutionarily conserved stressand damage-responses associated with the induction of overlapping profiles of gene expression. This argues for the existence of a core number of evolutionarily conserved genes regulating tissue damage control. Moreover, this might explain why overlapping stress- and damage-responses confer protection against apparently unrelated forms of stress and damage, a phenomenon known as hormesis. Among the those evolutionarily conserved genes are a number of effector genes that regulate iron metabolism and control the participation of iron in the production of free radicals leading to oxidative stress and tissue damage. In support of this notion, immune mediated inflammatory diseases are often associated with deregulated iron metabolism and oxidative stress and ii) stress responsive genes controlling iron metabolism exert anti-oxidant effects that confer tissue damage control in different immune mediated inflammatory conditions, including in infectious diseases.

Cancer targeted therapy and tumor heterogeneity: act locally, think globally.



Mohamed Bentires-Alj
FMI Basel, Switzerland

Each year over 1.5 million new cases of breast cancer occur among women worldwide and 500,000 women die from this disease. In most cases, metastasis is the cause of death. Indeed, while 98% of patients survive 5 years or more after being diagnosed with a localized (confined to the primary site) breast cancer, this number drops to 15-25% if the cancer has metastasized to distant organs. Curing metastatic breast cancer clearly represents an unmet medical need. Although progress has been made in broadly understanding breast tumor biology and progression to metastases, most of the relevant molecules and pathways remain undefined. The thread connecting the research in my lab is tumor heterogeneity. We assess mechanisms that influence normal and neoplastic breast stem cells, metastasis, and resistance to targeted therapies at the molecular, cellular, and whole organism levels.

The hypoxic response in Natural Killer cells: Linking cytoxicity and tumor immune surveillance to angiogenesis

Ewelina Krzywinska[1], Chahrazade Kantari-Mimoun[1], Magali Castells[1], Johannes Haubold[2], Dagmar Gotthardt[3], Yann Kerdiles[4], Ralph Klose[1], Joachim Fandrey[2], Veronika Sexl[3] and Christian Stockmann[1]
[1] *Institut National de la Santé et de la Recherche Médicale (INSERM), Unit 970, Paris Cardiovascular Research Center, Paris, France,*
[2] *Institut für Physiologie, Universitätsklinikum Essen, Universität Duisburg-Essen, Germany*
[3] *Institute for Pharmacology, Veterinary University of Vienna, Austria*
[4] *Centre d'Immunologie de Marseille-Luminy, Marseille, France.*

Innate immune cells, including the myeloid lineage, are critical for shaping immune responses within the tumor microenvironment by controlling angiogenesis and tumor oxygenation. Cellular adaptation to low oxygen depends on Hypoxia-inducible transcription factors (HIFs) which also play a pivotal role in inflammatory responses. NK cells unifying characteristics of innate and adaptive immunity, are cytotoxic innate lymphoid cells with a unique ability to instantly recognize and kill „aberrant" cancer cells while sparing „normal" cells. Owing to these tumoricidal features, NK cells are able to restrict primary tumor growth and limit metastatic spread. We observe that Natural Killer (NK) cells preferentially infiltrate into hypoxic zones of solid primary tumors and by genetic targeting of HIFs in NK cells, we define a crucial role of HIF-1α in NK cell function and cancer immune surveillance. HIF-1α-deficiency in NK cells impairs primary tumor growth as well as distant metastasis. This is due to reduced susceptibility of HIF-1α-deficient NK cells to tumor cell-derived inhibitory stimuli, resulting in improved recognition and killing of tumor cells.
Furthermore, we define the hypoxic response in NK cells as a critical mediator of tumor angiogenesis. Paradoxically, HIF-1α-deficiency in NK cells results in decreased expression of various angiostatic factors within the tumor microenvironment, resulting in unproductive tumor angiogenesis, characterized by immature, non-functional vessel and severe tumor hypoxia. This suggests that the hypoxic response in NK cells slows down overall tumor angiogenesis in order to allow for vessel formation in a more coordinated fashion.
In summary, we define HIF-1α as a critical mediator of NK cell effector function and cancer immune surveillance. Secondly, we show that HIF-1α in NK cells acts as a negative regulator of tumor angiogenesis that ensures the fine-tuning of the angiogenic response. These results indicate that targeting the hypoxic response in NK cells may represent a novel therapeutic avenue.

Targeting Bcl-2 proteins during hematopoietic stem cell transplantation

Matthias Kollek[1], Daniela Bertele[1], Gesina Voigt[1], Felix Krombholz[1], Verena Labi[2], Stephan Geley[3], Andreas Villunger[2], Ana Garcia-Saez[4] and Miriam Erlacher[1]
[1]Division of Pediatric Hematology and Oncology, University Medical Center Freiburg, Freiburg, Germany
[2] Division of Developmental Immunology, Biocenter, Innsbruck Medical University, Austria;
[3] Division of Molecular Pathophysiology, Biocenter, Innsbruck Medical University, Austria.
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Hematopoietic stem cell transplantation (HSCT) is the only curative treatment for many hematological and immunological diseases but hampered by the risk of graft failure or delayed engraftment. Clinical experience has shown that these problems can be overcome by transplantation of higher hematopoietic stem and progenitor cell (HSPC) numbers. This can be achieved through more efficient collection strategies or by ex vivo expansion but also through inhibition of cell death in donor HSPCs. A major aim of our group is to characterize lethal transplantation-associated stress signals and to inhibit apoptosis in donor HSPCs in order to increase their numbers and fitness.
We have identified two Bcl-2 proteins from the pro-apoptotic BH3-only subgroup, Bim and Bmf, to be central players in HSPC apoptosis induction during transplantation. Lack of either protein or overexpression of their antagonists Bcl-2 or Bcl-xL strongly increases HSPC competitiveness during transplantation, both in murine transplantation and human xenotransplantation models. Our data indicate that modulation of Bim or Bmf levels inhibits apoptosis in murine and human HSPCs and that the resulting extended life span is beneficial during HSCT. However, deletion of Bim or Bmf in donor cells causes autoimmunity and malignant transformation which dramatically reduces the life span of recipients. Thus, only transient apoptosis inhibition can be considered feasible for therapeutic use. Inhibiting apoptosis for 5-6 days indeed is sufficient to increase murine donor HSPC competitiveness in vitro and in vivo but does not increase the risk of malignant transformation. We are further characterizing pro-apoptotic as well as protective signals in donor HSPCs during HSCT in order to increase their fitness and to pave the way for novel approaches improving transplantation outcomes.

Transcription factor gene derepression as an oncogenic mechanism in AML.

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Cancer Research UK Manchester Institute

Through in silico and other analyses, we identified FOXC1 as expressed in at least 20% of human AML cases, but not in normal hematopoietic populations. FOXC1 expression in AML was almost exclusively associated with expression of the HOXA/B locus. Functional experiments demonstrated that FOXC1 contributes to a block in monocyte/macrophage differentiation and enhances clonogenic potential. In in vivo analyses, FOXC1 collaborates with HOXA9 to accelerate significantly the onset of symptomatic leukemia. A FOXC1-repressed gene set identified in murine leukemia exhibited quantitative repression in human AML in accordance with FOXC1 expression, and FOXC1high human AML cases exhibited reduced morphologic monocytic differentiation and inferior survival. Thus, FOXC1 is frequently derepressed to functional effect in human AML.

February 1st - Student Talks

Ncor1 balances conventional versus innate t cell development

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NCoR1 (nuclear receptor corepressor 1; encoded by the Ncor1 gene) has been initially identified as a regulator of nuclear receptor mediated repression. Interestingly, studies with NCoR1 knockout mice (which are embryonic lethal) revealed also important functional roles of NCoR1 during early embryonic development, such as neural cell differentiation, in the progression of erythrocytes and in developing fetal thymocytes. NCoR1 facilitates transcriptional repression through the recruitment of chromatin modifying enzymes and NCoR1 is recruited to target gene loci via binding to transcription factors. Among them, several members of the BTB zinc finger (BTB-ZF) transcription factor family (e.g. PLZF, BCL6 and MAZR), which are key regulators of T cell development and function, are in a complex with NCoR1. Together, this implies important roles for NCoR1 in T cells.
To study the role of NCoR1 in T cells, we crossed Ncor1F/F mice with different Cre-deleter transgenic lines (such as VaviCre, LckCre, Cd4Cre) to determine the role of NCoR1 at distinct T cell developmental stages and in peripheral T cells. Preliminary results indicate an essential role for NCoR1 during T cell development and in the generation of non-conventional (innate) T cells.

The soluble cytoplasmic tail of CD45 (ct-CD45) is an inhibitory factor in human adult serum that induces quiescent anergy in T cells

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The cytoplasmic tail of CD45 (ct-CD45) is proteolytically cleaved and released upon activation of human phagocytes. The soluble ct-CD45 was found to act on T cells as an inhibitory, cytokine-like factor that reduces T cell proliferation. Here we show, that ct-CD45 is abundant in human peripheral blood plasma from healthy adults compared to plasma derived from umbilical cord.blood. Plasma depleted of ct-CD45 enhanced T cell proliferation, while addition of exogenous ct-CD45 protein inhibited proliferation and cytokine production of human T lymphocytes in response to TCR signaling. Furthermore, T cells activated in the presence of ct-CD45 were rendered hyporesponsive to subsequent restimulation, which was reversible by exogenous IL-2 or IL-7, thus indicating an anergic state. However, microarray analysis did not indicate induction of any classical anergy-associated genes. Instead, we found induction of Schlafen family member 12 (SLFN12) and of Krueppel-like factor 2 (KLF2). Cell cycle analysis showed inhibition of cyclins expressed in the early G1 phase of the cell cycle. In summary, ct-CD45 triggers an anergy program in T cells, which is reversible by exogenous IL-2, acting independently of classical anergy factors. Our data suggests a cell cycle arrest in the early G1 phase, thus making it distinct from canonical T cell anergy.

Dau c 1, the Bet v 1-homolog in carrot, bears sensitizing activity: evidence at the T cell level

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Background: In contrast to other Bet v 1-related food allergens, the major carrot allergen, Dauc1, has been suggested to induce food allergy independently from Betv1.
Methods: Dau c 1-specific T cell lines (TCL) and clones (TCC) established from PBMC of birch pollen-allergic patients with carrot allergy were used to analyse T cell epitopes, allergen-induced cytokine secretion and the expression of the integrins alpha4beta7 and alpha4beta1 critical for gut and lung homing, respectively. mRNA expression of GATA3 and Tbet was analysed in sorted $CD3^+CD4^+CFSE^{low}$ cells upon stimulation of PBMC with either Dau c 1 or Bet v 1. Dau c 1 was subjected to endolysosomal degradation and the resulting fragments were sequenced by mass spectrometry.
Results: Among 14 distinct T cell-activating regions, Dau c 1 139-153 was recognized by 55% of the patients. Only 6/15 (40%) Dau c 1-specific TCL and 8/20 (40%) TCC reacted with Bet v 1. Bet v 1-non-reactive TCC expressed lower levels of the alpha4beta7-integrin and significantly higher levels of alpha4beta1-integrin than Bet v 1-positive TCC. In contrast to cross-reactive TCC, Bet v 1-non-reactive TCC were mainly Th1-like. A Th1-like response was also detected in Dau c 1-reactive $CD3^+CD4^+CFSE^{low}$ cells. Full-length Dau c 1 was detectable after 48 hours of endolysosomal degradation. Proteolytic fragments matched the T cell-activating regions.
Conclusion: Dau c 1 displays characteristics of sensitizing allergens, i.e. it possesses a major T cell-activating region and is stable to lysosomal proteolysis. Furthermore, we provide evidence for a Bet v 1-independent T cell response to Dau c 1 primed in the gut. These cellular insights confirm that the major carrot allergen has a special status among Bet v 1-related food allergens.

February 2nd - Plenary Talks

Regulation of IL-33 in Allergic Asthma

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Asthma is a chronic inflammatory disease of the lung whose incidence and prevalence has reached epidemic proportions in the last few decades. Although it is well accepted that aberrant type 2 immune responses are closely associated with susceptibility to the development of asthma, the mechanisms underlying these aberrant immune responses are not well understood. Based on the recent discovery that the epithelial-derived cytokine, IL-33, is a major regulator of CD4+ T cell differentiation, we have focused our efforts on identification of the innate immune pathways by which common allergens induce IL-33 in the airway epithelium. We have found that specific allergens such as house dust mite and peanuts induce epithelial IL-33 production through activation of the pattern recognition receptor, formyl-peptide receptor (FPR2). Specifically, we demonstrated that FPR2 ligation by epithelial cell-derived serum albumin A (SAA) results in innate lymphoid cell (ILC2) recruitment and secretion of large quantities of IL-13, thereby driving the asthma phenotype. Importantly, we have found that these pathways are dysregulated in epithelial cells from allergic individuals. In contrast, we have identified a novel PRR pathway, involving the C-type lectin receptor, dectin-1 that negatively regulates IL-33 production and the development of the allergic phenotype. The loss of this protective pathway is associated with increased risk of asthma. Taken together these studies provide valuable insights into disease pathogenesis and highlight unique opportunities for the development of novel therapeutic approaches to control this ever-increasing pulmonary disease.

Similarities and differences of human and rodent neocortical synapses, neurons and networks.

Gabór Tamás
Research Group for Cortical Microcircuits, Hungarian Academy of Sciences and University of Szeged, Hungary

Experiments on animal models showed that the efficacy of chemical transmission between neurons depends on several factors including the number, spatial distribution and size of synapses, presynaptic release mechanisms, postsynaptic membrane properties and synaptic plasticity. We recorded the first human synaptic connections which indicated species related differences in synaptic properties leading to altered signal propagation in human cortical microcircuits compared to animal models. The seminar will elucidate quantal and structural differences of human and rat neocortical synapses mechanistically explaining why single neurons of the human neocortex can trigger high frequency (~200 Hz) rhythmic activity in local networks. In turn, experiments will be presented from freely behaving animals detecting ~200 Hz rhythmic network episodes and the corresponding firing of identified interneurons and pyramidal cells during defined epochs of slow wave sleep. The suggestion that evolutionally conserved network episodes could be differentially recruited in mammalian species will be discussed.

Novel signaling pathways that regulate effector and memory T cell development.

Daniel Campbell
Benaroya Research Institute at Virginia Mason, Seattle

Upon antigen stimulation, T cells integrate signals from the T cell receptor, co-stimulatory molecules, and cytokines that control their clonal expansion and differentiation into short-lived effector cells or long-lived memory cells that help provide protection from re-infection. Correct interpretation of these signals is controlled by various signaling networks, and among these phosphoinositide 3-kinase (PI3K) signaling has a key function in reprogramming cellular metabolism and driving cell proliferation, redirecting cell migration, and controlling the differentiation of effector and memory cells. Despite the central role of PI3K in normal and pathogenic T cell responses, molecular control of PI3K activation during T cell stimulation remains poorly understood. We have shown that the adaptor protein B Cell Adaptor for PI3K (BCAP) has a critical role in T cell activation, proliferation and differentiation. Although not expressed in naïve T cells, BCAP (encoded by the Pik3ap1 gene) is rapidly upregulated on T cell activation, and our in vitro and in vivo experiments show that BCAP-deficient T cells have defects in antigen-receptor-induced proliferation, signaling and differentiation different effector and memory populations. These exciting new results support a model in which BCAP acts as an important adaptor protein that coordinates PI3K and other signals activity in activated T cells, thereby controlling their clonal expansion and effector/memory differentiation.

Prof. Dr. Steffen Massberg

is the head of the Medical Clinic at the Munich University Hospital. He is a specialist in the area of internal medicine, cardiology and intensive care. He has previously spent time working as a research assistant in the Department of Internal Medicine I (Campus Grosshadern) at the Hospital of the Ludwig Maximilian University of Munich.
In 2010 he became the senior physician and deputy director at the department for Heart & circulatory system diseases at the German Heart Centre Munich. He also has international experience as a DFG Heisenberg Fellow at Harvard Medical School in Boston.

Network designs for emotional behavior.

Wulf Haubensak
IMP Vienna, Austria

Emotional behaviors are central for survival: fear keeps us away from danger; reward-related emotions help to seize opportunities. For neuroscientists, they offer the unique opportunity to learn more about emotions as biomedically important part of our mental self and serve as robust entry points for studying the general functional design of the brain. Our laboratory adopts novel experimental and computational technologies to map und understand their underlying neuronal circuitry.
Combining circuit genetics, electrophysiology and imaging technologies in mice, we have identified a limbic circuit that writes Pavlovian fear experiences into long term memories. Optogenetic circuit mapping revealed strong reciprocal connectivity of amygdala and midbrain dopamine (DA). Site specific inhibition of this network, or DA, prevented long term plasticity and fear learning in amygdala. Collectively, we describe a minimal learning circuit that integrates glutamatergic teaching-, and DA prediction error-signals, as well as amygdala-to-midbrain-DA negative feedback, to gate fear learning at negatively valenced amygdala inputs. We believe that this compact design reflects the need for the efficient and robust prediction of threats.
In a computational initiative, we have explored the possibility to predict such functional networks from fusing publicly available genetic and brain data. To this end, we weighted brain gene expression data and connectomic information with functional genetic associations. We find that the functional genetic load is not distributed at random but accumulates in specific networks in the brain. This approach allows to extract candidate networks underlying specific brain functions in silico for their subsequent experimental characterization, complementing the experimental circuit physiological exploration of the brain.

February 2nd - Plenary Speaker

Epigenetics – could the past come back to haunt us?

Franziska Denk, *King's College London*

Adverse environmental events, such as injury, disease or stressful life situations, are some of the main risk factors for developing a chronic pain condition. Research over the past decades has revealed many details about the altered state of the nervous system once chronic pain is established: we know about neuronal hypersensitivity, both peripherally and centrally; we know about abnormal immune responses; and we know about altered brain function, in particular top-down modulatory processes.
Yet, it is still unclear why the same initial insult will lead to life altering pain in some individuals, but not others. Or indeed, why temporally constrained stimuli can have such long-lasting consequences. One possibility is that epigenetic mechanisms, such as histone modifications or DNA methylation, are involved. This talk will introduce attendees to the current state of research in this area, discussing possible hypotheses, as well as the already available evidence.

Pathophysiology of atopic dermatitis: An update

Prof. Thomas Bieber, MD, PhD, MDRA
Department of Dermatology and Allergy,
Christine-Kühne Center for Allergy, Research and Education,University of Bonn, Germany

Atopic eczema/dermatitis (AD) is a paradigmatic complex disease. The diversity of the clinical phenotype is reflecting many underlying aspects such as the genetic and epigenetic background affecting the innate and adaptive immune mechanisms, neuro-immunological and environmental factors including the microbiomic signals. In the focus of the current research are (i) aspects related to the genetically determined disturbance of the epidermal barrier function including Filaggrin, the protease-anti-protease system with SPUNK5/LEKTI as well as tight-junction structures such as Claudins; (ii) the role antigen-presenting cells including FceRI-expressing epidermal Langerhans cells (LC) and inflammatory dendritic epidermal cells (IDEC); (iii) different T cell populations which represent targets for current therapeutic approaches such as IL-4 and IL-13 producing Th2 cells, Th22 cells and more recently Th17 cells; (iv) the cross-talk of microbial agents with the innate and the adaptive immune systems and their role in the regulation of the immune response and the microbiomic diversity on the skin. Currently, besides understanding the pathophysiology of AD, substantial progress in the discovery of biomarkers with a predictive/prognostic value for the management of this chronic disease is an unmet need which can only be addressed in the context of biobank projects including large cohorts of AD patients. This approach will pave the way for precision medicine.

C-type lectin receptors in infection and immunity.

Teunis Geijtenbeek
Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands

Dendritic cells (DCs) are central players in the induction of innate and adaptive immunity to HIV-1. Innate sensing of pathogens by pattern recognition receptors (PRRs) triggers signaling pathways that lead to the induction of DC maturation, antiviral type I IFN responses and cytokine responses, and subsequent induction of specific T helper cell differentiation. C-type lectin receptors are an important family of PRRs that control early innate immune responses and consequently adaptive immunity. Notably, the CLR DC-SIGN induces carbohydrate specific signaling and thereby controls both antiviral innate immunity as well as adaptive immunity to different classes of pathogens. Our recent data have uncovered an important role for DC-SIGN in enhancing type I IFN responses to induce follicular T helper cells, which induce e strong antibody responses to fucose-expressing parasites. In contrast, several viruses including HIV-1 target DC-SIGN to escape innate antiviral immunity by preventing type I IFN responses. Identification of these mechanisms will greatly facilitate development of novel strategies to combat infections. Here I will discuss the role of innate signaling by DC-SIGN and how the signaling modulates innate and adaptive immune responses.

February 2nd - Student Talks

Comparison of five different damaging models of the respiratory epithelium

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Background: The respiratory epithelium with its tight junctions is an important barrier against inhaled exogenous factors. Damaged epithelium can be penetrated by allergens and pollutants more easily, thus facilitating allergic reactions and inflammation. Here we aimed to establish different models for damage in order to enable us to investigate protective factors for the epithelium. **Methods:** Three different cell culture models were investigated. A bronchial epithelial cell line (16HBE14o-) and primary human nasal epithelial cells were cultured in monolayers cultivated in six well plates and analysed by microscopy. Second, a transwell system was used to investigate permeability through the monolayer, using transepithelial resistance as an endpoint. Third, the xCELLigence DP system was employed for continuous real-time monitoring of impedance-based cell responses. The cellular response to the following damaging conditions was analysed: i) Physical damage by scratching the cell layer, ii) infection with human rhinovirus (RV), iii) incubation with standardised cigarette smoke extract and iv) exposure to the TH1 cytokine interferon-gamma (IFN-gamma) and v) exposure to house dust mite (HDM) extract. **Results:** The ability of cells to recover after physical damage by scratching within 24 (16HBE14o-) or 72 (primary cells) hours was shown both in cell culture and the xCELLigence DP system. Barrier function decreased in a time- and dose-dependent manner after infection of cells with RV, exposure to cigarette smoke extract, to IFN-gamma and to HDM extract.
Conclusion: We established and compared various models for damage of respiratory epithelial cells using three different cell culturing systems and five different damaging conditions.

Cross-talk between the probiotic strain *E. coli 083* and the host immune system and its consequences for the development of allergic airway inflammation

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Background: Supplementation with probiotic bacteria during pregnancy and early infancy has been associated with reduced risk to develop allergic diseases in later life. However, the cellular, molecular and also potential epigenetic mechanisms by which probiotic bacteria interact with the host immune system still remain unclear. **Methods:** The aim of this study is to investigate whether exposure to the probiotic strain *Escherichia coli O83* prevents allergic airway inflammation in adult mice as well as in the progeny of mice treated with this bacteria during gestation and lactation. In particular we will focus on whether epigenetic modifications contribute to a potential protective effect in the offspring. **Results:** Supplementation of adult mice with *E. coli 083* did not attenuate allergen-induced airway inflammation. Similarly, perinatal maternal application of this strain did not reduce the development of airway inflammation in offspring but decreased allergen-specific IgE levels were detected in both experimental conditions. In vitro stimulation of splenocytes and bone marrow-derived dendritic cells with *E. coli 083* resulted in increased production of IFN-γ and IL-10. Furthermore, we established the technique of chromatin immunoprecipitation by using an in vitro Th cell polarization model to be able to determine potential epigenetic changes in the progeny of E. coli-treated mothers. **Conclusions:** Supplementation with *E. coli O83* did not ameliorate allergic lung inflammation but reduced sensitization, both in adult mice, and in the offspring derived from treated mothers. In vitro characterization of *E. coli O83* revealed that this strain shifts the immune response towards Th1 and regulatory type.
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A Novel Extra-Dimensional Attentional Set-Shifting Task for Rodents to Explore Prefrontal Networks

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Background: Attention aids adaptive usage of the limited capacity of neural processing systems. An innate part of information processing is the attentional set, that facilitates selection of the relevant, and inhibits processing of distracting information. With assessing the capability of attentional set-shifting, it is possible to measure cognitive flexibility and executive functions. The most widely used neuropsychological task for the evaluation of these functions in humans is the Wisconsin Card Sorting Test, which requires the subject to alter the response strategy and use previously irrelevant information to solve a new set of problems. The test has proven clinical relevance, as multiple neuropsychiatric conditions report poor performance on it. However, similar tasks used for rodent models are limited because of their manual-based testing procedures. **Methods:** Water-deprived and head-fixed C57BL/6 mice were placed in a virtual reality environment and exposed to a decision-making task to retrieve small water reward. In addition silicon probe recordings were performed in the medial prefrontal cortex to address the underlying network mechanisms. **Results:** We present a novel behavioural task in which animals learn to discriminate two visual perceptual dimensions and they successfully switch their attention between them. Furthermore, the experimental set-up allows stable neural recordings and behavioural monitoring. **Conclusions:** We demonstrate that our extra-dimensional set shift task for head-fixed mice is an effective tool to study the molecular and cellular mechanisms within neuronal networks underlying executive functions. The understanding of the role prefrontal network operations in cognitive flexibility is invaluable for understanding numerous neuropsychiatric diseases, such as schizophrenia and depression.

The immune response against the timothy grass pollen allergen Phl p 5 in non-allergic humans

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Background: We examine a new hypothesis, which postulates that non-allergic individuals mount antigen-specific immune responses against rPhl p 5 and that different immune response types exist and maintain this healthy condition. Furthermore, we hypothesize that depending on the living environment, non-allergic immune responses can be different. Therefore, we assessed the immune status of non-allergic people living in a farming environment and non-allergic people living in an urban environment. **Methods:** PBMCs from non-allergic donors were expanded antigen-specifically with rPhl p 5. After enrichment of antigen-specific memory T cells, cytokine secretion and transcription factors, allowed identification of different T helper subsets. Antigen-specific IgE, IgG1, IgG4, and IgA antibody levels were measured by ELISA. **Results:** We could show that a TH1 biased immune response is the dominant subset in non-allergic townspeople and we could also confirm this finding by staining of TH1 associated transcription factor T-bet. On the other hand, farmers displayed less TH-1 responders and more TH-0 responders and as well a balanced expression of transcription factors FoxP3 and T-bet. Additionally, we detected significantly higher Phl p 5-specific IgG1 titers than IgG4 titers in both non-allergic groups. Interestingly, townspeople showed significantly increased Phl p 5-specific IgG4 titers and IgG seroconversion compared to famers. **Conclusions:** In summary, it can be stated that tolerance induction is not the only mechanism to maintain a non-allergic state but rather multiple mechanisms of naturally acquired protection exist and depending on the living environment different immune response types can establish and maintain a healthy non-allergic status.

The generation of Malondialdehyde-positive microvesicles

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Background: Malondialdehyde (MDA) is a product of fatty acid peroxidation which forms immunodominant adducts recognized by natural IgMs. Such adducts are found on apoptotic cells and microvesicles (MV). As both MVs and MDA-adducts have been implicated in inflammation, we hypothesize that MDA-positive MVs (MDA+MPs) are potentially harmful. We aim to identify the generation mechanism of MDA+ MVs and their biological function. Methods: Microvesicles were isolated from plasma or conditioned media by differential centrifugation, characterised by flow-cytometry and their pro-inflammatory potential in a monocytic and endothelial cell line (THP-1 and HUVECs) was analysed. **Results:** Stimulation of THP-1 cells with TLR-ligands for TLR2, 3, 4 and 9 led to the release of MDA+MVs, whereas stimulation with pro-inflammatory cytokines, such as TNFalpha and IL1alpha, or induction of apoptosis by a topoisomerase-I inhibitor (Camptothecin) did not affect MDA+MV generation. In a human endotoxinemia model, in which volunteers were injected with 2ng/ml LPS, circulating MDA+MV were significantly increased after 3 hours. To characterise functional effects of MDA+MVs, we separated MVs into a MDA+ and MDA- fraction by antibody-mediated depletion of MDA+MVs. We saw that the MDA+MV fraction derived from LPS-stimulated THP-1 cells had a greater potential to induce IL-1beta and IL8 release by THP-1 cells and HUVECs. **Conclusions:** We observed that TLR-ligands induced the generation of MDA+MVs by THP-1 cells and in the circulation in a human endotoxinemia model. MDA+MV generated under such conditions appeared to have an increased pro-inflammatory potential in vitro.

Mechanism of Low-Efficacy Substrate Efflux at the Human Serotonin Transporter

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Background: Amphetamines induce efflux of dopamine and serotonin from presynaptic neurons through their cognate transporters DAT and SERT respectively. However some compounds of the phenethylamine library (PAL) exhibit low efficacy in inducing neurotransmitter efflux when compared to amphetamines through unknown mechanisms. The aim of the study is to elucidate which conformational states of SERT and DAT do these 'partial substrates' trap during the transport cycle. **Methods:** Substrate induced structural transitions in SERT and DAT can be inferred from analyzing transporter associated currents: the peak current reflects substrate induced charge movement; the steady-state current indicates inward facing conformation visited by the cycling transporter. These currents were measured by whole cell patch clamping of HEK293 cells stably expressing hSERT. Currents induced by PAL-1045, a partial releaser for SERT, were compared to those induced by serotonin (5-HT) and full releasers PAL-287, PAL-1046 and para-Chloroamphetamine. **Results:** Reduced steady state amplitudes of currents through SERT with increasing concentrations of PAL compounds suggest that PALs bind to the inward and outward facing conformations of SERT with high affinity. Slower recovery of 5-HT induced peak currents on PAL-1045 application, when compared to the full releasers, also argues for longer dwell-time of PAL-1045 in its binding site which precludes intracellular serotonin binding and efflux. **Conclusions:** Taken together, our observations provide evidence for a mechanism resulting in low-efficacy substrate efflux through SERT in the presence of PAL-1045. The results have implications for the development of low-efficacy releasers as therapeutic agents for addiction therapy.

Impact of glial cell activation on synaptic plasticity in nociceptive pathways.

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Background: Synaptic long-term potentiation (LTP) at the first synapse in nociceptive pathways is a cellular model for enhanced pain sensitivity. Recent evidence suggests that solely factoring neuronal activity in terms of synaptic plasticity provides an incomplete understanding of the establishment of amplified nociceptive transmission. Although glial cells have emerged as modulators of synaptic plasticity, so far it is unknown whether their activation alone is sufficient to amplify synaptic strength. **Methods:** We used BzATP-induced P2X7 receptor-signalling to activate glial cells in the dorsal horn and studied the effect on synaptic transmission between nociceptive C-fibres and lamina I neurons in an electrophysiological approach. **Results:** Application of P2X7 receptor agonist BzATP induced a significant depression of synaptic transmission in all neurons recorded. We showed that this BzATP-induced depression was not mediated by P2X7 receptor-signalling but by the BzATP metabolite adenosine acting on inhibitory A1 receptors. Bath application of specific A1 receptor antagonist DPCPX not only reversed the depression, but further unmasked a rapid BzATP-induced facilitation of synaptic transmission. To exclude effects of abrupt adenosine withdrawal, we investigate P2X7 receptor-signalling under blockade of A1 receptors. Activation of glial P2X7 receptors under blockade of A1 receptor-signalling induced LTP in 60 % of all neurons tested. Blockade of P2X7 receptor-signalling by specific antagonist A-438079 completely prevented the BzATP-induced potentiation, whereas blockade of A1 receptor signalling alone had no effect on synaptic transmission. **Conclusions:** Activation of glial cells is sufficient to induce LTP at the first synapse in nociceptive pathways.

Shielding of the major mugwort pollen allergen Art v 1 inside of virus-like nanoparticles makes it invisible for B-lymphocytes in vivo.

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Background: Virus-like nanoparticles (VNP) are safe vaccine platforms, consisting of virus capsid proteins and a lipid envelope but lacking viral genome. Proteins of interest can be targeted to VNP by either C-terminally fusing them to a GPI anchor acceptor sequence – leading to surface expression – or, alternatively, by N-terminally fusing them to the viral matrix protein p15Gag – leading to their 'shielded' expression inside of VNP. Allergen-specific immunotherapy requires the repetitive delivery of potentially anaphylactogenic proteins to patients in order to achieve desired immunomodulatory effects. Consequently, safe containment strategies for unmodified allergens seem to be desirable. **Methods:** VNP expressing Art v 1 either on the surface or shielded inside particles were analyzed for their potential to activate T and B lymphocytes or effector cells along with mugwort extract in vitro and in vivo. **Results:** Degranulation of RBL cells sensitized with Art v 1-specific IgE occurred only upon exposure to VNP expressing surface exposed but not shielded allergen. In contrast, Art v 1 protein derived from both particles was well-presented to allergen-specific T cells, with shielded allergen exhibiting a 3.8±2.1-fold better stimulatory capacity compared to surface expressed allergen. Upon intranasal application into wildtype or Art v 1-specific 'allergy mice' VNP expressing surface exposed allergen induced significant titers of allergen-specific IgE, IgG1 and IgG2a, while VNP expressing shielded allergen entirely failed to do so. **Conclusion:** Shielding of allergens inside of VNP might represent a safe and versatile alternative for *in vivo* delivery of potentially anaphylactogenic proteins, while preserving their T cell stimulatory (modulatory?) capacity.

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NOD1 regulates DC functions by modulating IL-10 signaling

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Background: NOD1, a member of the NOD like receptor family, is well known to act as cytosolic pattern recognition receptor. One of the main outcomes of ligand sensing by NOD1 is the activation of NF-kappaB which results in the induction of a pro-inflammatory immune response mediating the host defense against bacterial infection. However, this study describes a novel, ligand independent function of NOD1 as modulator of IL-10 induced signaling in human monocyte derived dendritic cells (moDCs). **Methods/Results:** We show here that silencing of NOD1 in human moDCs supports the tolerogenic phenotype of IL-10 treated DCs. In moDCs deficient for NOD1, CD86 and the release of pro-inflammatory cytokines is significantly down-regulated, whereas the IL-10 target genes IL-10 and march1 are increased under these conditions. Moreover, co-culture experiments involving human moDCs and CD4+ T-cells indicate that IL-10 treated moDCs lacking NOD1 are more potent to induce the generation of FOXP3+CD25+ T-cells. To study the molecular mechanisms underlying these observations, we monitored IL-10-induced STAT1/3 activation. Whereas NOD1 silencing resulted in diminished IL-10-mediated STAT1 phosphorylation, STAT3-DNA binding was significantly enhanced. This may explain the observed increase in IL-10 target gene expression. Moreover, we demonstrate that NOD1 silencing is closely associated with reduced SOCS2 expression, indicating that SOCS2 might be involved in the regulation of IL-10-induced STAT1/3 signaling. **Conclusion:** Taken together, this study identifies NOD1 as regulator of IL-10 signaling and provides first evidence that NOD1 deficiency may contribute to a more pronounced tolerogenic phenotype of IL-10 stimulated moDCs.

Supported by the Austrian Science Fund (W1213) and the American Association of Immunologists (AAI).

February 2nd- Student Speaker

February 3rd - Plenary Talks

Neutrophil extracellular traps in inflammation and thrombosis

Denisa Wagner, PhD
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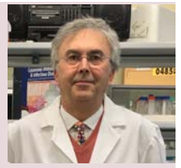
For many years, my lab has been studying the interplay between inflammation and thrombosis. These processes occur together, stimulate each other and share cellular and molecular components. The latest example of a common functional component is neutrophil extracellular traps (NETs). NETs are chromatin released together with toxic granular components from highly stimulated neutrophils. Originally found to trap/sequester invading pathogens, they were soon after discovered to be part of sterile inflammatory and thrombotic processes. NETs interact with von Willebrand Factor, which is also involved in platelet and leukocyte recruitment and is crucial for venous thrombus development after inferior vena cava stenosis. This will be discussed together with the role of NETs and the enzyme that generates them (PAD4) in animal models of deep vein thrombosis, myocardial infarction and in physiological wound healing. Interestingly, we observed that some diseases, such as cancer and diabetes prime neutrophils for NETosis. This interferes with wound healing in diabetes and contributes to cancer-associated thrombosis. The production of NETs may affect tumor biology promoting cancer progression.

Advances on the biology of polymorphonuclear neutrophils



Marco A. Cassatella
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In the last years, evidence has accumulated to unequivocally document that polymorphonuclear neutrophils are highly versatile and sophisticated cells, whose functions go far beyond the elimination of microorganisms. The notion that their longevity increases several-fold during inflammation has in fact changed the view under which neutrophils have long been considered. Accordingly, during their persistence in tissues, neutrophils have been shown to exert complex activities, including orchestration of the immune response and active induction of inflammation resolution. The notion that neutrophils shape the inflammatory/immune responses through de novo production of cytokines and/or release of preformed proinflammatory mediators, such as DAMPs and proteases, is also now well established. Finally, the demonstration that neutrophils exhibit complex crosstalk with components of the innate and adaptive immune system, which may contribute to the pathogenesis of numerous chronic inflammatory disorders, and emerging concepts on neutrophil heterogeneity and neutrophil plasticity, implying that, under pathological conditions, neutrophils may differentiate into discrete subsets defined by distinct phenotypic and functional profiles, has greatly renewed interest in these cells within the immunology community.
Based on these premises, I will summarize novel aspects of the biology of neutrophils that have been recently uncovered, including specific features on how neutrophil-derived cytokines are regulated at the molecular level.

Lysosomal Proteases in Cancer Progression

Christoph Peters[1,2,3] , Alejandro Gomez-Auli[1,3,4],
Oliver Schilling[1,2,3] and Thomas Reinheckel[1,2,3]
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Proteases and kinases are involved in tumor progression. Regulation of signaling pathways by kinases is reversible, whereas switches catalyzed by proteases are irreversible. Regulatory networks of proteases and kinases are interconnected in multiple ways and inhibition of kinases and proteases provides a broad spectrum of approaches for cancer therapy. We addressed the role of lysosomal cysteine proteases in cancer progression and were able to show, that absence of cathepsin L (Ctsl) enhances tumor growth and invasion in the K14-HPV16 mouse model of squamous cell carcinoma. In contrast absence of cathepsin B (Ctsb) as well as Ctsb and cathepsin Z (Ctsz) reduces the tumor burden in the MMTV-PyMT mouse model of breast cancer. Cathepsins in tumors can be provided by cancer cells as well as by cells of the tumor microenvironment, e.g. cathepsins from tumor associated macrophages (TAMs) contribute to cancer progression. Analyses of the secretome of PyMT cancer cell and TAM cocultures revealed an elevated abundance of the cellular repressor of E1A-stimulated genes 1 (CREG1) in cocultures of PyMT cancer cells with Ctsb and Ctsz deficient TAMs in comparison to wild type controls. CREG1 inhibits cell proliferation and promotes cellular differentiation. Upon treatment of breast cancer cells with recombinant CREG1, a significant reduction in proliferation, migration and invasion has been observed. Furthermore, in MMTV-PyMT mice overexpressing Ctsb a reduced CREG1 level has been detected in the mammary cancer tissue interstitial fluid. In summary, we have shown that cysteine cathepsins are involved in the regulation of tumor progression and that CREG1, a putative substrate of cysteine cathepsins, has anti-tumorigenic properties. Because of its increased expression in cells and tumors from mice deficient for both Ctsb and Ctsz, this protein could provide a molecular mechanism explaining why mice lacking Ctsb and Ctsz have a reduced tumor burden.

Two dimensional regulated protein interactions and force awakens T cell activation

Brian Evavold
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T cells are critical components of adaptive immune responses that when activated by antigen presenting cells can have positive (anti-infection, anti-tumor) or negative (autoimmune, allergic) outcomes. Our work focuses on the initial protein interactions between the T cell receptor (TCR) and peptide:MHC (pMHC) antigen since their binding kinetics controls the nature of T cell responses. The affinity and bond lifetime of TCR and pMHC interaction occurs in a two dimensional (2D) space as the proteins are embedded in opposing cell membranes. In addition, it is a dynamic environment that leads to the application of force to the TCR interactions with pMHC. We demonstrate that together the properties of 2D affinity and bond lifetime under force define T cell activation and provide novel insight into anti-infection versus autoimmune responses.

February 3rd - Plenary Speaker

February 3rd - Student Talks

Complement factor H deficiency dampens myeloid cell recruitment independent of its complement regulatory activity

Máté Kiss[1,2], Nikolina Papac-Miličević[1,2], Dimitrios Tsiantoulas[1,2], Tim Hendrikx[1,2], Laura Göderle[1,2], Mária Ozsvár-Kozma[1,2], Barbara Bartolini-Gritti[1,2], Clara Jana-Lui Busch[1,2], Christoph J. Binder[1,2]

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Background: Complement factor H (CFH) is the major regulator of the alternative complement activation. It is specialized in preventing host tissue damage from complement induced proinflammatory and cytolytic effects. In addition, we recenly found that CFH binds malondialdehyde (MDA), a prominent lipid peroxidation product with potent proinflammatory properties and thereby protects from the consequences of oxidative stress. Due to these functions we have hypothesized that CFH possesses a protective role in disease settings of acute and chronic sterile inflammation.
Methods: WT and Cfh-/- mice were intraperitoneally injected with 100 µl thioglycollate/g (body weight) to induce acute sterile inflammation. Peritoneal lavage was harvested 2, 24, 72 and 168 hours post injection and the number of recruited cells was determined along with the profiling of infiltrated macrophages by flow cytometry. C3-/- and Cfh-/-C3-/- mice were included in order to test the complement dependency of the observed effects. **Results:** In contrast to our expectations CFH deficiency led to a dramatic decrease in inflammatory cell recruitment. The number of both infiltrating neutrophils and macrophages was reduced by >50% in Cfh-/- mice compared to WT controls 24 and 72 hours post injection, respectively. Isolated Cfh-/- macrophages secreted more inflammatory chemokines, such as KC or MIP2-alpha, presumably as a compensatory mechanism for abrogated myeloid cell infiltration. All the above findings held true also on a C3 deficient background suggesting a complement-independent function of CFH in inflammatory cell recruitment.
Conclusion: We identified CFH as a key protein involved in myeloid cell recruitment in vivo via a mechanism independent of its complement regulatory ability.

Generation of allergen-specific T cell stimulator cells to investigate coinhibitory pathways in allergy

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Background: More than 25% of the population suffers from IgE-mediated immune reactions and its related symptoms. T lymphocytes have a crucial role in initiating and promoting allergies and their responses are tightly regulated by numerous activating and inhibitory signals. Currently, there is limited knowledge regarding the role of inhibitory pathways in allergen-specific T cells. **Methods:** To address this issue we have generated a novel type of an engineered, modular APC that can present allergenic peptides on MHC class II molecules to T cells from allergic individuals. The major birch and mugwort pollen allergens Bet v 1 and Art v 1 were used as model allergens. Human K562 cells were transfected to stably express HLA-DR1 or HLA-DR7 and fusion proteins of the invariant chain with allergenic peptides were expressed for endogenous MHCII loading. **Results:** Co-cultivation experiments with T cell reporter cell lines expressing allergen-specific T cell receptors (As-R) were performed to compare external peptide with endogenous MHCII loading, to evaluate MHC loading enhancers MLE and MHC acid stripping and also to investigate the antigen processing pathway. We found that overexpression of HLA-DM and addition of MLE increased peptide presentation upon exogenous loading of allergenic peptides. **Conclusions:** Our results indicate that allergen-specific T cell stimulator cells are useful tools to study the MHC class II presentation of allergenic peptides. By expressing coinhbitory ligands on allergen-specific T cells stimulator cells, we will be able to investigate the role of coinhibitory pathways in down-modulating the response of allergen-specific CD4+ T cells. *Supported by the Austrian Science Fund (FWF) project DK-APW01248FW (as part of the PhD program Molecular, Cellular and Clinical Allergology, MCCA of the Medical University of Vienna)*

The role of CD44 in the pathophysiology of chronic lymphocytic leukemia

Julia Christine Gutjahr[1,2], Tamara Girbl[1,2], Elisabeth Bayer[1,2], Richard Greil[1,2] and Tanja Nicole Hartmann[1,2]

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Background: Chronic lymphocytic leukemia (CLL) is a B cell malignancy with very heterogeneous clinical outcome. CLL cells that circulate in the peripheral blood are cell cycle arrested and need to home to lymphoid organs to receive various external stimuli for their proliferation and survival. CD44 is an adhesion molecule, which is structurally very diverse due alternative splicing and posttranslational modifications. The binding ability of the ubiquitously expressed CD44 and its ligands needs to be strictly controlled. This can be achieved by posttranslational modifications such as glycosylations, CD44variant expression, or CD44 clustering. **Methods**: Here we employed short term adoptive transfers of human leukemic cells to immune-deficient mice and developed a CLL mouse model with CD44 deficient B cells to investigate the contribution of CD44 in CLL cell localization. Further, we determined the prognostic potential of CD44 by screening the expression of CD44s and CD44v6 in blood samples from 250 CLL patients. **Results:** Adoptive transfers showed that the adhesion molecule CD44 supports the homing of CLL cells to bone marrow and spleen. Expression screening in CLL patient samples revealed substantial levels of CD44v6 in 5% of all cases. The vast majority of these cases were high risk patients harboring unmutated IgVH genes. Furthermore, these CD44v6 samples expressed diminished levels of CXCR4 and high levels of CD5, which was previously suggested as a phenotype for recently proliferated CLL cells. **Conclusion:** In summary, our data point to an important role of CD44 in the localization of CLL cells and a dynamic regulation of CD44s and CD44v expression upon CLL cell activation, which we will further elucidate.

Information Poster Sessions

February 2nd	**13:00 - 14:30**	**presenting authors present at their poster**
Allergy 1	P01 - P07	13:00 - 13:45
	P08 - P14	13:45 - 14:30
Allergy 2	P15 - P21	13:00 - 13:45
	P15 - P27	13:45 - 14:30
Inflammation	P28 - P32	13:00 - 13:45
Neurobiology	P33 - P35	13:45 - 14:30
Cancer 1	P36 - P39	13:45 - 14:30
February 3rd	**11:30 - 13:00**	
Cancer 2	P40 - P49	11:30 - 12:15
Vascular Biology	P50 - P53	12:15 - 13:00
Immunology 1	P54 - P60	11:30 - 12:15
	P61 - P66	12:15 - 13:00
Immunology 2	P67 - P72	11:30 - 12:15
	P73 - P77	12:15 - 13:00

POSTER SESSION
Horsaalzentrum AKH – Level 8
Medical University of Vienna
Währinger Gürtel 18-20, 1090 Vienna

Please note that the organizers cannot assume any liability for loss or damage of posters displayed in the poster area. The poster should be mounted between 8:00 and 9:00 in the morning on the day of presentation and need to be removed on the day of presentation. The posters should be removed until 19:00 hrs on Tuesday and between 16:10 hrs and 16:30 hrs on Wednesday.

FORMAT
The usable surface on the poster board will be 90 cm in width x 130 cm in height. Only adhesive tape can be used to mount posters. Material will be provided onsite.

GUIDED POSTER SESSIONS
During the guided poster sessions the presenters are required to stand by their poster to deliver a concise poster presentation of not more than 3 minutes and to answer questions from delegates.

Poster Session February 2nd

Allergy
Inflammation
Neurobiology
Cancer

ALLERGY 1

P01 Isabel Pablos — **Art v 1, Amb a 4 and Par h 1 are highly cross-reactive defensin-like proteins with similar structural features and distinct immunological properties.**
P02 Marta Ponce — **Assessing basophil activation pathways via flow cytometry in the context of food allergy.**
P03 Martín Candia — **Establishment of a cellular, fluorescent-based, peptide binding assay for the selection of altered peptide ligands of immunodominant peptides of major pollen allergens.**
P04 Jelena Gotovina — **Investigating the immunomodulatory potency of stress hormones interacting with molecular allergens .**
P05 Nazanin Samadi — **Phenotyping of allergen-reactive CD8+ T cells in type I allergy.**
P06 Yulia Dorofeeva — **Comparison of Par j 2.0101, a major allergen of Parietaria judaica pollen, produced in different expression systems.**
P07 Dominika Polak — **Neutrophils are potential apc in ige-mediated allergy.**
P08 Angelika Tscheppe — **Production of a recombinant hypoallergenic variant of the major peanut allergen Ara h 2 in the baculovirus insect cell system.**
P09 Azahara Rodríguez — **Development of sandwich ELISAs for the quantification of clinically relevant house dust mite allergens.**
P10 Chiara Palladino — **The interplay of Ara h 1 and peanut lipids in the allergic sensitization process.**
P11 Dubravka Smiljkovic — **The effects of Btk targeting drugs on IgE receptor-mediated signal transduction and activation of mast cells and basophils.**
P12 Gabriela Sánchez Acosta — **The role of phl p 5 specific igg antibodies for allergen presentation.**
P13 Huey-Jy Huang — **Towards a non-allergenic peptide mix containing the T cell epitopes of the clinically most relevant house dust mite allergens for tolerance induction.**
P14 Lukas Einhorn — **Generation of recombinant alpha chains of the high affinity IgE receptors of dogs, cats and horses to improve allergy diagnosis.**

ALLERGY 2

P15 Manuel Reithofer — **The role of CD8+ T-cells in allergy: A novel approach to stimulate CD8+ T-cells of allergic patients.**
P16 Manuel Reithofer — **Characterization of NET response to adjuvants used in allergy vaccines.**
P17 Mary A. A. Kodydek — **Identification of IgE epitopes of plant food allergens cross-reacting with the major birch pollen allergen, Bet v 1.**
P18 Olivia McKenna — **The role of Proteases in allergic sensitisation.**
P19 Pawel Dubiela — **Specificity of non specific lipid transfer proteins and influence of the ligands on their three dimensional structure.**
P20 Peter Tauber — **The 3-phosphoinositide-dependent kinase-1 targeting drug BX795 promotes interleukin-2 but shuts off T helper 1 and 2 cytokine secretion upon activation of allergen-specific T cells.**
P21 Pia Gattinger — **Towards the characterization of the allergenic activity of carbohydrate-reactive IgE.**
P22 Piotr Humeniuk — **Activation of iNKTs by food derived lipids.**
P23 Sergio Villazala — **The binding of monomeric IgE to CD23 on B cells induces intracellular signalling through ERK pathway.**
P24 Sherezade Moñino Romero — **Modulatory capacities of soluble Fc-epsilon RI in the IgE-mediated immune response.**
P25 Sheron Dzoro — **Construction of a phage display library from Escherichia coli to study IgE-reactive bacterial antigens.**
P26 Tanja Kalic — **Parvalbumin from Atlantic cod interacts with plasma membranes of intestinal and bronchial epithelial cells and induces differential gene expression of cytokines.**
P27 Yoan Machado — **Hypoallergenic Bet v 1.0101-Mannan neoglycoconjugates are highly immunogenic when applied via laserporated skin.**

INFLAMMATION

P28 Bettina Wanko — **Osteopontin modulates the number of T cells in adipose tissue of obese individuals.**
P29 Ci Zhu — **Interplay between Farnesoid X Receptor (FXR) and Epidermal Growth Factor Receptor (EGFR) during Hepatic Injury.**
P30 C.X. Lim — **Identification of a microRNA that regulates monocyte-derived dendritic cells.**
P31 Jörg Klufa — **Systemic metabolic defects caused by epidermal EGFR-deficiency.**
P32 Gabriel Stulnig — **The Role of Plasmacytoid Dendritic Cells in Imiquimod Induced Skin Inflammation and Melanoma Clearance in Mice.**

NEUROBIOLOGY

P33 A Tugrul Ozdemir — **Firing Patterns of Distinct Types of Neuron in Prefrontal Cortex during Working Memory and Cognitive Flexibility**.
P34 Sutirtha Ray — **Kv7 channels: Potential targets for antinociceptive action of Paracetamol.**
P35 Viktoria Hadschieff — **Epigenetic modulation of nociceptive transmission.**

CANCER

P36 Alexander D. Nardo — **Inhibition of human liver-cancer-cell tumorigenicity and metastasis by treatment with monoclonal antibodies against thrombin- and mmp-cleaved osteopontin.**
P37 Evelyn Hutterer — **CD18 expression in chronic lymphocytic leukemia is regulated by DNA methylation-dependent and -independent mechanisms.**
P38 Leo Edlinger — **A PAK2-STAT5 axis is key for tumor formation of BCR-ABL+ cells in vivo.**
P39 Tamara Scheidt — **Proteome- and Phosphoproteomeanalysis of oncogenic signaling pathways.**

Allergy

P01 Art v 1, Amb a 4 and Par h 1 are highly cross-reactive defensin-like proteins with similar structural features and distinct immunological properties

Isabel Pablos[1], Stephanie Eichhorn[1], Yoan Machado[1], Peter Briza[1], Christof Ebner[2], Jung-Won Park[3], Alain Didierlaurent[4], Naveen Arora[5], Stefan Vieths[6], Gabriele Gadermaier[1], Fatima Ferreira[1]

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Background: Art v 1, from mugwort (Artemisia vulgaris), Amb a 4 from ragweed (Ambrosia artemisiifolia) and Par h 1 from feverfew (Parthenium hysterophorus) are defensin-like protein and are important triggers of allergy. We aimed to produce the recombinant allergens for comprehensive physicochemical and immunological characterization. **Methods:** Proteins were expressed in E.coli and characterized using mass spectrometry, dynamic light scattering and spectroscopy. In vitro antigen uptake with BMDCs and endolysosomal degradation assay were performed. IgE sensitization experiments were assessed by ELISA using patients' sera from three countries. The allergenic activity was studied by mediator release assay. **Results:** The identity of purified proteins was confirmed by mass spectrometry. Recombinant proteins showed a similar size as determined by dynamic light scattering and the spectroscopy studies revealed comparable content of α-helices and β-sheets, indicating similar foldings. Amb a 4 and Art v 1 were more efficiently internalized by BMDCs than Par h 1. The allergens showed different proteolytic stability. The IgE reactivity of Art v 1 was higher in Austrian and Korean cohorts compared with Amb a 4 and Par h 1, while in the Canadian cohort all allergens showed similar reactivity. In cross-reactivity studies, Amb a 4 and Par h 1 showed a higher cross-reactivity between them compared to Art v 1. All three allergens triggered IgE-mediated basophil degranulation, demonstrating their allergenicity. **Conclusion:** Art v 1, Amb a 4 and Par h 1 have similar structural features but show different immunological behavior. The three allergens display different IgE sensitization profiles in patients'cohorts.

P02 Assessing basophil activation pathways via flow cytometry in the context of food allergy

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[2] Division of Immunology and Allergy, Food allergy and Anaphylaxis Program, The Department of Paediatrics, Hospital for Sick Children, The University of Toronto, Toronto, Canada.

Background: Food allergy occurs at a prevalence of up to 5% in children within the first years of life. Some food allergies are associated with a high likelihood of tolerance development up to the age of five whereas others are not. The reasons are not fully understood. Currently, markers used to confirm food allergy such as specific IgE levels are not appropriate to monitor tolerance development or define sensitized non-allergic individuals. Basophil Activation Test using CD63 as a readout parameter has been described to be superior in assessing tolerance in peanut sensitized, tolerant individuals as compared to other tests. The aim is to assess optimal kinetics of basophil activation pathways in vitro via flow cytometry in addition to CD63 measurement. **Methods:** Children´s basophils were evaluated for CD63 and CD203c surface expression as well as for phosphorylation of ERK1/2 and p38 MAPK, ALK and PLCgamma1 upon FcepsilonRI crosslinking using flow cytometry. **Results:** Kinetics of phosphorylation pathway demonstrate 1 minute (ERK1/2) and 3 minutes (p38) as optimal time points to measure IgE-related basophil activation. No PLCgamma1 phosphorylation was detected in basophils via FcepsilonRI. **Conclusions:** To understand the effect of the phosphorylation of these key intracellular proteins that contribute to basophil activation and therefore elicit allergic symptoms is of great interest and may allow a precise delineation of events taking place during desensitization and tolerance development.

P03 Establishment of a cellular, fluorescent-based, peptide binding assay for the selection of altered peptide ligands (APL) of immunodominant peptides of major pollen allergens

Martín Candia[1], Peter Tauber[1], Alina Neunkirchner[1], Doris Trapin[1], Sandra Rosskopf[1], Rafaela Campana[2], Peter Steinberger[1], Rudolf Valenta[2] and Winfried Pickl[1]

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CD4+ T lymphocyte activation requires T-cell antigen receptor-dependent recognition of immunogenic peptides bound to and presented by MHC-II molecules. Previous reports have shown that T-cell function can be modulated by altering the sequence of immunogenic peptides. We here used HLA-DR1+ K562 cells to establish a fast, flow cytometry-based competitive binding assay for the characterization of putative APL of the immunodominant Art v 123-36 peptide of the major mugwort pollen allergen Art v 1. Different concentrations of 25 Art v 123-36-derived peptides along with seven MHC loading enhancers (MLE) were pre-incubated with wild-type or HLA-DR1+ K562 cells 2 hours. Subsequently, incubation with biotinylated-HA306-318 (from haemagglutinin influenza A) reference peptide was performed and its specific binding was determined with phycoerythrin-labeled streptavidin by flow cytometry. Pre-incubation with 1-adamantaneethanol (100µM) led to a significant 5.38±0.04 fold increase in peptide binding ($p<0.05$). In the competitive assays two peptides with increased (IC50 competitor/wt ratio>1.50), eight with similar (ratio 0.50-1.50), and 15 with decreased (ratio<0.5) binding capabilities were identified. Functional evaluation in T cell proliferation and cytokine secretion assays identified two superagonists, one partial agonist and three bona fide antagonists. One superagonist revealed increased binding affinity, while this was similar to the wt for the partial agonist and antagonists. In summary, a robust and fast system to determine peptide binding affinity for HLA class II molecules on entire APC has been established and is currently used to identify APL with clinical relevance. Funded by the FWF: DK-W-1248-B13, SFB F4609-B19 and supported by Biomay AG and the MUV.

P04 Investigating the immunomodulatory potency of stress hormones interacting with molecular allergens

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Background: IgE-mediated allergy can be modulated by stress. Stress hormone adrenaline is an iron-binding molecule and therefore represents a siderophore. Considering that the major birch pollen allergen Bet v 1 may bind siderophore-iron complexes into its molecular pocket, we aimed to investigate whether Bet v 1 may interact with adrenaline. **Methods:** AutoDock Vina was used for in silico docking calculations. The Bet v 1 pocket was emptied by DFO dialysis (apo-Bet v 1) before further analyses. UV/VIS was used to assess whether Bet v 1 could sequester iron-adrenaline complexes. Further, PMA pretreated THP1-XBlue cells were used to investigate the effects of adrenaline stimulations on the NFkB pathway. **Results:** For Fe(adrenaline)3 docking into the pocket of Bet v 1 an affinity energy of -10,3 kcal/mol and Kd of 24 nM was calculated. In UV/VIS co-incubation of adrenaline with iron confirmed adrenaline as a siderophore. Incubation of Bet v 1 in the presence of adrenaline-iron complexes (holo-Bet v 1) led to an increase in the intensity at OD 280 nm compared to apo-Bet v 1 and caused a shift of the peak maximum to 530 nm compared to adrenaline-iron complex alone. Adrenaline promoted NFkB activation on macrophage-like cells using PMA preactivated THP1-XBlue cells at physiological concentrations (nM) whereas further increase of adrenaline abolished this effect. **Conclusions:** The major birch pollen allergen Bet v 1 is able to interact with Fe(adrenaline)3. The interaction of lipocalin allergens with adrenalin could have implications in the sensitization as well as effector phase of allergy.

P05 Phenotyping of allergen-reactive CD8+ T cells in type I allergy

Nazanin Samadi[1], Claudia Kitzmüller[1], Rene Geyeregger[2], Barbara Bohle[1], Beatrice Jahn-Schmid[1]

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Background: T cells play a main role in the induction and maintenance of IgE-mediated allergy. The function of CD4+ T cells in the pathophysiology of allergic disorders has been extensively investigated while the role of CD8+ T cells is still poorly understood and controversial. **Aim:** The aim of this project is to characterize allergen-specific CD8+ T cells in patients with different allergic manifestations (rhinoconjunctivitis, atopic dermatitis and atopic bronchial asthma). Different seasonal (birch pollen and grass pollen) and perennial allergens (cat dander and house dust mite) were included. **Methods:** PBMCs from allergic patients were stained with the proliferation dye efluor 670 and incubated with allergen. Proliferating CD3+CD8+ cells were then assessed for the expression of differentiation markers (CD27, CD28, CD45RO, CXCR3, CRTh2, PD-1), homing markers (CCR4, CD62L, CD29b), intracellular cytokines (IL-4, IL-5, IL-13, IL-17, IL-22 and IFN-γ, TNF-α), and cytotoxic proteins (granzyme B and perforin) by flow cytometry and compared to non-proliferating CD8+ cells. **Results:** We found allergen-reactive CD8+ T cells in all allergic manifestations. Moreover, largest numbers were detected upon stimulation with house dust mite and grass pollen

February 2nd - Poster session

extracts. Proliferating cells contained higher numbers of cells producing IL-4, granzyme B and perforin than non-proliferating CD8+ T cells. In addition, a significantly higher expression of CD27, CD45RO, CD62L, and CD29b was detected in allergen-reactive CD8+ T cells indicating central memory T cells of mucosal origin. **Conclusion:** Thus, we could demonstrate allergen-reactive IL-4$^+$CD8$^+$ T cells in different allergic manifestations which produce cytotoxic proteins. Their functional activity will be investigated in future experiments. Supported by the Austrian science fund, projects W1248 and SFB F4610.

P06 Comparison of Par j 2.0101, a major allergen of Parietaria judaica pollen, produced in different expression systems.

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Background: Parietaria judaica is one the most common pollen allergen sources in the Mediterranean area and with a long period of pollination from February to November. Par j 2.0101, a cysteine-rich, lipid transfer protein (LTP) with a molecular weight of 11.3 kDa, is the major allergen in Parietaria judaica recognized by more than 80% of allergics. **Methods:** A synthetic gene, codon-optimized for insect cells coding for Par j 2 including 6xHistag at the C-terminus sites was subcloned into pTM1 vector into the BamHI/SmaI sites (ATG: biosynthetics, Merzhausen, Germany). This construct was transformed into E. coli to generate high molecular weight recombinant bacmid DNA and then transfected into insect cells to obtain recombinant baculovirus for expression. To determine which system is the best choice to express rPar j 2 – comparison of the proteins, expressed in E.coli and in insect cells on the base of gel fitration, CD spectroscopy and ELISA experiments, was done. **Results:** The recombinant protein obtained by expression in baculovirus-infected insect cells and purified by Ni2+ metal ion affinity chromatography reacted with IgE from allergic donors. CD spectra of rPar j 2 from E.coli revealed a content in unordered conformations comparing rPar j 2 from insect cells. Sera samples from 26 parietaria allergic patients were exposed to extract, rPar j 2 expressed in E.coli and in insect cells. The results showed the prevalence of allergen-pecific IgE to rPar j 2 (i) comparing to rPar j (e). **Conclusions:** By expression in baculovirus-infected insect cells it was possible to obtain soluble, folded, IgE-eactive recombinant protein rPar j 2 for diagnosis and possibly for therapy of patients with Parietaria pollen allergy.

P07 Neutrophils are potential apc in ige-mediated allergy

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Background: Neutrophils are present in large numbers in allergic late-phase reactions. However, it is not yet clear whether they contribute to allergic inflammation. These professional phagocytes might present allergen to allergen-specific T cells since they express MHC class II molecules upon stimulation with certain cytokines, chemokines and bacterial factors, such as GM-CSF, TNF-alfa, IL-8, IFN-gamma and LPS, respectively. In fact, murine neutrophils have been shown to process and present antigens to CD4+ T-cells. **Aim:** To assess whether human neutrophils act as antigen-presenting cells for allergen-specific T-cells. **Methods:** Neutrophils isolated from the peripheral blood of allergic donors were cultured under different conditions and analyzed for the expression of MHC class II, CD40, CD80, and CD86, by flow cytometry. Surface binding, internalization and intracellular degradation of fluorescence-labelled Bet v 1 by neutrophils were compared with monocytes. Microsomal proteases were isolated from both cell types and incubated with Bet v 1. The resulting proteolytic fragments were sequenced using mass spectrometry. Finally, neutrophils and monocytes were cocultured with Bet v 1-specific T-cell cultures generated from birch-pollen allergic donors in the presence or absence of Bet v 1 and proliferative responses of T-cells were assessed. **Results: A** cocktail of IL-3, GM-CSF and IFN-gamma enhanced the expression of HLA class II and CD80 on neutrophils. Neutrophils effectively internalized Bet v 1 and their uptake and endolysosomal degradation of the allergen was faster than by monocytes. In addition, neutrophils processed longer peptides of Bet v 1 than monocytes. Neutrophils pulsed with Bet v 1 induced proliferation in Bet v 1- specific T- cells specific for different epitopes distributed over its entire amino acid sequence. However, monocytes were the more potent antigen-presenting cells. **Conclusions** Our data provide evidence that neutrophils may serve as antigen-presenting cells for allergen specific T-cells and thereby, play a role in the late phase reaction of IgE-mediated allergy. *Supported by the Austrian Science Funds, project W1248 and SFB F4610.*

P08 Production of a recombinant hypoallergenic variant of the major peanut allergen Ara h 2 in the baculovirus insect cell system

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Background: Peanut allergy is one of the most dangerous food allergies. The most important peanut allergen is Ara h 2. At present, no safe allergen-specific immunotherapy is available for clinical use. We aimed to produce a hypoallergenic mutant (mt) Ara h 2. **Methods:** Recombinant baculoviruses harbouring genes for in silico designed mtAra h 2 or the wild-type (wt) Ara h 2 with a hexahistidyl-tag were used to infect Trichoplusia ni BTI-TN5B1-4 ‘‘HighFive’’ cells. After purification from the supernatants, expression of the proteins was verified by Western blotting. Following determination of physicochemical characteristics, IgE-binding to purified natural (n) Ara h 2, wtAra h 2 and mtAra h 2 was tested by direct ELISA, Western blotting and inhibition ELISA. **Results:** Mass spectrometry confirmed the absence of post-translational modifications for the main wtAra h 2 fraction. The N-terminal amino acid sequence and folding of wtAra h 2 corresponded to the natural protein. For mtAra h 2, mass spectrometry and N-terminal sequencing yielded a mass corresponding to the predicted size and the correct N-terminus. CD spectrometry showed a high content of alpha-helices. Immunoblots of Ara h 2-sensitized patients indicated lower IgE-binding to mtAra h 2. In direct ELISA, allergic patients’ sera revealed a 20-50% reduced IgE-binding to mtAra h 2 compared with wtAra h 2. Also inhibition ELISAs showed significantly reduced IgE-binding of mtAra h 2. **Conclusions:** mtAra h 2 is a promising template for designing the next generation of hypoallergenic mutants. *Supported by the Austrian Science Fund doctoral program W1248-B13*

P09 Development of sandwich ELISAs for the quantification of clinically relevant house dust mite allergens

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Background: House dust mite (HDM) allergy affects more than 10% of the population in industrialized countries. Beside Der p 1 and Der p 2, the HDM allergens Der p 5, Der p 7, Der p 21 and Der p 23 have been identified as the clinically most important allergens with high allergenic activity. Assays for measuring allergen concentrations in environmental samples, diagnostic and therapeutic allergen extracts are available only for Der p 1 and Der p 2. **Methods:** Allergen-specific antibodies with defined specificities were obtained by immunizing rabbits with synthetic peptides derived from different portions of the allergens and with the complete recombinant allergens. The rabbit antisera were tested for allergen reactivity towards immobilized allergens and allergens in solution and used to build sandwich ELISAs based on capturing and detecting antisera with defined specificity. **Conclusion:** Using purified allergens for standardization will allow to quantify the natural allergens in biological samples. The sandwich ELISAs will be useful to measure and quantify the HDM allergens Der p 5, Der p 7, Der p 21 and Der p 23 in environmental samples, in allergen extracts used for challenge tests as well as in diagnostic and therapeutic allergen extracts
Supported by the FWF-funded PhD program MCCA, by the FWF projects F4605, F4602 and by a research grant from Biomay AG, Vienna, Austria.

P10 The interplay of Ara h 1 and peanut lipids in the allergic sensitization process

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Background: Peanuts contain a significant amount of lipids which might play a role together with peanut allergens, such as Ara h 1, in the allergic sensitization process. Some dietary lipids are known to take part in regulating inflammation and in influencing the generation of reactive oxygen species (ROS). ROS are known for stimulating Th2-like responses and to enhance antigen presentation by dendritic cells. Therefore, we aimed to assess whether peanut lipids were able to elicit ROS production in monocyte-derived dendritic cells (MoDCs) and thus to modulate the allergen-induced immune response. **Methods:** Ara h 1 was purified from roasted peanuts. Lipids were extracted from peanut with chloroform/methanol. MoDCs were obtained from PBMCs of non-allergic individuals and differentiated. MoDCs were treated with 10 µg/ml peanut lipids, Ara h 1, or both. Intracellular ROS generation in MoDCs was assessed by flow cytometry with the probe 5-(and-6)-chloromethyl-2´7´-dichlorodihydrofluorescein diacetate acetyl ester (CM-H2 DCFDA). TNF-α levels in the cell culture supernatant were measured by ELISA, in the presence or absence of the ROS scavengerN-acetyl-L-cysteine (NAC) at 5 mM. Results: Peanut lipids, but not Ara h 1, induced ROS production in MoDCs. Moreover, Ara h 1 induced the production of TNF-α. This upregulation was inhibited by the addition of peanut lipids. However, in cells treated with NAC, this Ara h 1-induced TNF- α production was unaffected by the presence of peanut lipids. **Conclusions:** Our data suggest that the production of ROS by peanut lipids modifies the response of MoDCs to peanut allergens.
Supported by W 1248-B13.

P11 The effects of Btk targeting drugs on IgE receptor-mediated signal transduction and activation of mast cells and basophils

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Background: Mast cells (MC) and basophils (BA) are key effector players in allergic inflammation. Both types of cells express high-affinity receptors for IgE (IgERI). Activation of MC and BA through IgERI is associated with activation of downstream signalling pathways and enhanced expression of cell-surface antigens such as CD63 or CD203c. Recently, the Bruton´s tyrosine kinase (BTK) has been identified as a new potential downstream-target in IgERI -cross-linked MC and BA. The aim of this study was to explore the effects of various BTK blockers on IgE-mediated histamine release, phosphorylation of downstream signalling targets and upregulation of cell-surface antigens. **Methods:** We examined human blood BA from 3 healthy donors and 8 patients allergic to Bet v 1, Der p 2, and/or Phl p 5 as well as the human mast cell line HMC-1 by flow cytometry. In addition, histamine release experiments were performed with BA. **Results:** We found that the BTK blocker Ibrutinib counteracts anti-IgE-induced and allergen-induced upregulation of CD63 and CD203c (IC50 <0.5 microM) and histamine release (IC50 <0.025 microM) in BA. The other two Btk inhibitors tested, AVL-292 and CNX-774, were also found to suppress IgE-mediated histamine release (IC50 <0.05 microM). Moreover, as determined by flow cytometry, all BTK blockers tested were found to inhibit phosphorylation of BTK in HMC-1 cells as well as in IgERI-cross-linked BA. **Conclusions:** All in all, our data show that Ibrutinib and other BTK inhibitors suppress anti-IgE-induced upregulation of CD63 and CD203c as well as IgE-mediated histamine release in BA at reasonable drug concentrations.
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P12 The role of phl p 5 specific igg antibodies for allergen presentation

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Background: Allergen-specific immunotherapy (AIT) is based on the administration of appropriate concentrations of allergen extracts. A beneficial response in patients has been associated with high productions of IgG4 and IgG1 antibodies, which compete with IgE for allergen binding. However, allergen-IgG complexes can also bind to FCgamma-receptors expressed on the surface of antigen-presenting cells (APC). This cross-linking may thereby increase allergen-uptake and eventually the number of HLA-peptide-complexes on the surface of these cells which may drive the resulting T cell response towards Th1. **Methods and results:** We will study the effects on the T cell level induced by the decrease of the IgE/IgG ratio using the major grass pollen allergen, Phlp5. This recombinant allergen was expressed and characterized and will be incubated with human Phlp5-specific monoclonal IgG1, IgG4 and IgE antibodies with identical paratop. In addition, sera from SIT-treated patients containing high levels of Phlp5-specific IgG will be used. Professional APCs will be isolated from whole blood samples in order to compare surface binding, internalization and processing of IgE-, IgG-bound and unbound Phlp5. To asses proliferative and cytokine responses, Phlp5 specific T cell lines and T cell clones will be produced and stimulated with APCs pulsed with antibody-loaded and unloaded Phlp5. Finally, these latter aspects will also be investigated by using naïve T cells. Together, this data will show if SIT-induced IgG antibodies may not only block IgE-mediated effects but also modulate allergen-specific T cell responses during the therapy.

P13 Towards a non-allergenic peptide mix containing the T cell epitopes of the clinically most relevant house dust mite allergens for tolerance induction

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Background: House dust mites are one of the most important allergen sources. Der p 1, Der p 2, Der p 5, Der p 7, Der p 21 and Der p 23 are the clinically most important house dust mite (HDM) allergens. The aim of this study was to define a mix of non-allergenic T cell epitope-containing peptides of these allergens for tolerance induction. **Methods:** According to the amino acid sequences of these allergens, we synthesized and purified 33 overlapping peptides covering the complete sequences of Der p 1, 2, 5, 7, 21 and 23. The peptides were tested for IgE and IgG reactivity with sera from HDM allergic patients in ELISA. PBMCs from 27 HDM allergic and 10 non-HDM allergic individuals were incubated with the synthetic peptides and T cell proliferation was measured using a CFSE dilution-based assay. **Results:** The peptides could be purified in large amounts. They lacked secondary structure but most of them remained soluble in physiological buffers. ELISA assays indicated that most peptides from Derp 1, 2, 5, 7, 21 and 23 lacked IgE reactivity and thus were non-allergenic. T cell proliferation assays identified 12 predominant epitopes in the Der p allergens. **Conclusions:** Our data indicate that a reasonable number of non-allergenic peptides including the sequences and thus T cell epitopes of the clinically most relevant house dust mite allergens can be defined for prevention of HDM allergy by tolerance induction.

P14 Generation of recombinant alpha chains of the high affinity IgE receptors of dogs, cats and horses to improve allergy diagnosis

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Background: Dogs, cats and horses are like humans affected by IgE-mediated allergies. For their diagnosis the alpha chain of the human high affinity IgE receptor FceRI is applied based on an interspecies amino acid homology of 54-64%. We hypothesized, that the IgE detection in allergic dogs, cats and horses could be improved by the use of species-specific recombinant FceRI-alpha chains. **Methods:** Canine, feline and equine recombinant(r) FceRI-alpha chains were expressed in CHO-DUKX B11 cells using a custom SV40_ Neo mammalian expression vector. 384 clones of each species were evaluated for their production of IgE-binding rFceRI-alpha by immunoblotting and Enzyme-Linked Immunosorbent Assay (ELISA) prior to isolation of rFceRI-alpha from selected clones via anti-FLAG M2 affinity purification. **Results:** According to results of quantitative ELISA concentrations of 600-1000µg/mL for the canine and equine, and of 80µg/mL for the feline rFceRI-alpha proteins could be achieved, rendering an approximately yield of 48mg canine, 80mg equine and 6,4mg feline recombinant protein. Further, in Immunoblots the three rFceRI-alpha proteins could be identified at the expected size of 55kDa. **Conclusion:** The recombinant species-specific rFceRI-alpha proteins may improve the allergy diagnoses in allergic cats, dogs or horses.
The study was supported by the Austrian Science Fund (FWF) grants SFB F4606-B19, W1248-B13 (MCCA), and in part by P23398-B11 and W1205-B09 (CCHD).

P15 The role of CD8$^+$ T-cells in allergy: A novel approach to stimulate CD8$^+$ T-cells of allergic patients

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Introduction: The role of CD4+ T-cells in allergy is well characterized, whereas the CD8+T-cells have been neglected in the studies so far. Contrary to T-helper cells which recognize exogenous peptides on MHC class II molecules on antigen-presenting cells (APC), CD8+ T-cells recognize mostly cytosolic peptides, degraded in the proteasome and presented by MHC class I molecules. CD8+ T-cells specific for exogenous antigens (like e.g. allergens) need the stimulation by antigen-presenting cells with the capacity to cross-present exogenously derived peptides. Effective cross-presentation is limited to certain dendritic cell subsets. To investigate the rare allergen-specific CD8 T-cells we intend to expand them in vitro in T-cell lines (TCL) derived from PBMC of allergic individuals. **Methods:** To optimize the induction of these TCL, we aim to establish artificial human allergen-presenting APC. We will express relevant allergens (Fel d 1, Der p 1 and Der p 2) in human transduced K562 cells expressing HLA-A2. To target them to the proteasome for processing, ubiquitin will be linked to the allergens by retroviral transduction. These cells will then be used to stimulate purified CD3+ or CD8+ T-cells

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of HLA-A2 positive allergic patients. Their stimulatory capacity will be compared to allergen-pulsed APC from PBMC and used to induce allergen-specific TCL for characterization of phenotype and function. **Results and Conclusion:** So far the allergen-presenting APC have been established and additionally a positive control cell line, presenting common viral peptides has been constructed. Preliminary data show proliferation of CD8+ T-cells in total PBMCs in response to the postitive control.

P16 Characterization of NET response to adjuvants used in allergy vaccines

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Introduction: Most subcutaneous allergy vaccines contain alum as adjuvant, and a few monophosphoryl lipid A (MPL), a TLR-4 agonist. For alum it has been shown in mice that neutrophil-derived DNA mediates adjuvant activity. Neutrophils have the ability to trap pathogens extracellularly by releasing DNA and granular material, so-called neutrophil extracellular traps (NETs). We intend to characterize the NET response of human neutrophils to alum and MPL and their possible role in the immune response induced by allergen-specific immunotherapy. **Methods:** Freshly isolated human neutrophils are seeded on coverslips and stimulated with NET-inducing factors including PMA and LPS in comparison to alum and MPL. Formation of NETs is evaluated by staining of DNA or granular proteins and fluorescence microscopy. Time course experiments to assess the amount of extracellular DNA are performed and elastase activity in supernatants determined. **Results:** The response to MPL showed expected similarity to the LPS-triggered NET-formation with DNA-fibers, granular myeloperoxidase, elastase or LL-37 sticking to them. In contrast, alum induced cloud-like NETs, also associated with vital nuclei and granular proteins. None of the adjuvants caused cell death after 3 hours, as it was observed with PMA. In time course experiments increased amounts of extracellular DNA was observed with alum. In supernatants increased extracellular elastase activity upon stimulation with both adjuvants was observed. **Conclusion:** The two adjuvants induce different distributions of extracellular DNA, which shows the typical features of NETs, DNA associated with granular proteins. In order to further investigate the stimulatory capacities of these NETs, co-cultivation experiments with APCs will be performed.

P17 Identification of IgE epitopes of plant food allergens cross-reacting with the major birch pollen allergen, Bet v1

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Background: Birch pollen allergic patients often complain of oral allergy syndrome (OAS) during consumption of certain plant food due to cross-reactivity of IgE antibodies specific for the major birch pollen allergen, Bet v 1, with the respective food allergens. We compared epitope recognition patterns responsible for this cross-reactivity with five homologous plant food allergens. **Methods:** Using inhibition ELISA assays, rabbit antisera against six peptides covering the entire Bet v 1 sequence were used to inhibit the binding of birch pollen allergic patients´ (n=33) IgE to rApi g 1 (celery), rAra h 8 (peanut), rBet v 1 (birch pollen), rDau c 1 (carrot), rMal d 1 (apple) and rPru av 1 (cherry). These patients had birch specific IgE levels of ≥10 kUA/l and also complained about symptoms of OAS to one or more of these Bet v 1 homologous food sources. **Results:** Twenty-three patients showed reactivity to rBet v 1, nineteen to rPru av 1, seventeen to rMal d 1, nine to rAra h 8, seven to rApi g 1 and four to rDau c 1. Inhibition results revealed different IgE epitope-containing patches on the allergens. Four major epitopes were identified in rBet v 1, two were common for rPru av 1 and rMal d 1. Allergens rAra h 8, rApi g 1 and rDau c 1 each consisted of only one IgE epitope-containing patch. **Conclusion:** Our study reveals Bet v 1 as the major IgE epitope-containing allergen which therefore appears to be the primary sensitizing molecule in patients suffering from OAS to Bet v 1 homologous food allergens.

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P18 The role of Proteases in allergic sensitisation

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Background: Proteases in allergen sources have been suggested to contribute to primary sensitisation to allergens and exacerbate allergic disorders. Major allergens, for example the house dust mite allergen Der p 1, have long been identified as key players in allergic diseases, showing a defined role of protease activity. However, patients also become sensitised and develop allergy to non-protease allergens such as Der p 2 from dust mites or the major birch pollen allergen, Bet v 1. **Objectives:** Hence, we are keen to investigate the role of pollen and bacterial derived proteases in sensitisation and allergy development to non-protease allergens. **Methods:** Focussing on birch pollen extract, we aim to characterise and purify key pollen derived proteases (PDPs). Preliminary experiments using zymography show existing gelatinase activity, further elucidation of which will be established using mass spectrometry and transcriptome analysis. The effects of PDPs on epithelial integrity and permeability will be analysed in vitro on a polarized monolayer by transepithelial electrical resistance (TEER) measurements. In addition, the influence of PDPs on DC loading and T-cell differentiation will be investigated in vitro using transwell co-culture systems with Ova-specific DO11 T-cells. To investigate the role of PDPs on immune-polarization in vivo, cytokine reporter mice for IL-4 (‘’4GET’’) and for IFN-γ (‘’GREAT’’) will be immunised with non-protease allergens (i.e Bet v 1), in combination with pollen or bacterial derived proteases. Moreover, these combinations of non-protease allergens and proteases will be analysed for their capacity to induce Th2 responses in adjuvant-free mouse models of allergic sensitization.

P19 Specificity of non specific lipid transfer proteins and influence of the ligands on their three dimensional structure

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Background: Plant non-specific lipid transfer proteins (nsLTPs) are relevant food allergens e.g. from peach (Pru p 3), hazelnut (Cor a 8) or walnut (Jug r 3). They share a conserved fold with an internal cavity. Different lipid–protein complexes showed that the tunnel adapts its volume while binding a broad range of hydrophobic molecules. **Methods:** The binding of lipids to Pru p 3, Cor a 8, and Jug r 3 was monitored by adding 10 µM 1,8-ANS and measuring the decrease of 1,8-ANS fluorescence. Furthermore, molecular dynamic analysis (MD) was applied to explore the nature of interaction between nsLTPs and tested ligands. Saturation transfer difference (STD) spectroscopy and W-LOGSY (Water-Ligand Observed via Gradient Spectroscopy) technique were applied to confirm results obtained in silico. **Results:** Due to pre-incubation of Pru p 3 with lipids a concentration dependent reduction of ANS binding was observed. Pru p 3 incubated (1:1; 1:10) with lauric acid showed 19% and 66% of ANS fluorescence reduction respectively, compared with Pru p 3 without lipids. For oleic acid (1:1; 1:10) reduction was 7% and 77%, respectively. Molecular dynamic analysis suggests changes in protein structure due to binding to certain ligands. Interaction between oleic acid and Pru p 3, moved the C-terminal loop out towards the surface of the molecule. NMR based experiments confirmed binding capacity observed in MD analysis. **Conclusions:** In this study, we observed differences in binding capacity of Pru p 3. Molecular dynamic simulation has shown that interaction between Pru p 3 and tested ligands can lead to conformational changes. The allergen–lipid interaction may act as a potential danger signal during the allergic sensitization phase or increase allergenicity during the effector phase.

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P20 The 3-phosphoinositide-dependent kinase-1 targeting drug BX795 promotes interleukin-2 but shuts off T helper 1 and 2 cytokine secretion upon activation of allergen-specific T cells

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Background: BX795, an inhibitor of 3-phosphoinositide-dependent kinase-1 (PDK-1), was investigated for its effects on viability, allergen-specific activation, growth and factor production of major mugwort (Artemisia vulgaris) pollen allergen-specific T lymphocytes derived from double transgenic allergy mice. **Methods:** We assessed proliferation and T cellular activation by measuring 3H-thymidine uptake and evaluation of CD69, CD25, CD154 and CD49e expression on CD3+CD4+ T cells. Factor production was determined by multiplexing of supernatants after T cell activation with titrated amounts of allergen. **Results:** Using the IC50 dose for proliferation inhibition, no alteration in viability of BX795-treated splenocytes was observed. Furthermore, BX795 treatment did not negatively affect activation marker expression studied. Noteworthy, BX795 almost completely inhibited IFN-gamma(72.9±10.0%, $p<0.01$) early on (24 hours) and inhibited IL-4 (88.3±6.5%, $p<0.05$), IL-13 (75.8±8.7%, $p<0.001$) and IL-10 (80.8±13.1%, $p<0.001$) secretion at 48 hours. Moreover, at 48 hours BX795 partially inhibited IL-5 (35.8±23.2%, $p<0.05$) and TNF-alpha45.2±24.8%, $p<0.05$) secretion with maximal inhibition seen after 72 hours (70.6±15.5% $p<0.001$ and, 68.0±17.8% $p<0.05$, respectively) BX795 did not influence IL-17 and GM-CSF levels, however, it significantly stimulated IL-2 secretion at 48 and 72 hours (2.3±0.6-fold and 4.7±1.4-fold, $p<0.01$ and $p<0.001$, respectively). Other substances tested, displaying an equal level of proliferation inhibition, such as the ERK2 inhibitor Vx-11e drastically inhibited IL-2 secretion. **In summary,** BX795 specifically shuts-off Th1 and Th2 cytokines while it strongly stimulates interleukin-2 secretion. A potential clinical application is currently being evaluated in in vivo experiments using double transgenic allergy mice.

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P21 Towards the characterization of the allergenic activity of carbohydrate-reactive IgE

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Background: A large number of allergens derived from insect venoms, moulds, pollen and plant-derived food are glycoproteins. Their Asparagine (N)-linked carbohydrates contain structural motifs which are not found on human glycoproteins. This N-linked glycans are part of the cross-reactive carbohydrate determinants (CCD) that can elicit IgE in about 20% of allergic patients. In order to investigate the allergenic activity of carbohydrates we have engineered N-linked glycosylation sites into the non-allergenic protein horse heart myoglobin (HHM). **Methods:** Sequences coding for one or two N-glycosylation sites were engineered into the 5’ end of the HHM cDNA and the proteins were expressed in baculovirus-infected High-FiveTM insect cells. A non-glycosylated version of HHM was tested for control purposes. The glycoproteins were analysed regarding fold and aggregation circular dichroism and gel filtration, respectively. IgE reactivity was assessed by ELISA and Immunoblotting. **Results:** HHM-glycovariants were expressed and purified from insect cells as monomeric and folded proteins. IgE from patients with bee and/or wasp, as well as pollen sensitization showed reactivity to the HHM-glycovariants but not to non-glycosylated HHM. **Conclusion:** The HHM-glycovariants can be useful as a marker for IgE-reactivity to carbohydrates and will allow to determine the allergenic activity of carbohydrate IgE epitopes.

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P22 Activation of iNKTs by food derived lipids

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Background: In contrast to conventional T lymphocytes invariant natural killer T lymphocytes (iNKTs), recognize chemically distinct CD1d-restricted antigens such as lipids. Therefore, iNKTs are a major subpopulation of T lymphocytes that recognize own or foreign lipid antigens. Upon activation iNKTs can either up- or downregulate immune responses by promoting the secretion of Th1, Th2, or immune regulatory cytokine patterns. So far, iNKTs have been identified as important players in different types of immune responses. However, the role of iNKTs in an allergic reaction is not well understood. **Methods:** Different food-derived lipid fractions from hazelnut, walnut and sunflower were prepared by standard extraction methods and preparative thin layer chromatography. In addition, synthetic lipids will be applied. To test whether lipid fractions contain iNKT cell antigens, we will use the mouse hybridoma cell lines DN3A4-1.2 and DN3A4-1.4 in a CD1d-dependent in vitro antigen presentation assay. Cells will be also tested in co-cultures of APCs/iNKTs/naive T lymphocytes. Upon in vitro activation of cells changes of surface marker expression and cytokine patterns will be assessed by FACS and ELISA analyses. **Results:** We established cultures of mouse hybridoma cell lines. Different food-derived lipid fractions from hazelnut, walnut and sunflower were prepared. First analyses employing the CD1d-dependent in vitro antigen presentation assay have been carried out. **Conclusions:** Using the model system described above we expect a better understanding of the effect of the lipid food matrix on the allergic immune response. In detail, we will get a deeper insight into the role of iNKTs upon activation via CD1d presented lipids and the functional interplay between iNKTs and T lymphocytes.

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P23 The binding of monomeric IgE to CD23 on B cells induces intracellular signalling through ERK pathway

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Background: CD23 is the low affinity receptor for IgE and is found mainly on B cells. IgE-allergen complexes bound to CD23 are internalised and subsequently presented to T lymphocytes. Despite its role in perpetuating allergic responses, little is known on CD23-mediated intracellular signalling. Increased levels of phosphorylated ERK, Fyn and AKT were reported in different cells lines upon the crosslinking of CD23 with specific anti-CD23 antibodies. However, the intracellular signalling followed by the binding of IgE and IgE-allergen complexes to CD23 has not been analysed. **Methods:** We studied the intracellular signalling cascades activated through CD23 using purified monoclonal human Bet v 1-specific IgE, monomeric Bet v 1 and a recombinant Bet v 1 trimer in a human Epstein-Barr virus-transformed B cell line expressing high levels of CD23. The induction of ERK phosphorylation was studied with specific antibody probes in B cell extracts via Western blotting. **Results:** Monomeric IgE was found to induce an increase in phosphorylated ERK after 4 minutes, attaining a peak after 15 minutes, whereas the binding of complexes, IgE-monomeric Bet v 1 and IgE-trimeric Bet v 1, to CD23 showed a later induction, after 8 minutes and reaching a maximum after 20 minutes. **Conclusions:** The binding of monomeric IgE alone to CD23 induces an intracellular signal, whose time course seemed to differ to the one initiated by IgE-Bet v 1 allergen complexes. This finding explains B cell activation by monomeric IgE, suggests differences in signalling induced by monomeric IgE versus IgE-allergen complexes and may represent a possible target for therapeutic intervention.

P24 Modulatory capacities of soluble Fc-epsilon RI in the IgE-mediated immune response

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Background: IgE-mediated allergies are potentially life threatening immunologic reactions towards otherwise harmless environmental antigens and serum IgE is the common marker for allergy diagnosis. However, allergen-specific IgE levels not always correlate with allergic reactions. The recently discovered soluble form of the high affinity receptor for IgE (sFcepsilonRI) present in serum may interfere with IgE levels. **Methods:** The modulatory capacities of sFcepsilonRI were analysed using a MelJuSo cell line stably transfected with functional FcepsilonRI. Cells were stimulated with chimeric IgE (cIgE) plus its specific ovalbumin (NP-OVAL). To measure the blocking capacity, FAB-like (Facilitated Antigen Binding) tests were performed with sFcepsilonRI collected from the supernatants, and this blocking capacity was compared with the effect of the humanized monoclonal anti-IgE antibody Omalizumab (OmAb). Change on FcepsilonRI surface expression and bound-IgE were measured by flow cytometry. Besides, serum sFcepsilonRI levels from defined food allergic patients from different regions were measured by ELISA. **Results:** sFcepsilonRI expression in the supernatant increased in a dose-dependent manner upon cIgE (5-30µg/mL) plus NP-OVAL (1-100µg/mL) stimulation. The blocking capacity of sFcepsilonRI reached 57-63% of inhibition in cIgE-FcepsilonRI binding, showing a similar effect to OmAb. There is a high positive correlation between total and complexed-sFcepsilonRI measured in patient’s serum, confirming that sFcepsilonRI maintains its binding affinity. **Conclusions:** sFcepsilonRI maintains the high IgE-binding affinity and inhibits IgE-FcepsilonRI binding. Thus sFcepsilonRI could be an important player in the complex signalling pathway of the allergic response.

P25 Construction of a phage display library from Escherichia coli to study IgE-reactive bacterial antigens.

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Background: We have found that up to 25% of patients suffering from atopic dermatitis (AD) display IgE reactivity against a variety of antigens from E. coli. This finding is unusual because E. coli is known as a natural, tolerogenic constituent of the human microbiome, and exposure to bacteria has been reported to have anti-allergenic activity in various studies. **Methods:** We have investigated by IgE inhibition experiments whether IgE reactivity to E. coli antigens is due to cross-reactivity with Staphyloccocus aureus or Malassezia sympodialis, which are frequent skin pathogens in AD. However, no evidence for relevant cross-reactivity was obtained. To identify the IgE-reactive components of E. coli, total genomic DNA was extracted from a commensal strain ATCC25922 and randomly fragmented by enzymatic restriction. Generated DNA fragments were ligated into a T7 phage vector to generate a T7 phage display library of E.coli genomic DNA. The constructed

library is currently being screened by biopanning with sera of AD patients, to identify IgE binding clones.

P26 Parvalbumin from Atlantic cod interacts with plasma membranes of intestinal and bronchial epithelial cells and induces differential gene expression of cytokines

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Background: Fish allergic patients experience rapid and severe IgE-mediated reactions following allergen exposure via gastrointestinal or respiratory routes. Food matrix components may contribute to the immune response. The role of epithelial cells in allergic sensitization and reaction is not well understood. We explored interactions of intestinal and bronchial epithelial cells with the major Atlantic cod allergen Gad m 1, with or without codfish-derived food matrix. **Methods:** Caco-2 and 16HBE14o- cells were used as in vitro models for human intestinal and bronchial epithelial cells, respectively. Confluent, polarized cells were treated with Gad m 1 with or without codfish-derived food matrix. Fluorescently labelled allergen was detected by confocal microscopy. mRNA levels of IL-6, IL-8 and TSLP were determined by qRT-PCR. **Results:** In Caco-2 cells, Gad m 1 bound to the apical plasma membrane, while in 16HBE14o- cells it localized to the lateral membrane domain (bellow ZO-1 level). These interactions were not influenced by codfish matrix. In both cell lines, treatments with Gad m 1 and codfish matrix induced differential gene expression of explored cytokines. **Conclusions:** Major codfish allergen Gad m 1 interacts with plasma membranes of intestinal and bronchial epithelial cells, but is not internalized. This interaction changes gene expression patterns of explored cytokines in the cells which is further modulated by food matrix components. Therefore, not only the allergen, but also the food matrix may contribute to allergic sensitization and reaction. *Supported by the Austrian Science Fund grants SFB 4608 and 4613 and the doctoral program W1248-B1.*

P27 Hypoallergenic Bet v 1.0101-Mannan neoglycoconjugates are highly immunogenic when applied via laserporated skin

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Background: Due to its unique immunological properties, the skin is an attractive target tissue for allergen-specific immunotherapy. However, transcutaneous immunotherapy requires formulations with low allergenic potential. The feasibility of efficiently targeting APCs through C-type lectin receptors has been recently demonstrated. Here, we coupled the major birch pollen allergen Bet v 1.0101 to mannan from S. cerevisiae and immunized mice with the conjugates via laserporated skin. **Methods:** Mannan was coupled to Bet v 1.0101 via mild periodate oxidation. Uptake of the neoglycoconjugates by APCs and their capacity for IgE crosslinking were investigated in vitro. Following immunization of mice via laserporated skin, immunogenicity and cytokine profiles were assessed. **Results:** Bet v 1.0101 glycoconjugates displayed up to 1000-fold reduction of IgE crosslinking capacity compared to the uncoupled allergen. In vitro uptake experiments using BMDCs revealed an increased uptake for Bet-Mannan neoglycoconjugates compared to free protein. Mice immunized transcutaneously via laserporated skin with Bet-Mannan neoglycoconjugates showed higher IgG1 and IgG2a antibodies than the ones immunized with the protein alone. Bet-Mannan neoglycoconjugates shifted the immune response towards a Th1/Th17 pattern evidenced by large amounts of IL-17 and IFN gamma secreted by Bet v 1.0101 re-stimulated splenocytes isolated from immunized mice. **Conclusions:** Coupling mannan to Bet v 1.0101 represents an attractive approach for allergen-specific cutaneous immunization. Future work has to be done to assess the therapeutic potential of these glycoconjugates in therapeutic and prophylactic models of allergy.

Inflammation

P28 Osteopontin modulates the number of T cells in adipose tissue of obese individuals.

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Background: Obesity results in a chronic low grade inflammation which contributes to cardio-metabolic diseases such as type 2 diabetes. Osteopontin (OPN) which is highly upregulated in obesity is rendered more active by proteolytic cleavage and acts as a driver of this inflammatory response. **Methods:** In this study the correlation between OPN and T cells in omental fat of obese individuals was analyzed by RT-PCR. An adhesion assay was set up to monitor the adhesion of fluorescently labeled human T cells to OPN coated on V-shaped wells. The viability of human T cells upon OPN stimulation was analyzed by measuring ATP levels and apoptosis was determined by Annexin V staining. **Results:** OPN strongly correlated with the pan T cell marker CD3 in adipose tissue of obese individuals. Furthermore, there was a significant correlation between CD8 and OPN as well as GATA3 and CD8. The adhesion of T cells to cleaved forms of OPN could be blocked with monoclonal anti-OPN antibodies and with sera of mice vaccinated with OPN-peptides. Additionally, full length and cleaved forms of OPN promoted the survival of T cells. **Conclusion:** In summary, there is a strong correlation between T cells and OPN in adipose tissue of obese individuals. The interaction between OPN and T cells can be specifically blocked. OPN affects the number of T cells in adipose tissue by acting as a prosurvival factor. Targeting OPN may pose a promising approach to reduce adipose tissue inflammation to prevent cardio-metabolic diseases.

P29 Interplay between Farnesoid X Receptor (FXR) and Epidermal Growth Factor Receptor (EGFR) during Hepatic Injury

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Background: BA-activated nuclear receptor FXR has anti-inflammatory properties. EGFR activation in hepatocytes promotes chemotaxis by upregulating CxCL5 and CxCL8. Therefore, we aimed to investigate whether anti-inflammatory effects of FXR counteract EGFR-mediated chemotaxis in hepatocytes. **Method:** RT-QPCR was used to assess hepatic expression of EGFR, inflammatory and fibrotic markers in LPS-injected mice and common bile duct ligated (CBDL) WT and EGFR Albumin Cre mice (EGFRLiverKO). Serum biochemistry was also assessed. In vitro studies were performed in HepG2 cells. **Result:** Compared to controls, EGFR mRNA expression was induced 12.8fold in livers of LPS-treated mice ($p<0.05$) and 2.4fold in CBDL mice ($p<0.05$). In line, in vitro findings showed that mRNA levels of EGFR and TNFα increased by 2.1fold ($p<0.05$) and 2.2fold ($p<0.05$), respectively, in HepG2 cells upon LPS stimulation. EGFRLiverKO mice showed a tendency for reduced cholestatic liver injury from CBDL than WT mice, as reflected by trends for lower serum levels of ALT (1634,67 vs 2078,00 U/L) and AST (3898,00 vs. 5502,00 U/L). Loss of EGFR also reduced Collagen1a1 mRNA expression by 46% in EGFRLiverKO mice compared with WT mice after CBDL ($p<0.05$). In vitro, FXR activation (via obeticholic acid) suppressed mRNA expression of EGFR by 43% and its downstream target CxCL5 by 52% after EGF treatment in HepG2 ($p<0.05$). **Conclusions:** In vivo, EGFR may promote inflammation and fibrogenesis during cholestasis. FXR activation may suppress gene expressions of EGFR and its downstream target CxCL5. However, it needs to be further tested if FXR activation may repress EGFR-mediated chemotaxis and inflammation in vivo.

P30 Identification of a microRNA that regulates monocyte-derived dendritic cells.

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Background: MicroRNAs are an important class of gene regulators that post-transcriptionally moduate gene expression and influence cell fate and function. While much is reported on its role in directing lymphoid cell development and activation during hematopoiesis, not much is known on how microRNAs affect dendritic cell differentiation and function. **Methods and Results:** We performed an array screen for microRNAs differentially expressed by human myeloid DC subsets. miR-181a/b was among the strongest regulated miRNAs in this screen. Using methods to lentivirally knock down or over-express miR-181a/b in human CD34+ monocytic precursors and in blood monocytes, we show that DC-SIGN+ monocyte-derived dendritic cells (moDCs) are positively regulated by this miRNA. DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3 grabbing non-integrin) is a C-type lectin pathogen binding receptor that is best known for its ability to bind HIV-gp120 with high specificity. In line with its down-regulation of DC-SIGN, moDCs which have miR-181a/b knocked-down also bind less to HIV-gp120. In vivo, murine moDCs marked by the expression of DC-SIGN arise after T cell activation or inflammatory stimuli (LPS or superantigen) and accumulate in T cell areas of lymph nodes. These cells are strongly diminished in miR-181a1b1a2b2 knock out mice. **Conclusions:** Hence, we show for the first time that a microRNA can regulate the development of moDCs marked by DC-SIGN which arise during inflammation not only in vitro but also in vivo.

P31 Systemic metabolic defects caused by epidermal EGFR-deficiency

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The epidermal growth factor receptor (EGFR) is an important regulator of epidermal function and homeostasis. Epidermal deletion of EGFR leads to severely perturbed skin differentiation and causes reduced animal growth and lethality during the first three weeks of life. The molecular cause why animals lacking EGFR in the epidermis die soon after birth is still unclear. It is known that these animals develop a severe skin inflammation and a skin barrier defect; however both of these pathological features become apparent only after mice lacking EGFR in the epidermis already show reduced growth and weight gain. In order to better understand the growth defect observed in these mice, metabolic parameters have been started to be analyzed revealing that epidermal loss of EGFR-signaling results in severely perturbed glucose metabolism and insulin levels in the blood of affected animals. Importantly, gene expression of metabolic regulators in livers of mice lacking EGFR in the epidermis indicates that glucose metabolism is deregulated already a few days after birth. In addition, the “starvation-marker” FGF-21 is highly expressed in these animals. To test whether excessive feeding protects animals with defective epidermal EGFR signaling from death, these mice were crossed in a leptin-deficient background and found that this fully rescues the lethality. Furthermore, animal growth as well as glucose and insulin levels are improved in such animals, demonstrating that the lethality caused by epidermal EGFR-deficiency stems from impaired food metabolization. We are currently searching for molecular mediators of the metabolic dysfunction of mice lacking EGFR in the epidermis. These findings will provide new insights into the complex consequences of epidermal Egfr-deficiency, which might also be relevant for cancer patients treated with Egfr inhibitors. Furthermore, results obtained by this study will lead to a better understanding of the role of the skin in the regulation of systemic metabolism.

P32 The Role of Plasmacytoid Dendritic Cells in Imiquimod Induced Skin Inflammation and Melanoma Clearance in Mice

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Imiquimod (Imi) is an agonist of toll like receptor 7/8 (TLR7/8), a pathogen recognition receptor that recognizes single stranded RNA. Imi exerts therapeutic anti-viral and anti-tumor effects in both mice and humans. Therapeutically, Imi is applied topically as a 5% cream formulation under the trademark Aldara. Previously, our group showed that Imi treatment leads to tumor clearance in a mouse model of melanoma. We showed that the anti-tumor effect of Imi is accompanied, among others, by the accumulation of plasmacytoid dendritic cells (pDCs). We could furthermore show that Imi activated pDCs acquire tumor killing effector properties by upregulating the cytolytic molecules TRAIL and granzyme B. By employing a transgenic mouse model to specifically deplete pDCs, we demonstrated that pDCs are crucial for the tumoricidal properties of Imi. In search for the molecular pathways conferring tumor-killing activities to Imi-stimulated pDCs, we found that pDC infiltration to Imi treated skin requires the chemokine CCL2. Thus, current studies are addressing the anti-tumor efficacy of Imi in CCL2-/- mice. Albeit the important effects of Imi in tumor immune biology, we and others have shown that repeated topical application of Imi on murine skin leads to skin inflammation and is used as an established mouse model of psoriasiform dermatitis. While addressing the function of pDCs in this process, we found that pDCs exert regulatory properties during Imi induced skin inflammation. Current studies are aimed at elucidating the mechanism by which pDCs modulate the severity of Imi mediated skin inflammation.

Neurobiology

P33 Firing Patterns of Distinct Types of Neuron in Prefrontal Cortex during Working Memory and Cognitive Flexibility

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Background: Prefrontal cortex plays role in content-dependent and goal-predictive behaviour, decision-making, spatial working memory, guiding or inhibiting future responses and cognitive flexibility. Distinct types of neuron contribute differentially to network activity to establish a spatiotemporal division of labour. Across and within different cortical layers, neurons have diverse projection profiles, with unique axonal and dendritic arborisations, expressing different transcription factors and ultimately serving different network operations. However, it remains elusive which types of excitatory and inhibitory neuron play a role in driving certain behaviour. **Methods:** We carry out single and large scale multiple unit recordings in freely moving rats during a working memory and rule switching task with juxtacellular technique, tetrodes, and silicon probes. **Results:** During the working memory task, prefrontal assemblies consisting of pyramidal cells as well as interneurons provide neuronal representations of the task sequence as well as for future choice. After a rule switch, the firing of these neurons change significantly and simultaneously leading to new cell assemblies. **Conclusion:** The diverse changes of firing patterns upon rule switch might be related to the existence of distinct types of pyramidal cell in the prefrontal cortex. By recording and juxtacellularly labelling these cells during the rule switching task, we aim to understand the diversity and interactions of neurons and reveal how they contribute in the neuronal circuitry to drive working memory and cognitive flexibility.

P34 Kv7 channels: Potential targets for antinociceptive action of Paracetamol

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Background: Paracetamol/ Acetaminophen (APAP) is a widely used analgesic whose mechanism of action remains controversial. Enhancement of currents through Kv7 potassium channels in dorsal root ganglion (DRG) neurons reduces excitability thereby providing analgesia. Therefore, effects of APAP and its metabolites NAPQI and AM404 on Kv7 channels were investigated. **Methods:** Currents through Kv7.2, 7.3, and 7.5 expressed in tsA201 cells were recorded using the perforated patch-clamp technique. **Results:** Currents through recombinant homomeric Kv7.2 and Kv7.5 channels were increased by NAPQI up to 250% and 400% of control, respectively, while those through Kv7.3 homomers were decreased down to 40%; both effects were irreversible and concentration-dependent. With Kv7.2/7.3 and Kv7.3/7.5 heteromers, currents were enhanced to 120% and 250%, respectively, by NAPQI concentration up to 3 µM, but depressed at higher concentrations, the effect being again irreversible. In a single point cysteine mutant of Kv7.2 (C492A) currents were enhanced by 3 µM NAPQI as in wild-type channels, but in a triple cysteine mutant of Kv7.2 (CCC150-152AAA) currents were reduced down to 20%, once again in an irreversible manner. Neither APAP itself (1 mM), nor AM404 (10 µM) affected currents through homomeric Kv7.2 and Kv7.3 channels. **Discussion:** These results indicate that the analgesic action of paracetamol may be explained by an enhancement of Kv7 currents by NAPQI as an active metabolite.

P35 Epigenetic modulation of nociceptive transmission

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Background: Chronic pain is a major clinical problem in western countries, and symptoms are often only marginally responsive to currently available medications. Recent studies propose antihyperalgetic effects of epigenetic modulators such as the histone deacetylase inhibitor (HDACi) suberoylanilide hydroxamic acid (SAHA). However, very little is known about the underlying mechanisms. Here, we study the effect of SAHA on synaptic long-term potentiation (LTP) at nociceptive synapses in the spinal cord dorsal horn, a well-established cellular model for pain amplification and some forms of hyperalgesia. **Methods:** To quantitatively assess synaptic strength, we record C-fiber-evoked field potentials in the superficial laminae of the dorsal horn in deeply anesthetized rats. LTP is induced by intra plantar injection of capsaicin or by abrupt withdrawal of the opiate remifentanil. To assess the effect on induction and maintenance of synaptic LTP, SAHA is applied systemically. **Results:** C-fiber-evoked field potentials are stable throughout a minimum recording period of at least four hours. The injection of capsaicin but not its solvent induced LTP at C-fiber synapses. Similarly, withdrawal from brief systemic remifentanil potentiated C-fiber-evoked field potentials in the superficial spinal cord dorsal horn.**Conclusions:** In the normal spinal cord, capsaicin injection as well as acute withdrawal from remifentanil induced potentiation of C-fiber-evoked field potentials. Ongoing experiments will reveal the effects of the epigenetic modulator SAHA on normal and enhanced nociceptive transmission. The therapeutic potential of this drug will be assessed in behavioural experiments.

Cancer

P36 Inhibition of human liver-cancer-cell tumorigenicity and metastasis by treatment with monoclonal antibodies against thrombin- and mmp-cleaved osteopontin

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Background: Hepatocellular carcinoma (HCC) is the most common form of liver cancer and is the cause of approximately 1 million deaths yearly. Osteopontin (OPN) is involved in promotion of cancer cells by regulating various facets of tumor progression such as cell proliferation, invasion, angiogenesis and metastasis. Overexpression of OPN has been found in a variety of cancers, including liver cancer. While OPN´s isoforms and their blockades are well investigated in cancer research, little is known about OPN´s cleavage products. Since MMPs and thrombin have been observed at the tumor site in several animal models, and since it has been shown that these cleavage products can be even more active than the full length OPN, we hypothesize that the treatment with our in-house produced antibodies against mmp- and thrombin-cleaved OPN will have a negative effect on the cancer-cell-line tumor progression and/or metastatization in human xenograft models. **Methods:** three strong-OPN-expressing and one OPN-non-expressing human liver cancer cell lines will be selected via qRT-PCR analyses and used in the further xenograft models of tumorigenicity and metastasis. In both studies, four treatment groups will be defined: CMIP005 21-5-4 (against thr-cOPN), CMIP003 9-3 (against mmp-cOPN), IgG CTRL, PBS. Tumor growth and establishment of micro-metastases in distal organs are the primary and secondary end points, respectively. **Conclusions:** the data obtained will elucidate whether the cleaved forms of OPN play a role in the pathophysiology of liver cancer and if the immunotargeting of them can be an effective therapy for HCC.

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P37 CD18 expression in chronic lymphocytic leukemia is regulated by DNA methylation-dependent and -independent mechanisms

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Background: The pathophysiology of chronic lymphocytic leukemia (CLL) strongly depends on interactions of CLL cells with the microenvironment, from which they receive pro-survival, activation- and proliferation-inducing signals. Normal lymphocyte recruitment to and retention within lymph nodes is tightly regulated by the integrin LFA-1 (CD11a/CD18). Despite this key role in immunology, little is known on the regulation of LFA-1 transcription. We previously found that the majority of CLL cells expressed reduced surface LFA-1 and identified CD18 as the rate-limiting subunit on protein and mRNA level. Here we investigated whether LFA-1 transcription can be regulated by DNA-methylation and if LFA-1 expression can be influenced by microenvironmental signals in CLL. **Methods:** Primary CLL PBMCs were co-cultured with murine fibroblasts and stimulated with IL2/CpG to induce CLL proliferation. Proliferative and non-proliferative fractions of viable CLL cells were sorted for subsequent DNA-methylation analysis by bisulfite conversion, nested PCR, cloning and sequencing. **Results:** High basal LFA-1 levels correlated with unfavorable prognostic markers CD38, CD49d and trisomy 12. In the latter subgroup, this was accompanied by an unmethylated CD18 gene promoter. Higher CD18 protein levels correlated with lower levels of promoter methylation in all samples. Upon co-culture CD18 expression increased in activated and proliferating CLL subfractions, however not due to promoter demethylation. **Conclusions:** Our data indicate DNA-methylation responsible for higher basal LFA-1 levels in trisomy 12 CLL. However, this mechanism has no causative role in the up-regulation of LFA-1 expression upon CLL cell activation. Further investigations will allow identification of alternative mechanisms regulating LFA-1 expression in CLL.

P38 A PAK2-STAT5 axis is key for tumor formation of BCR-ABL+ cells in vivo

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Background:STAT5 is an essential transcription factor required for disease initiation and disease progression by BCR-ABL oncogenes. STAT5 regulates survival, proliferation, and therapeutic responses in chronic myeloid leukemia (CML) and B acute lymphoblastic leukemia (B-ALL). We could previously show that – beside the well described tyrosine phosphorylation site on Y699 – a mutation of a serine to alanine (S779A) significantly increases diseases latency in BCR-ABL-mediated B-ALL showing the importance of this phosphorylation site. Subsequently, we identified PAK1 and PAK2 as upstream kinases of S779 phosphorylation. Therefore, we here aimed to investigate the role and potential differences between PAK1 and PAK2 in BCR-ABL+ cells.**Materials and methods:**Knockdown of PAK1 and/or PAK2 was performed in human BCR-ABL+ cells, and differences in cell cycle and growth were assessed in vitro. In addition, we injected the cells subcutaneously into immunocompromised mice and monitored tumor growth. **Results:**No significant differences in cell cycle characteristics were found in vitro upon single knockdown of PAK1 or PAK2, whereas knockdown of both PAK1 and PAK2 was incompatible with cell survival. Interestingly, tumor volume and tumor weight were drastically reduced when PAK2 was knocked down while PAK1 knockdown had no effect in vivo. **Conclusions:While** PAK1 and PAK2 compensate each other for growth and survival of BCR-ABL+ cells in vitro, only PAK2 is required for survival and growth in vivo.

P39 Proteome- and Phosphoproteomeanalysis of oncogenic signaling pathways

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Background: The wholistic approach of functional proteomics using HPLC-MS based techniques has become one of the most important research tools to investigate the protein and phosphoprotein composition of cells and especially tumor cells. Since phosphorylation serves as an important control mechanism, phosphoproteomic approaches are used to identify phosphorylated proteins involved in signaling cascades such as the tumorigenic signaling of the Hedgehog (Hh) pathway. Within this approach we are focussing on the molecular interplay of kinases and phosphatases involved in GLI code signaling using specific pathway activators and kinase inhibitors.

Methods: After treatment, human medulloblastoma cells (DAOY cells) are lysed by using ultrasonication and the protein content is isolated by precipitation and centrifugation. The intact proteins are reduced and alkylated before proteolytic digestion. For phosphoproteomics additional enrichment steps such as weak cation-exchange chromatography (WCX), electrostatic repulsion hydrophilic interaction liquid chromatography (ERLIC), and metal oxide affinity chromatography (MOAC) are performed. Thereafter ion-pair reversed-phase high-performance liquid chromatography -mass spectrometry (IP-RP-HPLC-MS) is used to characterize the protein composition. Peptide identification is performed by tandem mass spectrometry (MS2) using data dependent acquisition. Data analysis and interpretation is performed using the proteome discoverer along with the ingenuity pathway analysis software.

Expected results: The comprehensive view provided by proteomics and phosphoproteomics should help to better understand the mechanisms of action involved in the function of the GLI proteins as a zinc-finger transcription factor. The identification of regulated phosphoproteins that are not yet known to be related to the Hh pathway will enlighten the complexity of the Hh signaling.

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Immunology

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Cancer

P40 EGFR signaling in the interfollicular epidermis controls hair follicle morphogenesis

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Background: The epidermal growth factor receptor (EGFR) is involved in many different cancer types, which led to development of a variety of EGFR inhibitors as cancer therapeutics. However, cancer patients treated with EGFR inhibitors frequently develop acneiform skin toxicities, and hair alterations like trichomegaly and follicle degeneration. **Methods:** We analyzed mice lacking the EGFR in order to study the role of EGFR signaling in hair follicle (HF) development in more detail by using EGFRfl/fl K5Cre mice (delete in interfollicular epidermis (IFE) and outer root sheath (ORS)) and EGFRfl/fl LGR5 Cre mice (delete in ORS). We performed histological and flow cytometric analysis, including FACS based cell sorting of IFE and ORS keratinocytes and RNAseq to identify targets, which contribute to the abnormalities observed in EGFR deficient skin. **Results:** Our results show that lack of EGFR in IFE and ORS results in a delay of HF morphogenesis, which further leads to defective hair layer formation and short and curled hair similar to patients receiving anti-EGFR therapies. Moreover, EGFR-deficient skin show increased DNA damage, reduced survival, and impaired differentiation. Interestingly, additional deletion of p53 from keratinocytes does not rescue the phenotype of EGFRfl/fl K5Cre mice. Lack of EGFR in the ORS only results in a mild phenotype characterized by curly hair. **Conclusions:** Accumulation of DNA damage and subsequent p53-mediated cell death is not the leading cause for the defects in hair morphogenesis. The strong defects only observed when deleting the EGFR in the ORS and IFE together provide evidence that IFE-derived signals contribute to hair abnormalities.

P41 The role of the protocadherin Mucdhl in colorectal cancer

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Protocadherins constitute the largest subgroup of the cadherin protein superfamily and are frequently downregulated in human cancers suggesting a negative role in oncogenesis. The protocadherin Mucdhl is a transmembrane protein that is located in the microvillar brush border of enterocytes, cholangiocytes and kidney epithelial cells. Mucdhl crosslinks microvilli and has been implicated in regulation of beta-Catenin activity. We are interested in Mucdhl functions in colorectal cancer. We found that Mucdhl expression is downregulated in altered crypt foci, adenomas, carcinomas and colorectal liver metastasis. We further demonstrate a tumor-suppressive role of Mucdhl in colorectal cancer using transplantation experiments of cell lines with gain or loss of Mucdhl function. We generated Mucdhl knock-out mice to further investigate Mucdhl functions in autochthonous colorectal tumors. Knock-outs were viable and did not show an overt intestinal phenotype but displayed shortening of microvillus length. Formation of colorectal cancer, induced with the chemical Azoxymethane/Dextransulfate protocol, was not affected in Mucdhl knock-out mice but the number of aggressive carcinomas invading the muscularis mucosa was substantially increased. These data suggest that Mucdhl is a metastasis suppressor gene in colorectal cancer. We are currently using intestinal organoid cultures, cotransfection experiments and RNASeq of RNA, isolated from intestinal epithelial cells, to unravel molecular function of Mucdhl in colon cancer metastasis.

P42 Hedgehog/GLI and Interleukin-6/STAT3 Signal Integration in Basal Cell Carcinoma

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Background: Inappropriate activation of the Hedgehog (HH)/GLI signaling is involved in basal cell carcinoma (BCC) development of the skin, one of the most commonly diagnosed human cancers. Targeting HH/GLI signaling therapeutically is accompanied by severe side effects, rapid development of drug resistance and disease relapse raising the need for improved anti-HH therapy. Identifying synergistic pathway interactions promoting HH-driven oncogenesis may reveal rational combination treatments based on simultaneous inhibition of cooperative oncogenic signals. **Methods:** We applied a small-scale, candidate-based screen for pathways synergizing with HH/GLI in oncogenic transformation. We investigated the functional requirement of cooperative pathways promoting oncogenic HH/GLI signaling by means of genetic and chemical perturbation experiments along with molecular studies addressing pathway interactions. **Results:** We identified the Interleukin-6 (IL6)/JAK2/STAT3 signaling pathway as a novel cooperative signal synergizing with HH/GLI in oncogenic transformation of human epidermal cells and accounting for HH-IL6 target gene regulation and increased oncogenicity. We provide evidence that signal integration occurs at the level of common HH-IL6 target gene promoters. Of note, genetic deletion of Il6ra in the skin of Ptch-deficient mice reduced BCC-like lesions supporting the in vivo relevance of our data. **Conclusion:** The data reveal IL6/JAK2/STAT3 signaling as a novel positive modulator of HH/GLI signaling in cancer and suggest that rationale-based combination treatments relying on the simultaneous inhibition of both pathways may be a promising strategy to target HH-dependent BCC.

P43 The role of STAT1 in colitis-associated colorectal cancer

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Background: The transcription factor STAT1 is activated by interferon (IFN) signaling and promotes immune responses against pathogens and tumor cells. Apart from these functions in the immune system, STAT1 is considered as a tumor suppressor that integrates anti-proliferative and pro-apoptotic functions of IFNs. It can prevent expansion of neoplastic cell types by activating the transcription of genes encoding for caspases, death receptors, death ligands and iNOS. Furthermore, it negatively regulates cell cycle progression via regulation of p21waf/cip1, p27Kip1, c-myc and cyclin genes. However, several studies indicated that STAT1 might also exhibit pro-tumorigenic functions. **Methods:** We investigated the function of STAT1 in colitis associated colorectal cancer and employed mice with conditional inactivation of STAT1 in the intestinal epithelium (STAT1ΔIEC). Colitis was induced by the Dextrane sodium sulfate (DSS) protocol. Body weight loss and the extent of colitis were recorded. Colorectal tumors were induced by the chemical Azoxymethane/Dextrane sodium sulfate (AOM/DSS) protocol and tumor parameters were assessed in male and female STAT1deltaIEC and control mice. **Results:** We show that body weight loss and colitis score are higher in male control mice compared to STAT1deltaIEC male mice. Male control mice also have reduced tumor load and tumor multiplicity. Tumors that occur in these mice are of a lower grade. There are no differences in colitis or colitis-associated tumorigenesis in female mice. Human data also shows that presence of STAT1 in tumor cells confers survival advantage in males only**. Conclusion:** Our studies demonstrate that STAT1 acts as a gender specific tumor suppressor in colorectal cancer of mice and humans.

P44 ^{99m}Tc@DTPA-can225IgG – a new SPECT tracer for EGFR+ tumors in canines.

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Monoclonal antibody therapies are well established in clinical oncology, however, only 2 out of 19 FDA-approved anti-tumor antibodies are labeled with a radionuclide even though this allows detection of primary and distant metastatic lesions via SPECT or PET, as well as precise radiotherapy of them. To promote radioimmunotherapy of solid tumors, this study exploits the canine recombinant anti-EGFR IgG antibody „can225“ as a lead compound for comparative diagnostic studies in canine patients.

Can225IgG was labeled with DTPA and subsequently radiolabeled with ^{99m}Tc. Stability of ^{99m}Tc@DTPA-can225IgG was monitored in various buffers and in canine cancer patient serum over 4h, in order to simulate transport and in vivo conditions. Binding to human and canine EGFR was evaluated by

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immunoblots and autoradiography of canine mammary carcinoma sections. Specificity to recombinant and cell surface-expressed EGFR was retained in DTPA-can225IgG and ^{99m}Tc@DTPA-can225IgG. The antibody conjugate was stable in isotonic NaCl, TBS and NaOAc buffers for up to 4 hours, however, specificity of the antibody decreased upon exposure to acidic pH in 0.9% NaCl. Furthermore, the compound ^{99m}Tc@DTPA-can225IgG was also stable in sera of 5 canine mammary carcinoma patients. Antibody binding to EGFR+ tumor sections could be confirmed by autoradiography.
Based on our data we conclude that our lead compound ^{99m}Tc@DTPA-can225IgG passed the in vitro evaluation of specificity and stability. It is thus ready for in vivo studies in xenograft mouse models as well as, due to its canine origin, in dog cancer patients.

P45 Cell-type specific role of EGFR in liver diseases

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The Epidermal Growth Factor Receptor (EGFR, also known as ErbB1) is a member of the ErbB family of tyrosine kinases. EGFR is highly expressed in the adult liver and has been proposed to play an important role during liver development, function and regeneration. Our group has previously shown in a study of partial hepatectomy that EGFR is a critical regulator of hepatocyte proliferation in the initial phase of liver regeneration and that EGFR plays a protective role in hepatocytes. Surprisingly, in a study of toxic liver fibrosis, we did not observe any histopathological differences in the absence of EGFR. Furthermore, EGFR overexpression was described in 40-70% of human hepatocellular carcinomas. In a recent study, we discovered that EGFR is expressed in Kupffer cells/liver macrophages and that presence of EGFR-positive liver macrophages in HCC is associated with poor patient survival. Therefore, we are currently investigating the role of EGFR in immune-mediated models of liver injury.

P46 Signaling and processing of Helicobacter pylori CagA in B cells

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Background: CagA is the main pathogenic factor of the human pathogen Helicobacter pylori (Hp), which is strongly associated with gastric cancer and MALT lymphoma. CagA is translocated into gastric epithelial cells where it undergoes tyrosine phosphorylation and interferes with cellular function. Since lymphocytes are stimulated during inflammation in the gastric mucosa, Hp is also able to translocate CagA directly into B cells. However, less is known about the pathogenic mechanism of Hp and the connected signaling of CagA in B cells. **Methods:** We established MEC1 cells (B-cell line) as a new infection model to study the interaction between Hp and B cells. In kinetic experiments, MEC1 cells were infected with Hp wildtype and a cagA-deletion strain. Injection of CagA and activated non-receptor tyrosine kinases were analyzed by Western blotting. To investigate CagA cleavage, various truncated CagA constructs were used in overexpression experiments. **Results:** We observed that CagA is injected and strongly phosphorylated after infection with Hp. To determine upstream signaling that led to the phosphorylation of CagA in MEC1 cells, specific inhibitors were used to target Src and Abl family kinases. We observed that both kinases need to be inhibited to abolish CagA phosphorylation. Furthermore we could show that CagA is specifically cleaved between Asparagine residues into distinct fragments, whose functions are completely unknown. **Conclusion:** We could provide a suitable infection model to further investigate the complex signaling network with CagA as a key molecule in B cells, which is crucially important to understand the Hp mediated gastric diseases such as gastric MALT lymphoma.

P47 The role of Epidermal Growth Factor Receptor in c-Fos-dependent osteosarcoma formation

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The Epidermal Growth Factor Receptor (EGFR) is overexpressed or mutated in human carcinomas and glioblastomas, which are tumors of epithelial and glial origin, respectively. Recent studies from our laboratory using EGFR knockout mice (Egfr-/-) have also shown that EGFR plays a role in bone development and osteoblast function. In analogy, it is therefore likely that EGFR is also involved in the development of bone tumors and few publications have indeed reported EGFR overexpression in human osteosarcomas.
Here we show that Egfrf/f Runx2-Cre mice (EgfrΔOb) which lack the EGFR in osteo-chondroprogenitor cells develop an increased zone of hypertrophic chondrocytes in long bones resulting in impaired bone formation. When bred to c-fos transgenic mice (H2-c-fosLTR) that develop osteosarcomas with 100% penetrance, EgfrΔOb mice show reduced tumor incidence and burden. On the molecular level, tumors from EgfrΔOb mice exhibit decreased c-Fos and Cyclin D1 levels. In vitro experiments in primary bone tumor cells isolated from H2-c-fosLTR mice further show that EGFR inhibition leads to reduced c-Fos mRNA and Protein expression. Taken together our data suggest an essential role of EGFR signaling during both development and progression of c-Fos-dependent osteosarcomas.

P48 Functional alterations of homing receptors in chronic lymphocytic leukaemia by tissue-specific factors and therapy

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Background: Chronic lymphocytic leukaemia (CLL) is characterized by the accumulation of malignant CD19+/CD5+ B cells. Survival and proliferation of CLL cells is highly dependent on the lymphoid tumour microenvironment. Therapeutic targeting of the interactions between CLL cells and the supportive microenvironment is one of the most promising approaches to control the disease. Therefore, investigating the process of homing, a highly regulated mechanism involving a number of receptors, is of utmost importance. Eμ-TCL1 mice are the most widely used murine model, developing a disorder resembling the human CLL. Here, we started to investigate the alterations of major homing receptors on CLL and T cells from TCL1 transgenic mice, compared to wild type controls. **Methods:** We used flow cytometry to determine the expression of the most important homing receptors (CXCR4, CCR7, L-selectin, VLA-4 and LFA-1) on T cells, CLL cells or normal B cells, from TCL1 transgenic or wild type mice. **Results:** Homing receptors seem to be differentially expressed in the TCL1 transgenic and wild type mice. **Conclusions:** Changes in the expression of homing receptors in CLL cells have been extensively studied in the human disease, but remain unrevealed in the TCL1 mouse model. By investigating the homing receptors in the murine disorder, we are one step closer to establishing an appropriate mouse model in clinical practice. In the future, we also aim to explore changes in the expression of homing receptors during the progression of the disease, upon (serial) transplantation and following treatment with well-established therapies.

P49 Lysyl Oxidase-Catalyzed Stabilization of Proteins

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The human lysyl oxidase is a copper-dependent amine oxidase localized in the extracellular matrix (ECM). LOX catalyzes the covalent crosslinking of tropocollagen and tropoelastin fibers by oxidizing specific lysine residues in vivo. The resulting peptidyl aldehydes spontaneously condense with nearby epsilon-groups of peptidyl lysines or other aldehydes, which leads to the formation of intra- and intermolecular covalent crosslinks. These crosslinks stabilize polymeric collagen and elastin fibers in the ECM, thereby determining the elastic and mechanic characteristics of the ECM. Several pathological processes have been associated with the dysregulation of LOX expression, mostly characterized by a disequilibrium between the synthesis / repair and the destruction of the ECM, e.g. in tumor progression and metastasis. Despite intense efforts, the 3D structure of LOX has remained elusive. Therefore this study will concentrate on the expression and purification, as well on the biochemical and structural characterization of human LOX to close this knowledge gap, which is inevitable to further elucidate structure / function relationships in order to be able to gain a better understanding of the mechanisms involved in pathogenesis. This will provide a basis for the promotion of research in LOX for biotherapeutic and biotechnological applications.

Vascular Biology

P50 Platelet RNA profile changes after in vitro exposure to E. coli

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Background: The contribution of platelets to hemostasis as well as to immune processes makes them essential for the immune and the vascular system. Several studies have shown that platelets are able to interact with E. coli, whereby platelets can get activated and consequently trigger thrombotic events. The extent of platelet activation by bacteria is not only influenced by the bacterial strain but also seems to depend on the individual platelet make up. **Aims:** Our aim is to study platelet responses following exposure to E. coli bacteria. We are focusing on understanding the common as well as the individual changes in the RNA expression profiles of different donors. **Methods:** Citrated blood of healthy donors was used to manually isolate platelets by OptiPrep density gradient centrifugation. The washed platelets were incubated with the well characterized E. coli K12 strain in 1:5 platelet-bacteria ratio. Platelet RNA was isolated applying the Trizol method, followed by cDNA preparation using the SMARTer RNA-Seq Kit. A quality control of the cDNA amplification was performed on Bioanalyzer 2100. The Hiseq 2500 Illumina platform was used for RNA sequencing. Data analysis was carried out with R, edgeR and MISO (Mixture of Isoforms model) for the splicing quantification. **Results:** The data quality allows us to accomplish the detailed data analysis, which is currently in progress. From the preliminary results we could already identify up- and downregulated groups of genes and changes in RNA splicing. We will be able to present the conclusive data on the poster.

P51 Resolution of venous thrombosis is impaired in the absence of IgM

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Background: Venous thromboembolism (VTE) is a major health problem with an annual incidence of 0,75 to 2,69 per 1000 individuals in the general population. Recurrence or non-resolution occurs in up to 25% of cases. Thrombus persistence can lead to chronic thromboembolic pulmonary hypertension (CTEPH) or post-thrombotic syndrome (PTS). It is unclear which mechanisms underlie thrombus non-resultion. Splenectomy is a risk factor for CTEPH and was also shown to delay venous thrombus resolution in mice. Besides acting as a filter, the spleen plays a role in B cell maturation and is required for the maintenance of peritoneal B1a cells, which spontaneously secrete IgM. These natural antibodies are protective in atherosclerosis, and might also promote thrombus resolution. **Methods:** To address the effect of IgM on venous thrombus resolution, we employed a mouse model of stagnant flow venous thrombosis. Mice deficient in secreted IgM (sIgM-/-) and wildtype controls (sIgM+/+) were subjected to subtotal ligation of the inferior vena cava (IVC). 3, 7, 14 or 28 days after IVC ligation, mice were sacrificed to harvest thrombi. In addition, we used high-frequency ultrasound to measure thrombus size. **Results:** Thrombi harvested from sIgM-/- mice 3 days after IVC ligation were significantly smaller than thrombi harvested from sIgM+/+ littermates. In the further course of the experiment, sIgM-/- mice were characterized by bigger thrombi than their wildtype littermates, indicating delayed thrombus resolution. **Conclusion:** Our experiments suggest an important role for natural IgM in venous thrombus resolution.

P52 Interaction of platelets with dendritic cells

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Background: The essential role of platelets in haemostasis is well-established and undisputed. However, it is increasingly evident that platelets also possess other functions. They have been shown to contribute to inflammation, immune functions, lymphatic development, tissue regeneration as well as various pathological conditions such as atherosclerosis and tumour growth. Platelets communicate with various cell types, of which neutrophils, monocytes and endothelial cells have been studied most intensively. However, the relationship with dendritic cells remains under-explored. Indeed, a handful of studies indicates that there is both cell-to-cell interaction and interaction with soluble components. Up to now, the findings are controversial and incomplete. **Methods:** Washed platelets are either activated by TRAP or ADP or inhibited by theophylline and adenosine. Immature dendritic cells are differentiated from blood monocytes by culture with IL-4 and GM-CSF. The two cells types are co-incubated in an allogeneic culture for 2 days, with or without LPS. **Results:** Relative changes in the expression of markers on both platelets and dendritic cells are quantified by flow cytometry. Ongoing experiments will also determine the changes in release of cytokines known to effect the polarization of T helper cells by dendritic cells. **Conclusion:** We plan to elucidate whether the interaction of platelets with dendritic cells influences the polarization of T cells via interaction with dendritic cells. Future experiments will also aim to determine if platelets are capable of shuttle antigens to dendritic cells, as it has been previously shown in mice.

P53 NDSK-II domain of fibrinogen is one of the gatekeepers of thrombus resolution

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Background: Recent data suggest that thrombus resolution is driven by leukocyte recruitment and thrombus angiogenesis. An effective inhibition of leukocyte transmigration in vitro is mediated by naturally occurring peptide $B\beta_{15-42}$, which is a competitive inhibitor of the interaction between fibrin fragment N-terminal disulfide knot (NDSK)-II and vascular endothelial cell cadherin (VE-cadherin). To understand the contribution of leukocytes to thrombus resolution, we investigated the effect of $B\beta_{15-42}$ (FX06) in a murine stagnant flow venous thrombosis model. **Methods:** Study groups of 8-12 weeks old C57/BL6 mice were injected i.p. over various time periods with $B\beta_{15-42}$ or saline. Thrombus was induced by subtotal inferior vena cava (IVC) ligation. The effect of FX06 on endothelial cell sprouting was investigated. $B\beta_{15-42}$ concentration was investigated in chronic human thrombi by Western blotting. **Results:** FX06 delayed thrombus resolution after IVC ligation. Thrombi of all treated groups were larger than controls. We observed a significant decrease of macrophage recruitment into thrombi, and diminished microvessel density. $B\beta_{15-42}$ had no significant effect on endothelial proliferation and sprouting *in vitro*. First measurements of $B\beta_{15-42}$ in red clot of human cases of chronic thrombosis indicate higher concentrations compared with control thrombus. **Conclusion:** Our data suggest that $B\beta_{15-42}$ misguides thrombus resolution by inhibiting VE-cadherin mediated leukocyte migration. The data point to the importance of leukocytes in vascular remodeling after thrombosis.

Immunology

P54 Identification of monogenic causes of chronic early-onset diarrhea using next generation sequencing

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Background: Chronic diarrhea during infancy can be a manifestation of a variety of underlying conditions, including early-onset inflammatory bowel disease (EO-IBD) or non-inflammatory diseases such as congenital chloride diarrhea. Some of these conditions can be attributed to single gene defects as evidenced recently with the identification of human IL10-receptor deficiency. Our laboratory focuses on the identification of the monogenic causes for such disorders using high-throughput genomic approaches such as next generation sequencing (NGS) **Method:** We here studied a patient from a consanguineous background presenting with an early-onset chronic non-mucoid and non-bloody diarrhea. Affymetrix SNP Array 6.0 was used for homozygosity mapping. Exome capture was done using Illumina Nextera Rapid Capture Exome Kit and high-throughput sequencing was performed on Illumina HiSeq 2000. Results: We identified a novel nonsense mutation in the gene diacylglycerol-acyltransferase-1 (DGAT1). The mutation we identified segregated in an autosomal recessive manner. The protein DGAT1 is involved in the terminal step of triglyceride synthesis. A recent report on deleterious biallelic splice-site mutation in DGAT1 causing similar phenotype has been the only report on this disorder to date. We aim to further characterize how defective DGAT1 protein lead to the described phenotype, which is currently poorly understood. **Conclusion:** Our findings illustrate that chronic early-onset conditions affecting the homeostasis of the gut can be monogenic in nature, and exemplifies the need to correctly identify the molecular causes of early-onset diarrhea in order to enable appropriate and timely intervention for the severe clinical phenotypes accompanying chronic diarrhea.

P55 NOD-like receptor expression in human MSCs

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Background: Mesenchymal stem cells (MSCs) are a heterogenic population of stromal progenitor cells which can be isolated from various tissues. MSCs have the potential to differentiate into parenchymal cells of the mesoderm for instance osteocytes, chondrocytes, adipocytes and cells of the connective tissue. In addition, they also have immune-modulatory capacities, which are enhanced in a pro-inflammatory environment. Recently, it has been shown that the immune-suppressive role of MSCs can be modulated by differential stimulation via Toll like receptors. The present study aims at investigating the presence and function of NOD-like receptors (NLRs), another family of pattern recognition receptors, in human MSCs. **Methods:** Screening for NOD-like receptor (NLR) expression of MSCs derived from the white adipose tissue (WAT-MSCs) was performed by means of q-RT-PCR. ELISA, q-RT-PCR and knock down experiments were utilized to investigate the possible functions of specific NLR-members for the anti-inflammatory potential of WAT-MSCs. **Results:** Four out of 22 NLRs are constitutively expressed in WAT-MSCs namely NOD1, NLRP1, NLRX1 and NAIP. Treatment of AT-MSCs with high doses of IFN-gamma resulted in an increased expression of IDO, IL-6, IP-10/CXCL10 as well as NOD1. NOD1-induced expression by IFN-gamma correlated with the expression of IDO and IP-10/CXCL10 over several passages. Furthermore, NOD1 silencing by siRNA contributed to a decrease in IDO and IP-10/CXCL10 expression levels. **Conclusion:** NOD1, NLRP1, NLRX1 and NAIP are stably expressed in WAT-MSCs. The data suggests that NOD1 is connected to IDO and IP-10/CXCL10 expression levels and that NOD1 may be involved in the suppressive activity of WAT-MSCs.

P56 Solute Carriers: Proteins at the Interface of Host Metabolism and Viral Life Cycle

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Background. Host factor requirements for many classes of viruses are yet to be unraveled. Replication of the viral genome and synthesis of viral proteins inside the host cell are associated with altered, often enhanced cellular metabolism and increased demand in nutrients and specific molecules. With some 400 identified members in humans, the solute carriers (SLC) represent the largest family of trans-membrane proteins dedicated to the transport of small molecules, such as amino acids, sugars, nucleotides and ions.Herein we aim to characterize the role of host SLCs in viral replication as well as confirm their function as new regulatory group of proteins in the antiviral immune response. **Results:** Upon integration of the multiple large-scale datasets from recent genome-wide screens, a group of around 20 SLC proteins has been identified to have a function linked to viral replication or immune response. We systematically inactivated these genes in the HAP1 cell line using the CRISPR-Cas9 system. A primary screen performed using the Influenza A/WSN/33 strain suggests that mutations in several of the SLC genes from our pool substantially affect the susceptibility of these cells to infection. We plan to carry on further characterization of the most interesting SLC candidates in order to dissect their role in the viral life cycle. Moreover, we will study the protein-protein interactions of their gene products and will try to identify their natural cargo that may be critical during the viral life-cycle. **Conclusions:** Together, this "viral transportome" may offer new insights into possible strategies to pharmacologically interfere with viral infections.

P57 Immunoregulatory molecules in T cell activation and differentiation

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Background: Immune responses are tightly controlled to maintain immune homeostasis. This is governed by a fine balance between responding effector T cells (Teff) and regulatory T cells (Treg), which can suppress the former. Several molecules can interfere with the activation and differentiation of Teff and Treg cells. Among the most important regulatory molecules found on T cells are CTLA-4 and PD-1, both members of the CD28 family. **Hypothesis:** CTLA-4 limits CD28 signalling and PD-1 limits TCR signalling. Thus these molecules play a significant role in controlling an appropriate balance between Teff and Treg cells, and without them the immune response could turn into a pathologic inflammatory response mediated by autoreactive T cells. **Methods:** We will use two tetracycline-inducible systems to express ovalbumin in the epidermis of transgenic mice. Ova-expression is either driven by Keratin 5 (expressed in the basal cell layer) or involucrin promoter (expressed in the upper cell layers of the epidermis). In these mice we can follow the cellular response of adoptively transferred naive ovalbumin-specific CD4+ T cells (DO11.10) in vivo and the clinical inflammatory response. We will specifically analyse the response of T cells deficient in CTLA-4 or PD-1. **Preliminary results (PhD-thesis started in Oct 2015):** CTLA-4 and PD-1 deficiency leads to non-resolving clinical disease and the absence of CTLA-4 impairs Treg cell differentiation and Treg skin-homing. **Outlook:** We will dissect the biochemical pathways that govern T cell differentiation. Manipulating the Teff-Treg balance may enable us to develop treatment approaches for autoimmune disorders, allergic responses or cancer.

P58 Expression and purification of a folded recombinant surface protein subunit F2 of human respiratory syncytial virus (HRSV)

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Introduction: HRSV is an important causal agent of severe respiratory tract infections in children, elderly and immune compromised persons. There is still no active vaccine available for HRSV and there is a lack of diagnostic tests based on defined viral antigens and epitopes. Although the viral surface protein subunit F2 seems to be the key factor responsible for HRSV host specificity most studies have focused onto the F1 subunit. **Materials and Methods:** The subunit F2 was expressed in E. coli using a codon-optimized synthetic gene as His-tagged recombinant protein and purified by Ni-affinity chromatography. The purity and correct identity of the protein was analyzed by SDS-PAGE followed by Western-blotting using a monoclonal anti-His-tag antibody. Finally, the subunit was characterized regarding physicochemical properties by circular dichroism spectroscopy and MALDI-TOF analysis. **Results and Conclusion:** Recombinant F2 protein subunit could be expressed in good yields and was purified to homogeneity as a folded recombinant protein. This protein should be useful to study virus-host interactions and HRSV-specific immune responses, for the diagnosis of HRSV infections and eventually for the development of new treatment approaches.

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P59 Generation of a monocytic reporter cell line with high sensitivity towards selected toll-like receptor ligands

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Background: Toll-like receptors (TLRs) are triggered by evolutionary conserved pathogen- or stress-associated molecular patterns and constitute a central first-line defence mechanism of innate immunity. Upon ligand engagement, TLRs form homo- or heterodimers and relay signals via distinct cytoplasmic adaptor proteins, like MyD88, culminating in the activation of the transcription factor nuclear factor-κB (NF-κB). **Results:** In the current study, we are monitoring TLR signalling to NF-κB by using THP-1 cells, a well-characterized human monocytic cell line. These cells are widely used in macrophage differentiation studies and are known to express all TLRs (TLR1-9). Here we describe the generation of a THP-1 based reporter cell line, which stably expresses a NF-κB-inducible enhanced green fluorescent protein (eGFP) reporter gene. We studied the sensitivity of the resultant reporter cells for ligands of different TLRs and observed a strong reactivity towards LPS (TLR4), FSL-1 (TLR2/6), Pam3CSK4 (TLR2/1) and MALP2 (TLR2/6). Low picogram amounts of these stimuli could be detected and moreover the eGFP expression level depended on the concentration of TLR-ligands. Although we could confirm expression of all TLR in THP-1 cells, we observed no reactivity towards high concentrations of ligands for TLR3, 5, 7 and 8. **Conclusion:** Our THP-1 reporter cell line is a valuable and highly sensitive tool to measure selected TLR-ligands. There is a wide range of possibilities for its application including the detection of endotoxin contamination in protein preparations and other samples.

P60 Investigation of bio-nano interactions via metabolome and proteome analysis

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Nanoparticles are materials offering specific properties due to their small particle size range from usually 1 - 100 nm. They are present in numerous industrial products such as tyres, sun creams, tooth pastes, or lithium ion batteries. To the current knowledge some of them are toxic, while others are not, which is the reason for the need of risk assessment, performed in case-by-case studies. This can be done by investigating the effects of a specific species of nanoparticles on a cell system or a model organism. In this relation, proteomic and metabolomic profiling represents a promising approach to get an overview of alterations within cells or organisms caused by the exposure to nanoparticles on a molecular level.

In the current study Enchytraeus crypticus serves as model system, which is an ecologically important soil organism due to its activity in decomposition and bioturbation. By exposure to different concentrations of CuO nanoparticles and CuCl2, respectively, for specific periods of time, disparities concerning effects among the treatment conditions themselves, as well as in comparison to the untreated control groups may be found. Metabolome as well as proteome samples were measured and differentially quantified in MS2 experiments using high-performance liquid chromatography-quadrupole-Orbitrap mass spectrometry. Data evaluation was performed using bioinformatic workflows implementing the software tools OpenMS, KNIME, MaxQuant, Perseus and Ingenuity Pathway Analysis to identify significantly regulated proteins, metabolites, and molecular pathways.

P61 Antigen dose defines T cell fate in vivo: effector versus regulatory T cells

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Background: Immune homeostasis is governed by a fine balance of pathogenic effector T cells (Teff) and suppressive regulatory T cells (Treg). In addition of thymic Treg peripherally induced Treg (pTreg) can be generated in response to tissue antigen. However, the mechanisms controlling the generation of pTreg in vivo are still poorly understood. In this project we want to test the Hypothesis that low doses and chronic antigen exposure, favors pTreg generation over effector T cell differentiation. **Methods:** We chose to use two tetracycline-inducible systems for the expression of ovalbumin (Ova) in skin of transgenic mice. Keratin 5 promoter (K5) is expressed in the basal cell layer and involucrin promoter (INV) in the upper layers of the epidermis. In these mice we can follow adoptively transferred naïve Ova-specific T CD4+ cells (DO11.10) in vivo. **Results:** We find that K5-Ova-expression leads to the differentiation of both, Teff and pTreg cells, while INV-Ova completely blocks pTreg differentiation. Consequently, K5 results in self-resolving skin inflammation while INV leads to fatal disease. We found that INV leads to higher expression levels, which we can titrate by dilution of tetracycline. This reduces proliferation, restores pTreg cell differentiation, hampers the production of effector cytokines and reduces TCR signaling. Alternatively, diminishing TCR signal strength at full dox dose with rapamycin also rescues pTreg generation. **Conclusion:** Taken together, our findings suggests that antigen load and initial TCR signal strength can be crucial in the decision of Teff versus pTreg differentiation with remarkable consequences on clinical outcome.

P62 IgE Autoreactivity in Bullous Pemphigoid

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Background: Bullous pemphigoid (BP) is an auto-immune disease typically associated with old age. It is characterized by bullae at the dermal-epidermal junction (DEJ), thought to be induced by the binding of auto-antibodies. These antibodies can recruit inflammatory cells through complement activation, culminating in the proteolytic destruction of cell adhesion structures. While IgG has been the class consistently associated with the disease, some studies point to a potential involvement of IgE. **Methods:** The presence of self-reactive IgE was evaluated in the sera and skin of 33 BP patients via western blot, ELISA and immunofluorescence.**Results:** IgE was detected in the perilesional skin of 22/33 (67%) BP patients. This IgE was not found at the DEJ, but instead on the surface of mast cells and eosinophils, most likely bound as an immune complex. We have evidence that the high-affinity receptor for IgE is the primary molecule involved in this interaction and that eosinophils are expressing FcepsilonRI in BP patients. Furthermore, via immunoblotting, we have demonstrated peripheral BP IgE reactivity against antigens with approximately 60, 120, 180 and 230 kD. These likely represent intra- and extra-cellular domains of BP180 and the full-length BP180 and BP230 proteins, respectively. **Conclusions:** Our data suggests a role for IgE in the pathogenesis of at least a subset of BP patients. Given that the clinical picture of BP consists of erythema and bullae, appearing alone or concomitantly, an association between self-reactive IgE and urticarial-like lesions is plausible and suggests an alternative pathway of disease pathogenesis, perhaps independent from IgG and complement.

P63 Proteolytic Processing of Allergens and their Relevance in Antigen Presentation – A study outline

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Antigen processing by endolysosomal proteases (e.g. Cathepsins, legumain) is a crucial step in producing appropriate peptides for MHC II presentation to initiate Th1/Th2 response. However, the molecular mechanisms of the involved allergen recognition by the proteases still remain to be elucidated. Birch pollen (Betula verrucosa), notably the allergen Bet v 1 is affecting up to hundred millions of people. Among the isoforms, Bet v 1a is considered as the major allergenic isoform that can elicit the production of IgE antibody (Th2 response). Recent evidences show that the processing of Bet v 1a and Bet v 1d by Cathepsin S is depending on their microenvironment pH. Therefore, we aim to stabilize the Bet v 1a/Cathepsin S complex for characterization of the generation of antigenic peptides; to explore the antigenic peptides generated using an in vitro endosome model (Cathepsin S, L and Legumain); to test for and characterize the presentation of the identified antigenic peptide pool on MHCII. These experiments could increase our understanding on what makes an allergen an allergen from several aspects, ranging from the structural interaction of Bet v 1 and cathepsin S to the role of endolysosomal enzymes in allergenicity and immunogenicity of Bet v 1. The microenvironment, e.g., pH as well as ligand binding to Bet v 1, could also influence the antigen processing. Transpeptidation / peptide resynthesis in antigen processing as catalyzed by legumain can possibly generate non-canonical peptides that could lead to entirely new aspects in antigen presentation.

P64 The role and mechanism of CD40/CD40L expression on different T-cell subsets

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Background: Several T-cell costimulatory pathways have been identified that play a critical role in promoting transplant rejection. Their function on distinct T cell subsets, however, remains incompletely defined. Therefore we aim to investigate the role of CD40 and CD40L in specific T cell subsets, in particular

in conventional T cells versus regulatory T cells. **Methods:** The expression of CD40 and CD40L was analyzed on T-cell subsets in vitro. Therefore B6 splenocytes were cultured under different experimental conditions, with anti-CD3/CD28, PMA/Ionomycin being used as stimulators. CD40+/- and CD40L+/- T cells were further analyzed by FACS for their different expression behavior of selected markers. **Results:** Preliminary protein kinetic studies demonstrate that CD40L is transiently and inducibly expressed in both CD3+CD4 (85%) and CD8 (29%) subsets with maximum expression after 6 hours of PMA/I activation. Notably, 40-50% of CD3+CD4+Foxp3+ cells expressed CD40L after 6h anti-CD3/anti-CD28 or PMA/I activation. While Helios and CD62L were expressed more frequently within FoxP3+ CD40L negative cells (Helios CD40L- 72%, CD40L+ 44.6%; CD62L CD40L- 22%, CD40L+ 10.6%), ICOS expression was higher on CD40L+ (36.4%) than CD40L- (28%). Only a small subset of CD4 and CD8 T-cells (~1%) expressed CD40 and within those around 7-8% were single positive for CCR6, 13-24% for CXCR3 and around 67-76% double negative for both. **Conclusion:** This preliminary in vitro study reveals a time and stimulus-dependent induction of CD40L on distinct T-cell subsets, including FoxP3 Tregs, whose functional relevance is investigated in ongoing studies.

P65 The role of nuclear receptor corepressor 1 (Ncor1) in peripheral T cells

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Nuclear receptor corepressor 1 (Ncor1) has been originally identified as a corepressor of nuclear receptor-mediated gene repression. The repressive activity of Ncor1, and its homologue Smrt (Ncor2), is mediated via recruitment of chromatin complexes that include chromatin modifying enzymes such as histone deacetylases (HDACs). Studies with Ncor1 knockout mice (which are embryonic lethal) revealed important functions for Ncor1 during early embryonic development, such as neural cell differentiation, erythropoiesis and a block at the DN stage in developing fetal thymocytes, highlighting its essential role for development and differentiation. Beyond transcription factors of the nuclear receptor family, Ncor1 interacts also with several members of the BTB zinc finger (BTB-ZF) transcription factor family such as PLZF, BCL6 and MAZR, which are key regulators of T cell development and function. Together, this implies important roles for Ncor1 in T cells. To test this hypothesis, we have generated mice with a T cell-specific deletion of Ncor1 (using Cd4Cre). Preliminary results indicating altered T cell activation and cytokine production will be presented.

P66 Intrinsic features of protein antigen contribute to immune modulation

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Background: The skin accommodates multiple dendritic cell (DC) subsets that influence downstream immune effector functions in different ways. The underlying mechanisms involve differential sensitivity to, and subset-specific orchestration of pathogen-associated or host-derived danger signals. In contrast, protein antigens are traditionally regarded as passive target structures and their role in shaping immune reactions have been largely left unnoticed. **Methods and results:** Recently, we observed that two commonly-used model antigens, i.e. E.Coli betagalactosidase (βGal) and hen egg ovalbumin (OVA) when expressed in the skin under else identical conditions, are handled by distinct DC subsets in fundamentally different ways. Because in our experimental setting (gene gun immunization) all other parameters were the same, we think that these differences originate from intrinsic properties of the antigens. **Conclusion (Aim of the study):** Here, we will investigate whether the individual DC subsets have a particular selectivity for different protein antigens and in this way direct the modulation of the subsequent immune reaction. This would have important implications for the understanding of skin immunity and diseases, and vaccine development.

P67 Regulation of allergen-specific immune responses through the human members of the T cell immunoglobulin and mucin domain (TIM) family

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Background: The T cell immunoglobulin and mucin domain (TIM) genes have been implicated as allergy and asthma susceptibility genes. In humans this family comprises three molecules, TIM-1, 3 and 4. Human TIM-1 was demonstrated to be associated with protection against atopic diseases and able to mediate increased T cell proliferation and IL-4 production in Th2 cells. TIM-3 has been shown to down-regulate Th1 and Th17 cytokines, indicating that TIMs play a crucial role in T cell immunity particularly Th2 responses. Furthermore, TIMs have been shown to mediate uptake of apoptotic cells via binding of phosphatidyl serine. Moreover, TIMs act as genuine immunomodulatory receptors: TIM-1 has been reported to bind several ligands, namely TIM-4, CD300b and P-selectin and TIM-3 was proposed to act as cellular receptor for CEACAM1.**Methods:** We analyze the interaction of human TIM-molecules with their ligands and their role in T cell stimulation and regulation. We stimulate Jurkat T-cell reporter lines that stably express TIM-molecules to determine their activation in response to TIM-ligands. Our next steps will be to study the effects of TIM-ligation on proliferation and cytokine production of allergen-specific T cells receiving Signal 1 in the context of TIM-signals. **Results:** Our results confirm interaction of TIM-molecules with some of the proposed ligands and point to a weak interaction between TIM-1 and TIM-3. Stimulation of TIM-1 expressing Jurkat in presence of their ligands did not affect reporter activation. **Conclusion:** Our reporter assays do not give evidence for a role of TIM-1 in T cell activation.

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P68 Bidirectional polarization of T cell function via CD43

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Background: CD43 is one of the abundant cell surface glycoproteins expressed on T cells. Several studies with contradicting results have suggested that CD43 not only acts as a potent T cell co-stimulator but also negatively regulates T cell activation. Co-receptors, are crucial in shaping T cell immunity, as being either co-stimulatory or co-inhibitory, they decide the fate of T cell function. **Results:** To further investigate role of CD43 in T cell activation and subsequent function, peripheral blood T cells were activated via two distinct CD43 epitopes recognized by monoclonal antibodies (mAbs) CD43-6E5 (T6E5-act) and CD43-10G7 (T10G7-act) along with TCR signaling. T cell co-stimulation via either epitope on CD43 could potently induce T cell activation and proliferation. However, T cell co-stimulation via distinct CD43 epitopes, differentially regulated activation of downstream signaling pathways, T cell cytokine production and also subsequent effector function. T10G7-act produced lower levels of inflammatory cytokines, but higher levels of regulatory cytokines, TGF-beta and IL-35 and also responded poorly in re-stimulation assay compared to T6E5-act or to T cells activated via CD28 (TCD28-act). Furthermore, T10G7-act acquired cytokine-independent T cell suppressive function. T10G7-act did not directly inhibit responder T cells, but rather exhibited their effect via dendritic cells when added to allogenic mixed leukocyte reaction. **Conclusion:** Together our data suggests a unique role of CD43 in bidirectional polarization of T cell immunity, a positive co-stimulatory role and a negative role in down-modulation of immune response, depending on its targeted epitope.

P69 A humanized mouse model to detect immune regulation in skin graft rejection in a genetherapy setting of Epidermolysis Bullosa (EB)

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Background: To compensate loss of function mutations that cause genetic diseases replacement therapies (gene-, cell-, and protein therapy) aim at introducing neo-antigens. Here we investigate the gene therapy setting of recessive dystrophic EB (RDEB), which is caused by mutations in the gene coding for the skin protein type VII collagen (C7). Approximately 40% of all patients with RDEB are deficient in C7 and are excluded from any replacement therapy because these individuals are likely to immunologically recognize the therapeutic full-length protein as a foreign/neo-antigen and reject it. **Hypothesis:** Antigen-specific pTreg will suppress inflammation and rejection and thus confer stable tolerance towards the neo-antigen. **Aims:** We aim to generate neo-antigen-specific pTreg within a humanized mouse model to modulate and suppress the tissue specific response to that neo-antigen. **Approach:** Retrovirally corrected skin grafts and PBMCs of RDEB patients will be co-transplanted to immuno-deficient NSG recipient mice. We will then follow the antigen-specific response towards C7 and manipulate it using small molecule drugs and biologica (e.g. IL2 complexes, Rapamycin). **Results:** In a full-thickness skin grafting setting human CD45+ and especially CD8+ T cells expand in the presence of alloantigen. Generated organotypic skin equivalents express human C7 in the basal membrane zone. **Conclusion:** We have established a humanized mouse model that encompasses all components

(i.e. immune cells and skin) to detect antigen specific T cell responses towards a neo-skin-antigen and can serve as a tool to investigate immune modulation with the aim to generate pTreg and study their suppressive activity and stability in vivo.

P70 Molecular imaging of the antigen recognition dynamics in CD8$^+$ cytotoxic T-cells

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Cytotoxic T-cells (CTLs) are of paramount importance for the immune defense against viruses and tumors. Remarkably, CTLs can detect with their low affinity T-cell antigen receptors (TCRs) the presence of even a single antigenic peptide-loaded MHC molecule I (pMHCI) among thousands of structurally related yet non-stimulatory pMHCs (Purbhoo et al. 2004). How they achieve this is not clear but appears to depend at least in part on the special binding conditions within the special constraints of the immunological synapse, the area of contact between a T-cell and an antigen presenting cell. Here receptors and their ligands are not only pre-oriented, but they are often enriched in specific membrane domains and also subjected to cellular forces. To relate these cell biological parameters to T-cell antigen sensitivity in a more comprehensive manner we are monitoring TCR-pMHC binding in nascent synapses with the use of molecular imaging modalities. We confront TCR transgenic CTLs with a glass-supported lipid bilayer (SLB) functionalized with pMHCI, adhesion and co-stimulatory molecules. This allows us to conduct (single molecule) measurements in noise-attenuated Total Internal Reflection (TIRF) mode, to control for ligand composition and density to quantitate their specific influence on TCR-pMHCI binding and TCR-proximal downstream signaling. We also plan to assess the role of CD8 co-receptor engagement with the use of pMHCI mutants, which are deficient in CD8 binding. We expect to gain novel insights into cell biological and molecular processes underlying the phenomenal sensitivity of CTLs towards antigen.

P71 The role of B-cells and humoral immunity in the lung

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Background: B-cells contribute substantially to the total pool of immune cells in healthy lungs. Yet, compared to other cell types, relatively little is known about the characteristics and functions of pulmonary B-cells. The aim of this project to understand to which degree B-cells contribute to lung homeostasis and to host defense against acute bacterial infections.
Methods: Flow cytometry was used to identify cellular populations of C57BL/6 wildtype and B-cell deficient mice at baseline and after infection. Mice were infected with S. pneumoniae and CFUs from different organs were counted after 22 or 48h. ELISAs were used to quantify presence of S. pneumoniae specific IgM and IgG antibodies in serum.
Results: We found that the B-cell population accounted for about 20% of total viable immune cells within the lungs of healthy adult C57/BL6 mice. Around 60% of these cells exhibit an IgDhigh/IgMlow/ to intermed surface marker phenotype, while surface markers compatible with B1 cells were expressed on 1% of total lung B-cells. We discovered that B-cell deficient mice showed increased CFU counts in lung tissues 48h after induction of pneumococcal infection. These mice also showed increased neutrophil influx 22h after infection. By using ELISA plates coated with S. pneumoniae, we could show the presence of S. pneumoniae binding natural IgM antibodies in serum of uninfected WT mice, which were absent in B-cell deficient mice.
Conclusions: B-cells are among the largest leukocyte populations in the lung of C57BL/6 mice. Lack of B-cells appears to be detrimental during acute pneumococcal pneumonia.

P72 The Role of ALK3 in Langerhans Cells

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Langerhans Cells (LCs) represent the epidermal subset of dendritic cells. They are thought to perform two distinct functions as they can act both pro- and anti-inflammatory. Their differentiation and activation is dependent on TGF-beta 1. Recent in-vitro experiments by our group could demonstrate that human LCs can be generated without TGF-beta 1 in the presence of BMP-7. These data suggest that Alk3, a receptor for both BMP-7 and TGF-beta 1, is necessary for the differentiation and proliferation of LCs but is insufficient for anti-inflammatory signals. In collaboration with the group of Maria Sibilia we generated two conditional knock-out mouse lines to test this hypothesis in vivo. By using the Alk3-flox CD11c-cre model we will test the impact of Alk3-deletion on the function of LCs. The Alk3-flox vav-cre model will be utilized to observe the effect of Alk3-deletion in LC differentiation. Our preliminary experiments show that CD11c-cre Alk3-flox mice display a normal LC-network in the epidermis and no spontaneous inflammatory skin phenotype. However, by using a model of Imiquimod-induced skin inflammation, we could demonstrate that Alk3 deletion leads to a stronger, longer lasting skin inflammation. In a migration assay, LCs from these knock-out mice migrate faster than from wildtype mice and the emigrated LCs show a trend to a higher expression of MHCII and CD86. Taken together, our data suggest that Alk3 plays an anti-inflammatory role during acute skin inflammation, but is not critical during skin homeostasis under steady state.

P73 Role of the AP-1 protein c-Jun in Imiquimod mediated tumor clearance.

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Cancer is one of the leading causes of death in the industrialized world. Every third diagnosed cancer is a skin cancer. Imiquimod (IMQ) is an immune modifying compound used as a 5 % cream formulation (Aldara) to treat warts and basal cell carcinomas (BCC). The mechanism of action of IMQ relies on the activation of Toll like receptor 7/8 (TLR7/8) expressing immune cells, prominently a subtype of dendritic cells called plasmacytoid dendritic cells (pDCs). pDCs are Type I interferon producing innate immune cells. We have recently shown that if activated they can be converted into tumor killing cells. The tumor killing ability of pDCs relies on the production of lytic molecules like Granzyme B (Gzmb). The production of these tumor killing molecules in pDCs as well as other pro-inflammatory molecules like tumor necrosis factor alpha (TNF-α) are controlled by a defined subset of transcription factors like interferon regulator factor 7 (IRF 7). Another family of immune regulators is the AP-1 family whose role in pDCs and IMQ mediated tumor clearance is poorly understood. In order to investigate the role of c-Jun in pDC development and function, we are employing mice harbouring floxed c-Jun alleles to delete c-Jun in all bone marrow (BM)-derived cells with the poly I:C inducible Mx-Cre transgenic line. Our results indicate that c-Jun is dispensable for the development and maturation of pDCs. Furthermore, we could show that c-Jun is an important factor for the production of Interleukin-6 (IL-6) and Interferon beta (IFN-β) in IMQ stimulated pDCs.

P74 Generation of IL-9-producing T cells from healthy human skin explant cultures

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Background: Th9 cells are a subset of T cells which play an important role in several diseases including atopic and other inflammatory skin diseases. **Methods:** We employed a well-established skin explant culture method that allowed us to investigate the effects of an IL-9 skewing milieu on skin resident T cells. Skin biopsies from healthy donors were cultured on cell foam matrices (grids) in the presence of either IL-2 and IL-15 (standard condition) or IL-2, IL-4 and TGF-beta (Th9-promoting condition). **Results:** Both culture conditions favored the proliferation of CD3+ T cells (90-98%). Standard conditions yielded more T cells (1.2x10^6/grid) as compared to Th9-promoting conditions (0.8x10^6/grid). We found significantly more Ki-67+ T cells at standard conditions as compared to Th9-promoting conditions indicating that cell division contributes to overall cell counts. More CD4+ T cells could be identified in cultures at Th9-promoting conditions (40.8%) compared to standard conditions (24.3 %) after 4 weeks. More CD8+ cells were present at standard conditions (55.2%) than at Th9-promoting conditions (46.7 %). IL-9-producing T cells emerged at week 2 (6%) and increased until week 5 (27%) when cultured at Th9-promoting conditions. No IL-9-producing T cells could be identified at week 5 when cells were cultured at standard conditions. CLA could be readily identified on T cells cultured on grids compared to those without grids independent of the medium used indicating that grids were essential to retain CLA on T cells. **Conclusions:** IL-2, IL-4 and TGF-beta promote the development of IL-9-producing T cells from healthy human skin.

P75 Examining virus-recognizing receptors in Langerhans cells following human skin barrier disruption and stimulation with synthetic RNA

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Background: Keratinocytes and LCs ascertain a potent skin barrier against environmental threats. To better understand whether LCs respond functionally to viral antigens, we investigated whether PRRs recognizing double stranded RNA (TLR3, RIG-1, MDA5, PKR) can be regulated/activated in LCs upon recognition of poly(I:C) using a human ex vivo skin culture model. **Materials and Methods:** To efficiently disrupt the physical epidermal barrier which mainly consists of the stratum corneum, normal human skin obtained from plastic surgery, was stripped sequentially 10 times to remove this layer. Punch biopsies were then placed in 24 well culture plates and PBS (control) or poly(I:C), a potent inducer of a strong inflammatory response in several cell types, was epicutaneously applied. Samples were harvested after 24 and 48 hours of incubation. Cryosections and epidermal sheets were prepared and analyzed for PRR expression in skin cells using immunofluorescence. **Results:** We found that poly(I:C) upregulated TLR3 but not PKR in keratinocytes and failed to upregulate/induce TLR3 and PKR in LCs. In contrast, in the presence of poly(I:C), MDA5 was strongly upregulated in some resident and emigrating LCs compared to controls. Keratinocytes failed to express MDA5. **Conclusion:** Our data suggest that not TLR3 but MDA5 may play a key role in the innate immune response of LCs to viral infection. This model will now allow us to define the PRR pathways which will be activated in LCs as well as in keratinocytes after contact with allergens.

P76 STAT1 isoform specific functions in infectious and inflammatory diseases

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Signal transducer and activator of transcription (STAT1) is a member of the JAK/STAT signaling pathway and is essential for signaling by all types of interferons (IFNs). STAT1 is crucial for the defence against bacterial and viral infections but also contributes to immunopathologies. STAT1 exists as two alternatively spliced isoforms, STAT1a and STAT1b, differing in the C-terminal transactivation domain, which is absent in the STAT1b isoform. Accordingly, STAT1b was considered to be transcriptionally inactive if activated as homodimers, i.e. in response to IFN-gamma, and to potentially exert dominant negative functions. Using mice that only express either STAT1a (Stat1a/a) or STAT1b (Stat1b/b) we have recently shown that STAT1b is transcriptionally active and capable of mediating IFN-gamma-dependent defence against systemic Listeria monocytogenes infections, although with lower efficiency than STAT1a. To dissect the contribution of the individual STAT1 isoforms to intestinal antibacterial immunity, we challenged mice intragastically with the enteropathogen Citrobacter rodentium. Bacterial load in the spleen was increased in Stat1-/- and Stat1b/b compared to Stat1a/a mice, whereas it was unaffected by the absence of either one, or both, isoforms in the intestine. To study inflammatory functions of STAT1 isoforms, we challenged mice with high-dose lipopolysaccharide. Stat1b/b mice showed an intermediate survival between Stat1-/- and Stat1a/a mice, suggesting that STAT1b is less immunopathogenic than STAT1a. Furthermore, our data clearly show that STAT1b is not a dominant negative factor, as no differences between Stat1+/+ and Stat1a/a were observed in innate immunity. Ongoing experiments are directed to assess STAT1 isoform specificity in adaptive immunity.

P77 The role of Stat3 in polarization of tumor-associated macrophages

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Activation of the transcription factor STAT3 in tumor and immune cells is important for immune escape of many human cancers. We demonstrated that formation of azoxymethane/dextransulfate (AOM/DSS)-induced colorectal cancers was strongly suppressed in mice lacking STAT3 in myeloid cells/macrophages. Gene expression profiling showed that STAT3-deficient macrophages were M1 polarized and triggered strong anti-tumor T cell responses in the tumor stroma. Macrophage polarization is influenced by TLR signaling indicating that the intestinal microbiome influences anti-tumor immune responses in colon cancer. We used in vitro macrophage cultures to gain insight into tumor – stroma interactions modulated by STAT3 activation in myeloid cells. As a cellular source, we isolated bone marrow cells from mice with conditional deletion of STAT3 in the myeloid compartment and differentiated them in vitro into mature F4/80+ CD11b+ macrophages. The macrophages were then stimulated with TLR ligands to assess STAT3-dependent functions in macrophage polarization. We demonstrate that STAT3-deficient macrophages are strongly activated by TLR4, TLR2/6, TLR3 and TLR7/8 ligands, but did not differentially respond to TLR5 ligands when compared with control macrophages. Differential activation of STAT3-deficient macrophages was also reflected by increased expression of M1 polarization markers and altered phagocytosis. STAT3 inhibitors are considered for treatment of colorectal cancer. Our data suggest that certain TLR ligands, present within the intestinal microbiome, might influence pharmacological effects on macrophage polarization and therapeutic response.

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