2nd International Workshop on
Inflammation and Immunity

"Walk the Line"

Immunoregulation:
between Host Defense and Pathology

March 18-19, 2010
General Hospital of Vienna, Austria
Lecture Hall 2, Hörsaalzentrum Level 8
Währinger Gürtel 18-20
1090 Vienna, Austria

Differentiation of Immune Cells

Mechanisms of Dysregulated Immune Responses

Crosstalk between the Innate & Adaptive Immune System

Mechanisms of Tolerance

Program & Abstracts

Organized by the Students of the IAI-PhD Program of the MUW

http://www.meduniwien.ac.at/phd-iai

FWF PHD-PROGRAM INFLAMMATION AND IMMUNITY MEDICAL UNIVERSITY OF VIENNA

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TABLE OF CONTENTS

PHD-PROGRAM
INFLAMMATION AND IMMUNITY

The PhD and MD/PhD Program Inflammation and Immunity (IAI) aims at revealing novel mechanisms controlling the development and function of immune cells in health and disease and train excellent young researchers with a new qualification profile in basic, translational and clinical research. The IAI PhD program is closely linked to two Special Research Programs (SFB-F18 and SFB-F23) funded by the Austrian Science Fund (FWF) and several Austrian (GEN-AU, CDL) and European (NoE, RTN, Strep) networks.

Our overall scientific objective is to understand the detailed events and molecular mechanisms associated with inflammatory and immunological diseases. Genes, molecules, isolated cells and tissues as well as whole organisms are all being exploited as model systems. Thereby mouse immunologists will closely interact with human immunologists and establish a close interaction between basic and clinical science. We provide synergistic expertise in the fields of molecular biology, cell biology, mouse genetics, immunology, allergology, infectiology and immunopharmacology, also at the translational level. The major critical advantage of working in this interactive network will be the transfer of molecular observations made in vitro to the clinical treatment of immune and inflammatory diseases in patients. Access to patient material will be provided by the clinical research groups present within the IAI program. This interface between basic and clinical research is of particular importance for the success of the IAI PhD program.

The IAI PhD Program will concentrate its research efforts in the following four areas:

* Basic aspects of Immunity
* Inflammatory diseases
* Infectiology and Vaccinology
* Allergy, Hypersensitivity and Transplantation

Our scientific goals will be implemented by a state-of-the art career development plan which includes comprehensive educational training in the field of IAI, special lectures on career awareness, collaborative programs with international universities and research institutions, exchange of research tools and technologies, teaching and special scientific workshops. This will offer IAI PhD students all the prerequisites for a future successful career in academia, industry or any field of health care.

http://www.meduniwien.ac.at/phd-iai/
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Greeting from the IAI PhD Students

The doctoral program "Inflammation and Immunity" was established under the academic umbrella of the Medical University of Vienna in 2007. The ambition of the program is to provide young scientists, not only with the opportunity to conduct scientific investigations in their desired field of interest (ranging from fundamental research in the areas of immunology and cancer, to more applied aspects of Dermatology, Allergology, Virology and Transplantation), but also aims to impart the necessary skills required for a successful scientific career.

As part of this training, we students have the opportunity to organize an international workshop. The aim of the workshop is to be up-to-date with the latest developments in the field and also to provide young researcher and other distinguished scientists from Austria an occasion to meet and interact with prominent international researchers.

The first workshop "You Give me Fever" held last year, centered on current topics in inflammation and was endowed with huge success, thanks to the never ending efforts of our colleagues and distinguished guests. Over 200 participants were in eager attendance for the keynote lecture delivered by Sir Ravinder Maini and other talks by fellow excellent speakers. Thus, the expectations from this year's workshop are high.

We are confident that our eminent guest speakers of this years' workshop will present you with a fascinating insight about their peerless work in the field of Immunoregulation: between Host Defense and Pathology. We look forward to their inspiring lectures and further vivid exchange.

With this, we extend you a warm welcome to our second international workshop "Walk the Line"! We wish you a pleasant stay here in Vienna and thank you for your kind participation at our workshop.

The organizing committee

Greeting from the Rector of the Medical University – Wolfgang Schütz

Dear colleagues,

Our PhD Curriculas give the students the possibility to work in an international scientific atmosphere with challenges and exercises that are necessary to remain in the very competitive international field of medical science. I am very proud to say that we have already four high rated PhD Programs that also play an important part of a new Career model at the Medical University. This Career Model respects the individual ability and inclination of the practicing scientist and doctor.

Our postgraduate programs and their high acceptance by the students – national and international – are the perfect way to continue the tradition of the highly known and internationally respected Viennese Medical School.

I wish all of you who are representing the young and enthusiastic scientist a rewarding, pleasant und inspiring workshop!

Wolfgang Schütz
Rector Medical University of Vienna

Greeting from the Head of IAI PhD-Program - Maria Sibilia

Welcome to the 2nd International Workshop of the Doctoral Program Inflammation and Immunity (IAI)
"Walk the Line: Immunoregulation: between Host Defense and Pathology"

About three years ago the Doctoral Program “Inflammation and Immunity“ (IAI) has been implemented as an educational excellence platform in basic medical research at the Medical University of Vienna (MUW). Since then the MUW as a major European Center of biomedical research and education provides the infrastructure for students from all over the world. Meanwhile 19 students from 8 countries have been accepted into the international IAI PhD program after passing a competitive selection procedure. They are conducting their research in the laboratories of the 8 faculty members that are part of the IAI PhD program. Our aim is to strengthen the research interactions and to extend our collaborative network beyond laboratory walls and different research disciplines, as well as language gaps and cultural differences. Each year the IAI PhD students organize an international workshop which should contribute to facilitate our endeavors.

The topic chosen by our students for the 2nd workshop is Immunoregulation. The research in this area is of worldwide medical need and is important to accelerate the process from basic discoveries to new therapeutic strategies for the cure of many immunological disorders such as autoimmune diseases and infections. The students have invited internationally well-known experts and world leading scientists in the field and we are looking forward to an active exchange of opinions, stimulating discussions and exciting scientific interactions for the next two days.

In the name of all faculty members I would like to thank the students for putting together such an exciting program and to all the speakers for accepting the invitation and traveling to Vienna to make this event possible. I wish all the IAI students and all participants of the workshop two exciting days.

Finally, we would like to express our gratitude towards the Austrian Science Fund (FWF) and the MUW for financing and supporting our PhD program and to all companies for co-sponsoring this workshop.

Maria Sibilia (IAI PhD program coordinator)

and

all PIs of the program:
Barbara Bohle, Wilfried Ellmeier, Franz X. Heinz, Georg Stingl, Herbert Strobl, Rudi Valenta, Thomas Wekerle

"Walk the Line": Immunoregulation: between Host Defense and Pathology

Thursday, March 18, 2010

08:30 – 09:00 Registration

09:00 – 09:15 Welcome

SESSION I: DIFFERENTIATION OF IMMUNE CELLS

Chair: Sriram Srivatsa, Roland Tschismarov

09:15 – 10:15 **Mechanism of myeloid-derived suppressor cell differentiation in cancer**
Dmitry Gabrilovich

10:15 – 11:00 Student talks: Barbara Drobits, Wolf Gebhardt, Madeleine Kalb

11:00 – 11:30 *Coffee break*

11:30 – 12:30 **Functional adaptation of thymic stromal cells for T cell repertoire selection**
Ludger Klein

12:30 – 14:00 *Lunch Break*

SESSION II: MECHANISMSM OF DYSREGULATED IMMUNE RESPONSES

Chair: Brinda Subbarayal, Jiang Zhou

14:00 – 15:00 **Intestinal homeostasis: a balancing act between effector and regulatory T cells**
Fiona Powrie

15:00 – 15:30 Student talks: Katarzyna Niespodziana, Véronique Schulten

15:30 – 16:00 *Coffee Break*

16:00 – 17:00 **Walking the line between pathology and tolerance: manipulation of T lymphocytes in human allergic disease**
Mark Larché

POSTER PRESENTATION AND GET-TOGETHER

17:00 – 18:00 **Poster presentation: hallway of Hörsaalzentrum Level 8**

Friday, March 19, 2010

08:30 – 09:00 Registration

SESSION III: CROSSTALK BETWEEN THE INNATE AND ADAPTIVE IMMUNE SYSTEM

Chair: Elisabeth Glitzner, Gregor Eisenwort

09:00 – 10:00 **Cell biology of antigen presentation by dendritic cells**
Ira Mellman

10:00 – 10:30 Student talks: Thomas Bauer, Afitap Derya Köprülü

10:30 – 11:00 *Coffee Break*

11:00 – 12:00 **The Monocyte - A Masterful Jack in all Tracks**
Steffen Jung

12:00 – 13:30 *Lunch Break*

SESSION IV: MECHANISMS OF TOLERANCE

Chair: Karin Hock, Bharani Srinivasan

13:30 – 14:30 **to be announced**
Harald von Boehmer

14:30 – 15:00 Student talks: Anastasia Abramova, Haley Ramsey

15:00 – 15:30 *Coffee Break*

15:30 – 16:30 **On the Induction of priming and tolerance**
Maries van den Broek

16:30 – 17:00 Closing Remarks

ON THE INDUCTION OF PRIMING AND TOLERANCE

Maries van den Broek

Department of Oncology, University Hospital Zurich
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$CD8^+$ cytotoxic T cells are crucial to protect the vertebrate host against infections and cancer. They recognize infected or altered cells through specific peptides presented on the surface of those cells in the context of MHC class I molecules. Although nucleated cells express MHC class I molecules and therefore can be recognized by activated $CD8^+$ T cells, only very specialized cells – dendritic cells – can activate naïve $CD8^+$ T cells.

Dendritic cells (DC) are present in all tissues and continuously sample the environment. In a steady state, DC have a resting or immature phenotype, whereas this rapidly changes to a mature/activated phenotype in response to inflammation or infection. In order to activate naïve and memory $CD8^+$ T cells, DC must have an activated or mature phenotype, a status that results from inflammatory stimuli including infections. The exact reason why DC are superior to all other known cell types with respect to the activation of T cells is not precisely known, but their localization, their ability to cross-present and the expression of certain co-stimulatory molecules contribute.

Local ablative radiotherapy (RT) is an efficient treatment for cancer and it is thought that the induction of apoptosis in tumour cells is the main mode of action. However, we found that the therapeutic effect of RT crucially depends on mobilization of tumor-specific $CD8^+$ T cells and continued antigen presentation by DC.

The same cell type that is crucial for the induction of immunity, however, can also turn off or prevent immunity and thus is the main mediator of peripheral T cell tolerance, a condition that protects us from autoimmune diseases but also hampers immunity against cancer. We show here that steady state DC induce peripheral T cell tolerance in an antigen-specific and T cell-intrinsic fashion and we present evidence for involvement of CD70, PD-1, CTLA-4 and $FoxP3^+$ regulatory T cells in this process.

TO BE ANNOUNCED

Harald von Boehmer

Harvard Medical School
Boston, MA, USA

MECHANISM OF MYELOID-DERIVED SUPPRESSOR CELL DIFFERENTIATION IN CANCER

Dmitry Gabrilovich

H. Lee Moffitt Cancer Center & Research Institute
Tampa, FL, USA

Myeloid-derived suppressor cells (MDSC) represent an intrinsic part of myeloid cell lineage and comprised of myeloid progenitors and precursors of myeloid cells. In healthy host upon generation in bone marrow immature myeloid cells (IMC) quickly differentiate into mature granulocytes, macrophages, or dendritic cells. In a number of pathological conditions (cancer, various infections diseases, sepsis, trauma, bone marrow transplantation, autoimmune abnormalities) increased production of IMC is associated with partial block of their differentiation and most importantly pathological activation of these cells manifests in up-regulation of arginase, inducible nitric oxide synthase (iNOS) and NO production, increased level of reactive oxygen species (ROS). This results in expansion of IMC with immune suppressive activity. Accumulation of MDSC was detected in practically all mouse tumor models and in patients with different types of cancer. In mice, MDSCs are characterized by the co-expression of myeloid lineage differentiation antigen Gr1 and CD11b. In humans, MDSC are currently defined as CD14-CD11b+ cells or more narrowly as cells that express the common myeloid marker CD33 but lack the expression of markers of mature myeloid and lymphoid cells and the MHC class II molecule HLA-DR. Recently, the morphological heterogeneity of these cells in mice was defined more precisely based on the expression of cell-surface markers Ly6G and Ly6C. Granulocytic MDSCs have a CD11b+Ly6G+Ly6Clow phenotype, whereas MDSCs with monocytic morphology are CD11b+Ly6G-Ly6Chigh. These two subpopulations may have different functions. Accumulation of MDSC is caused by different soluble factors. Recent studies have demonstrated that factors implicated in regulating the expansion of MDSCs can be provisionally split into two main groups with partially overlapping activity. The first group includes factors that are produced primarily by tumor cells and promote the expansion of MDSC through myelopoiesis stimulation, which is associated with inhibition of myeloid-cell differentiation. These factors include stem-cell factor (SCF), macrophage colony-stimulating factor (M-CSF), IL-6, granulocyte/macrophage colony-stimulating factor (GM-CSF) and vascular endothelial growth factor (VEGF) and others. Signalling pathways triggered by most of these factors in MDSCs converge on signal transducer and activator of transcription 3 (STAT3). One of the potential targets for STAT3 was recently identified as S100A8/A9 proteins. Accumulation of these proteins in myeloid progenitors prevents their differentiation and results in expansion of MDSC. The second group of factors is produced primarily by activated T cells and tumor stroma and directly activates MDSC. These factors, which include IFN gamma, IL-13, IL-4 and TGFβ, among others, activate several different signaling pathways in MDSCs that involve STAT6, STAT1, and NF-kB. Most studies have shown that the immune-suppressive function of MDSCs requires direct cell–cell contact, which indicates that they operate either through cell-surface receptors and/or through the release of short-lived soluble mediators. Currently, a number of clinical trials explore the possibility of regulating immune responses in cancer by depleting or inactivating MDSC in cancer patients.

THE MONOCYTE - A MASTERFUL JACK IN ALL TRACKS

Steffen Jung

Weizmann Institute of Science
Rehovot, Israel

Peripheral blood monocytes play a central role in the myeloid cell compartment by providing a critical link between the bone marrow, as major site of adult hematopoiesis, and peripheral, terminally differentiated mononuclear phagocytes comprising macrophages and dendritic cells. Yet, emerging evidence suggests that these ephemeral mobile cells also function in their own rights: Thus monocytes seem directly involved in the establishment and resolution of inflammatory reactions, remodeling upon tissue injury and angiogenesis. Monocyte studies recently gained momentum with the definition of dedicated bone marrow-resident monocyte precursors, the MDPs. Moreover, the identification of discrete Ly6Clo and Ly6Chi monocyte populations in the mouse allows to probe for differential functions of their human correlates. Here, I will summarize the recent advance in our understanding of monocyte origins, subset dynamics and monocyte fates and present recent results from our laboratories based on adoptive monocyte transfer approaches.

FUNCTIONAL ADAPTATION OF THYMIC STROMAL CELLS FOR T CELL REPERTOIRE SELECTION

Ludger Klein

Institute for Immunology, University of Munich
Munich, Germany

During intrathymic generation of the T cell repertoire, a series of selection processes ensures that only self-MHC (Major Histocompatibility Complex) restricted and self-tolerant T cells are allowed to survive and become part of the mature T cell repertoire. Interactions with MHC ligands on the surface of thymic epithelial cells (TECs) play a pivotal role in the decision-making of developing thymocytes. Work in our lab focuses on the identification of distinct cell-biological features of TECs that predispose them to serve in part non-redundant functions in thymocyte education. For instance, medullary (m)TECs express a plethora of peripheral tissue antigens (PTAs), a property that obviously has evolved to make these self-antigens "visible" to developing thymocytes for tolerance induction. Through this phenomenon, also called “promiscuous gene expression”, mTECs serve an essential function in central tolerance, as evident from the occurrence of spontaneous autoimmunity in humans and mice that carry a mutation in the autoimmune regulator (Aire) gene. Because “promiscuously expressed” self-antigens may be transferred to and presented by dendritic cells (DCs), it is unclear whether mTECs, besides being an “antigen reservoir”, also serve a mandatory function as bona fide antigen presenting cells. To address this issue, we reduced MHC class II on mTECs through transgenic expression of a C2TA-specific “designer miRNA”. This resulted in an enlarged polyclonal CD4 single-positive compartment and, among thymocytes specific for model-antigens expressed in mTECs, enhanced selection of regulatory T cells (Treg) at the expense of deletion. Taken together, our data document an autonomous contribution of mTECs to negative selection of CD4 T cells that in quantitative terms may be similar to the corresponding role of DCs. Furthermore, our findings support an avidity model of Foxp3+ regulatory T cell development versus deletion.

WALKING THE LINE BETWEEN PATHOLOGY AND TOLERANCE: MANIPULATION OF T LYMPHOCYTES IN HUMAN ALLERGIC DISEASE

Mark Larché

McMaster University
Hamilton, Ontario, Canada

The cellular and molecular mechanisms that determine the conditions under which T lymphocytes become activated, or tolerized, by encounter with cognate antigen remain incompletely understood. Using synthetic peptide sequences (T cell epitopes) administered to human subjects with allergic disease, we have induced both activation and tolerance of peptide-specific T cells. In allergic asthmatic individuals, systemic or local challenge with T cell epitopes from the sensitizing antigen/allergen can result in the activation of allergen-specific T cells leading directly to exacerbation of disease within a few hours. Subsequently, however, tolerance is established to the same epitopes in a process that requires several days to become complete. Thus, the induction of disease exacerbations and immunological tolerance are temporally distinct and can be dissociated. We find that it is possible to induce profound allergen-specific tolerance, without exacerbating disease and vice versa, by targeting T cells of the same specificity. We have exploited these clinical models in an attempt to better understand the cellular and molecular parameters that determine how T lymphocytes "walk the line" between pathology and tolerance.

Intradermal injection of T cell epitopes can give rise to MHC-restricted airway narrowing, in allergic asthmatic individuals, manifest 2 to 3 hours after challenge. This exacerbation of disease peaks approximately 6 hours after challenge and resolves within 24 hours. Kinetically identical reactions can be elicited via inhalation of nebulized epitopes. In subjects challenged intradermally (systemically), re-challenge with the same dose of peptides two weeks later fails to elicit any change in airway caliber, implying the induction of tolerance. In contrast, re-challenge via the inhaled route (local) results in the reiteration of the original reaction suggesting that tolerance is not established via this route.

The induction of T cell-dependent airway narrowing is dose-dependent. It is therefore possible to induce tolerance in the absence of disease exacerbations by delivery of low doses of peptides. Furthermore the induction of disease exacerbations appears to be dependent upon clinical severity of disease. For example we have not observed the induction of airway narrowing (late asthmatic reaction) in over 100 individuals with allergic rhinitis +/-mild asthma treated with multiple doses of peptide. In contrast, equivalent doses of peptide frequently elicit such reactions in subjects with more severe disease. Paradoxically, whilst the peptides administered are incapable of cross-linking specific IgE to activate mast cells and basophils, the likelihood of an asthmatic individual developing airway narrowing is closely related to levels of allergen-specific IgE in the serum.

We have exploited the ability of low dose peptide challenge to induce tolerance in order to develop peptide-based vaccines for allergic disease. Clinical trials have demonstrated significant improvements in a broad array of clinically-related outcomes, including the ability to tolerate prolonged airborne exposure to allergen. Using peripheral blood mononuclear cells (PBMC) collected before and after therapy we have begun to dissect the immunological mechanisms of tolerance, demonstrating reduced TH1/TH2 cytokines, increased IL-10 and the presence of allergen-specific regulatory T cells within the CD4+ compartment.

In summary, we have established interventional clinical models in which allergen-specific T cells are targeted in order to induce and study disease exacerbations and/or immunological tolerance.

CELL BIOLOGY OF ANTIGEN PRESENTATION BY DENDRITIC CELLS

Ira Mellman

Genentech, Inc.
South San Francisco, CA, USA

Dendritic cells (DCs) sit at the interface between the innate and adaptive arms of the immune response. Equipped with virtually all of the molecular sensors that innate cells posses to detect microbial products and inflammatory insults, DCs act upon this information not to activate cytotoxic responses but rather to integrate these signals for the purposes of activating antigen-specific immunity. The complex response of DCs to these activation signals is referred to as “maturation”, and involves a wholesale cellular reorganization that converts DCs from cells adapted for antigen capture to cells adept at antigen processing, presentation, and T cell stimulation. For the past several years, my group has studied the cell biological mechanisms responsible for DC maturation, and in the process learned much about how these cells regulate immunity, control the balance between immunogenic and tolerogenic responses, and increase the efficiency of viral and bacterial sensing. We will review what we have learned about these processes, focusing first on the mechanisms controlling the redistribution of MHC class II molecules from lysosomes in immature DCs to the plasma membrane in mature DCs by regulated ubiquitination, emphasizing how the length of ubiquitin chains plays an essential role in the process. The role of maturation in regulating endocytosis, antigen processing, and virus infection will also be considered, including evidence that infected DCs may actually be unable to present viral antigens efficiently, stressing the importance of “cross presentation” in the normal immune response. Finally, we will also discuss new findings showing how DC lysosomes are adapted for facilitating the egress of microbial products to the cytosol, where they can reach essential cytosolic sensors such as NOD1 and NOD2.

INTESTINAL HOMEOSTASIS: A BALANCING ACT BETWEEN EFFECTOR AND REGULATORY T CELLS

Fiona Powrie

Translational Gastroenterology Unit, Experimental Medicine Division - Nuffield Dept of Medicine
University of Oxford
Oxford, UK

In inflammatory bowel disease (IBD), a chronic debilitating inflammatory disease of the gastrointestinal tract, there is a breakdown in intestinal homeostasis resulting in aberrant inflammatory responses to intestinal bacteria. Under normal circumstances the immune response in the intestine is a delicate balance between effector and regulatory T cell responses (TR) . Results from this laboratory have shown that naturally occurring regulatory T cells play an important role in controlling intestinal inflammation. More recent studies have identified the gut as a preferential site for differentiation of Foxp3+ TR cells and that functionally distinct DC subsets promote TR development via a TGF-beta and retinoic acid dependent mechanism. By contrast during inflammation, IL-23 produced by intestinal DC restrains regulatory T cell development promoting intestinal inflammation. This presentation will focus on the role of IL23R and Stat-3 signalling pathways in influencing the balance between tolerance and immunity in the intestine.

THE ROLE OF THE TRANSCRIPTION FACTOR MAZR IN MAST CELLS

Anastasia Abramova[1], S. Sakaguchi, A. Schebesta, N. Boucheron, U. Schmidt, M. Kneidinger, P. Valent and W. Ellmeier

[1] *Center for Physiology and Pathophysiology, Institute of Immunology, Medical University of Vienna, Austria*

Mast cells are key players in normal and pathophysiological type I hypersensitivity reactions. This includes different types of diseases such as allergic rhinitis, allergic asthma, and systemic anaphylaxis. Their activation has to be tightly controlled. Mast cells are classically activated upon Fc□RI stimulation although alternative modes of activation exist. The signaling pathways that are induced via Fc□RI receptor triggering have been studied intensively and a large number of signaling molecules and pathways that positively and/or negatively regulate mast cell activation have been identified. However, much less is known about transcription factors that control the activation and function of mast cells.

Our laboratory recently identified the transcription factor MAZR as an important regulator of Cd8 gene expression in thymocytes, and the current analysis of MAZR-deficient mice revealed that MAZR is part of the transcription factor network that controls CD4/CD8 cell fate decision of DP thymocytes. However, MAZR is also expressed in mast cells and preliminary studies with MAZR–/– bone marrow-derived mast cells indicate impaired early and late mast cell effector functions in the absence of MAZR. BMMC of MAZR deficient mice show normal development, cell counts, morphology, cell surface markers and calcium flux. However, upon FC□RI crosslinking induced activation they exhibit impaired histamine release, crucial part of the early effector function, as well as impaired late effector functions such as cytokine and leukotriene production. Bypassing the FC□RI via PMA/Ionomycin activation rescues the defect in cytokine production, which indicates that MAZR is a crucial regulator of mast cell function. The aim of my PhD project is to investigate the exact mechanism of MAZR involvement in mast cell function as a potential target for a therapeutic intervention in allergies.

TGF- β1 REGULATES THE EPIDERMAL LANGERHANS CELL NETWORK VIA THE TAM RECEPTOR SYSTEM

Thomas Bauer[1], F. Saller, A. Brisset, J. Jurkin, N. Yasmin, A. Angelillo-Scherrer and H. Strobl

[1] *Institute of Immunology, Medical University of Vienna, Austria*

Langerhans cells (LCs) are dendritic cells (DCs) that form a dense network in the epidermis of the skin. Interestingly, as also described for microglia, the pool of LCs is maintained in a tissue-autonomous way. Thus among all hematopoetic cells LC homeostasis, in the steady state, occurs independently of the bone marrow. Their development is dependent on the cytokine transforming growth factor β1 (TGF- β 1) and the macrophage colony stimulating factor receptor (M-CSFR). However the mechanism of LC homeostasis remains unidentified. We hereby report the upregulation of Axl, a member of the Tyro3, Axl and Mer (TAM) receptor tyrosine kinase (RTK) family during LC differentiation from human CD34+ hematopoietic progenitor/ stem cells. The induced expression of Axl and the maintenance of Mer, another TAM member, are downstream events of TGF- β1 signaling. Gas6 and Protein S, the ligands of the TAM receptor system are expressed by keratinocytes in human skin. Furthermore, TAM receptor knockout (KO) mice show a severe reduction in LC numbers. Interestingly lack of Tyro3 and Mer alone also greatly reduces LCs indicating a cooperative action of these receptors in vivo. In accordance with these data, the Gas6 KO mice also display a significantly impaired LC network in the skin. Conversely ectopic Axl expression significantly promotes LC differentiation and activation of autocrine produced Gas6 and Protein S by vitamin K supplemented media strongly increases LC numbers in an in vitro LC differentiation model. We could further show that engagement of TAM receptor signaling by addition of Gas6 activates the extracellular signal-regulated kinase (ERK) and induces proliferation of LC progenitors. Collectively these data identify the expression of the TAM receptors and their ligands in the skin and show that TGF- β1 utilizes this system to establish an intact LC network in the epidermis.

ROLE OF IMIQUIMOD IN ANTI-TUMOR IMMUNE RESPONSES

Barbara Drobits[1], M. Holcmann[1], R. Grundtner[1] and M. Sibilia[1]

[1] *Institute for Cancer Research, Medical University of Vienna*

The immune response modifier Imiquimod, a ligand for TLR7, has been shown to exert anti-viral and anti-tumor activities. Treatment of melanomas with Imiquimod leads to tumor regression, accompanied by increased tumor infiltration of plasmacytoid dendritic cells (pDCs), a DC subpopulation expressing TLR7. So far, the mechanism how Imiquimod mediates anti-tumor activity is poorly understood.
In this study, we investigated if pDCs or other cell types e.g. skin cells play an active role in anti-tumor immune responses mediated by Imiquimod.
Although TLR7/8 expression was not detectable in the skin, in vitro stimulation of keratinocytes from wild-type, TLR7 and MyD88 knock-out mice with Imiquimod led to activation of the MAPK pathway accompanied with increased apoptosis. Moreover, we found increased IL-6 levels in primary dermal cultures upon Imiquimod treatment.
Interestingly, we could show that TLR7 and MyD88 expression is required for the tumoricidal effect of Imiquimod in bone marrow-derived cells. To get further insight into which leukocyte population is responsible for tumor regression, we depleted various immune cells, such as CD4+, CD8+, CD25+ and NK1.1+ cells, in Imiquimod treated, melanoma bearing mice. The anti-tumor effect mediated by Imiquimod is impaired in the absence of CD4+ and CD8+ cells, whereas depletion of NK-cells resulted in a strong Imiquimod effect. Moreover spontaneous tumor regression after CD4+- or CD25+- cell depletion was observed, even in the absence of Imiquimod.
In summary, we could show that the tumoricidal effect of Imiquimod strictly depends on TLR7 and MyD88 expression on immune cells. The recruitment of immune cells to the site of treatment may be mediated by IL-6 independent of TLR7 expression. It seems that CD4+
and CD8+ cells are required for the anti-tumor response mediated by Imiquimod. Whether pDCs are essential for Imiquimod action is currently under investigation.

IDENTIFICATION OF PROTEIN COMPLEXES THAT CONTROL CORECEPTOR GENE EXPRESSION AND CD4/CD8 CELL FATE DECISIONS DURING T CELL DIFFERENTIATION

Wolf Gebhardt[1], K. Bennett[2], B. Superti-Furga[2] and W. Ellmeier[1]

[1] *Institute of Immunology, Medical University of Vienna, Lazarettgasse 19, 1090 Vienna, Austria;* [2] *Center for Molecular Medicine of the Austrian Academy of Sciences, Lazarettgasse 19, 1090 Vienna, Austria*

T cell differentiation into either CD4 helper (TH) or CD8 cytotoxic (TC) T cell lineage from CD4+ CD8+ double-positive (DP) thymocytes is critical for the outcome of an immune response. It has been shown that the functional phenotype of the differentiated T cell lineage correlates with the expression of the coreceptor molecules CD4 and CD8, which are required for T cell development and proper antigen recognition by T cells. Thus, it is conceivable that common factors regulate Cd4 and Cd8 gene expression and the molecular decisions that promote either the TH or the TC lineage, respectively. Therefore, the identification of cis-regulatory elements and trans-acting factors involved in the epigenetic and transcriptional regulation of the coreceptor molecules may help to understand the molecular basis of CD4/CD8 lineage commitment. The control of Cd4 and Cd8 gene activities rests upon a complex interplay of cis-regulatory and trans-acting elements. The BTB domain protein MAZR was shown to bind to Cd8 enhancers and to negatively regulate Cd8 expression. ThPOK, another protein of the BTB family, was found to be necessary and sufficient to impose the TH lineage fate on DP T cells. Both MAZR and ThPOK are thought to elicit their functions in cooperation with other proteins. Here, a tandem affinity purification was used to extract MAZR and ThPOK protein complexes in a CD4+ CD8+ T cell line for identification of associated proteins by mass-spectrometry. The catalytic subunit of BAF complexes, BRG1, and HDAC1 were identified to associate both with MAZR and ThPOK proteins in-vitro and in-vivo, indicating a close interplay between this family of transcription factors and epigenetic regulation of locus accessibility. Interestingly, among many putative interactors, the mass spectrometry analysis revealed the association of MAZR and ThPOK with a RING domain protein, which is highly expressed in CD4+ cells. To examine the pertinence of this novel factor to the process of CD4/CD8 T cell differentiation, a functional in-vivo analysis is performed, applying BM reconstitution and RNAi assays to experimentally manipulate the expression.

THE FUNCTIONAL SPECTRUM OF TLR7/8-TRIGGERED DENDRITIC CELL CYTOTOXICITY

Madeleine Kalb[1], G. Stary[1], F. Koszik[1] and G. Stingl[1]

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Dendritic cells (DCs) do not only exhibit the unique capacity to evoke primary immune responses by presenting antigens to naïve T cells, but may also acquire Toll-like receptor (TLR)-triggered cytotoxic molecules. Whereas TLR7/8- and TLR9-stimulated plasmacytoid DCs (pDCs) isolated from human peripheral blood express tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), TLR7/8-stimulated myeloid DCs (mDCs) express perforin and granzyme B, but not TRAIL. We now wanted to gain a better understanding of the molecular players involved in DC cytotoxicity. Using a range of TLR 1-9 agonists we show that the induction of cytotoxic molecules is subtype-specific and only inducible with intracellular, but not extracellular TLR agonists. In both pDCs and mDCs cytotoxic molecule expression was accompanied by phenotypic maturation. This did not prohibit the induction of an intact immune response as determined in an MLR. In the case of pDCs, but not mDCs, TLR7/8-induced killer molecule expression was IFN-alpha-dependent. Consistently, TRAIL-expression on pDCs could be induced by IFN-alpha stimulation. At a functional level both TLR7/8- and IFN-alpha-stimulated pDCs killed Jurkat T cells in a TRAIL-, IFN-alpha- and cell contact-dependent fashion. In contrast, TLR7/8-stimulated mDCs lysed the MHC Ilow tumor cell line K562 in a perforin-dependent fashion, but much less efficiently their HLA-A2-transfected counterpart. Since natural killer (NK) cells are able to kill HLA-A2-transfected K562 cells and mDCs lack the phenotypic profile of NK cells, these findings indicate that, although they use the same killing mode, NK cells and mDCs recognize different targets.
In conclusion our data demonstrate two distinct mechanisms by which pDCs and mDCs elicit their tumoricidal activity, pointing to an as yet underappreciated powerful innate defense line in infectious and tumor immunity.

THE ROLE OF TEC FAMILY KINASES IN MACROPHAGES

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Macrophages are important in innate and acquired immunity. Failures are associated with inflammatory and autoimmune diseases. Understanding their stimulation is the basis for therapeutic targeting. Members of the Tec kinase family (Bmx, Btk, Itk, Rlk and Tec), expressed in the haematopoietic system, constitute the second largest family of non-receptor tyrosine kinases. Mutations in btk represent the source of human X-linked agammaglobulinemia (XLA). A mutation in the murine btk gene accounts for a similar syndrome, X-linked immunodeficiency (xid). Although the Tec family members Tec, Btk and Bmx are expressed in monocytes/macrophages, little is known about their function there. Tec kinases become activated upon signaling via divers receptors including antigen receptors, receptor tyrosine kinases or TLRs. Several studies in XLA or xid macrophages and in monocyte/macrophage cell lines implicated roles for Tec kinases in TLR signaling and as well as other macrophage effector functions like phagocytosis. Inspired by these findings, we aim to determine the role of Tec kinases in bone marrow-derived macrophages (BMM), during macrophage activation and in other macrophage functions such as recruitment or phagocytosis. In a comprehensive functional analysis of TLR-mediated BMM activation from mice deficient for one or more of the Tec family members in vitro, we reveal which of the kinases play a role in which TLR pathway. Based on the results of this analysis, we set the goal to further study how Tec kinases regulate the respective signaling cascades. Our study will contribute insights into the role of Tec kinases in this important cell population of the innate immune system.

A CAT VACCINE BASED ON CARRIER-BOUND FEL D 1-DERIVED PEPTIDES FOR BY-PASSING ALLERGEN-SPECIFIC IGE AND T CELL REACTIVITY

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Allergen-specific immunotherapy (SIT) is the only disease-modifying therapy for IgE-mediated allergy but can induce IgE- and T cell-dependent side effects. We have developed a vaccine for cat allergy based on a recombinant fusion protein consisting of the PreS domain of Hepatitis B Virus (HBV) and two surface-exposed peptides derived from the major cat allergen, Fel d 1. The peptides were selected from the Fel d 1 sequence to eliminate allergen-specific IgE and T cell reactivity. Using a murine model, it is demonstrated that the vaccine induces allergen-specific IgG antibodies which inhibit allergic patients IgE reactivity to similar extent as IgG antibodies induced by vaccination with complete Fel d 1. According to the hapten-carrier principle described by Benacerraf the T cell help for the Fel d 1-specific IgG responses was derived from the HBV-carrier protein. Unlike the complete Fel d 1, the carrier-based vaccine did not induce reaginic Fel d 1-specific IgE antibodies indicating lack of allergenicity. The Fel d 1-specific IgG response could be augmented by pre-immunization with the HBV carrier protein and boosted without apparent need for T cell help by an isolated Fel d 1-derived peptide without HBV carrier. The vaccine can be easily produced by recombinant expression in E. coli in large quantities. It differs from the earlier described Fel d 1 T cell-epitope-based vaccines because it eliminates Fel d 1-derived T cell epitopes to a large extent. Furthermore, it lacks IgE-related allergenic activity but induces Fel d 1-specific protective IgG responses and thus should be useful for side-effect-free therapeutic and eventually prophylactic vaccination against cat allergy.

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ESTABLISHMENT OF A MURINE MODEL OF MIXED CHIMERISM AND TRANSPLANTATION TOLERANCE IN T CELL SENSITIZED RECIPIENTS

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While many tolerance protocols employing chimerism have shown to work well in small animal models with the help of co-stimulation blockers, translation to large animal and non-human primate models has been less successful. One probable cause for these failures may have been the numbers of previously primed T cells, acting to a subsequent challenge involving familiar or cross-reactive antigens. Current murine models of transplantation employ the use of specific pathogen free mice, effectively maintaining their naive immune repertoire, which falls short of modelling an immune history seen in clinical models. The establishment and therapeutic challenging of such a model is of high interest to the field of chimerism and transplantation tolerance.

In light of this issue, our study involved the adoptive transfer of sensitized T cells, in order to establish a murine model of preceding recipient sensitization to better mimic the clinical setting. Sensitized allospecific T cells were first generated via a mismatched tail skin graft from Balb/C (H-2d) onto C57BL/6 (H-2b) mice. Once sensitization was confirmed by anti-donor IgG levels, lymph and splenic T cells were harvested. A non-myeoablative chimerism protocol of 20x106 Balb/c bone marrow cells (BMC), using costimulation blockade consisting of CTLA4Ig and anti-CD154, was employed for this model. Administration of 30x106 T cells from sensitized B6 mice abrogated the induction of chimerism despite the current protocol (5/22 chimeras vs. 22/28 chimeras for control group, p= 0.01). Preliminary data has shown that anti-LFA, a therapeutically relevant T cell costimulation and migration blocker, when applied to this presensitized recipient model, has the ability to promote chimerism, despite transfer of sensitization in comparison to T cell sensitized control group (3/5 chimeras vs. 0/5 chimeras). Donor skin graft acceptance is currently being followed in anti-LFA-1 treated mice.

Further studies planned include challenging this model of preceding recipient sensitization against therapeutic protocols involving other drugs of clinical relevance, such as Infliximab and Bortezomib. The further development and investigation of this model would be of great interest for use in mixed chimerism and tolerance studies, better mimicking the clinical situation.

ANALYSIS OF THE CELLULAR CROSS-REACTIVITY BETWEEN NON-SPECIFIC LIPID TRANSFER PROTEINS FROM HAZELNUT AND PEACH

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Non-specific lipid transfer proteins (nsLTPs) are important plant food allergens in the Mediterranean area associated with symptoms ranging from oral allergy syndrome (OAS) to anaphylactic shock. The cross-reactivity between nsLTPs from different foods is discussed to be limited. We aimed to analyse the allergic T cell response to Cor a 8, the nsLTP from hazelnut and its potential cross reactivity with Pru p 3, the nsLTP from peach. First, PBMC from 16 Cor a 8 and Pru p 3-sensitized, food allergic individuals were stimulated with rCor a 8 to generate allergen-specific T-cell lines (TCL). These cultures were stimulated with dodeka peptides representing the entire amino acid sequence of Cor a 8 to identify its T-cell epitopes. Several T-cell activating regions were identified spreading along the entire molecule. In contrast to Pru p 3, which contains the immunodominant epitope Pru p 361-75, no immunodominant epitope was identified in Cor a 8.
Cross-reactivity between Cor a 8 and Pru p 3 was examined using allergen-specific TCL generated with either rCor a 8 or nPru p 3. Out of 16 Cor a 8 generated TCL, 10 proliferated in response to Pru p 3 whereas only 2/12 Pru p 3 generated TCL cross-reacted with Cor a 8. We currently investigate which T-cell epitopes in both allergens are involved in cross-reactivity.

Analysing the T-cell response and cellular cross-reactivity between members of the nsLTP family will provide a basis for the development of therapeutic approaches to treat this life-threatening food allergy.

POSTER PRESENTATION

Trop2 is a novel marker for Langerhans cells
Gregor Eisenwort, E. Glitzner, J. Jurkin and H. Strobl

The role of dendritic cells in psoriasis
Elisabeth Glitzner, M. Holcmann, B. Drobits, H. B.Schönthaler, E. F. Wagner and M. Sibilia

Rejection of bone marrow triggered by donor T cells is associated with an increase in IL-17A and IL-6
Karin Hock, N. Pilat, U. Baranyi, C. Klaus, M. Gattringer, H. Ramsey, F. Muehlbacher and T. Wekerle

Specificity of T-cell Responses after Tick-borne Encephalitis Virus Infection and Vaccination
Julia Schwaiger, J. H. Aberle, K. Stiasny and F. X. Heinz

Purification protocol for the enrichment of wheat antigens involved in active celiac disease
Bharani Srinivasan, C. Constantin, I. Swoboda, M. Focke Tejkl, I. Mittermann, H. Vogelsang, W. D. Huber and R. Valenta

Role of Epidermal Growth Factor Receptor in inflammation induced colorectal cancers
Sriram Srivatsa, H. Lanaya, S. Rost and M. Sibilia

Analysis of the blocking capacity of birch pollen specific-immunotherapy induced antibodies on cross reactive food allergens.
Brinda Subbarayal, N. W. de Jong, C. Ebner, N. Reider, R. G. van Wijk and B. Bohle

Molecular analysis of Hdac1 function in $CD8^+$ T cells
Roland Tschismarov, N. Boucheron, R. Grausenburger, P. Matthias, C. Seiser amd W. Ellmeier

Recombinant proteins for the Analysis of the Humoral Immune Response to Yellow Fever Vaccination
Oksana Vratskikh, J. Zlatkovic, K. Stiasny amd F. X. Heinz.

Phenotypic and functional characterization of Th1 and Th17 cells in the peripheral blood and skin of psoriatic patients
Jiang Zhou, F. Koszik, P. M. Brunner and G. Stingl

Registration

Registration will take place at the registration desk, which is located in the admission area in Hörsaalzentrum Level 8 of the General Hospital. Registration is open from 8:30 to 9:00 and during breaks. Admission to all scientific sessions is free.

Language

Official workshop language is English.

Congress Venue

General Hospital Vienna, Austria
Lecture Hall 2, Hörsaalzentrum Level 8
Währinger Gürtel 18-20
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Access to the Venue