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Chance favours only the prepared mind
Louis Pasteur



Recently we have celebrated the 40th anniversary of our Institute with a scientific symposium at the historical building of the Society of Physicians in Vienna. The intention of this event was to recapitulate essential developments since 1967 as well as to reconcile the achievements in scientific areas where members of the Institute had contributed significantly. Prof. Georg Stingl has called to our mind the circumstances of the foundation of our Institute as the first academic institution in German speaking countries explicitly devoted to the scientific specialty Immunology in his brilliant lecture on "Borschkegasse 8a - A remarkable experiment".

He portrayed the important personalities who decisively influenced our Institute so that it was possible to assume a key position in the scientific community of the Medical University of Vienna. The scientific part was devoted to the main topics of the research focuses of the last decades. Christophe Caux, Michael Neuberger, Fiona Powrie and Mirjam Heemskerk gave exceptional presentations on their particular field of expertise and very impressively demonstrated the enormous achievements in these immunological areas. The programme was rounded off by Reinhold Schmidt, who accentuated the position of the Institute in the international immunological community.

A recent far-reaching event has been the reorganization of the Medical University of Vienna, which brought about the formation of larger organization units. According to the development plan of the Medical University of Vienna a main goal is to unite institutions with a strong focus on immunological research under the umbrella of a superordinated organization unit in order to bundle overlapping activities. After the allocation of the Institute of Immunology in 2007 very recently the Institute of Hygiene and Medical Microbiology has become part of the Centre of Physiology, Pathophysiology and Immunology and as such will strengthen our research activities also with regard to infection-related activities.

One of the main upcoming challenges of the near future will be to realize the relocation of the Institute, which is imminent because of reconstruction and long overdue adaptation of the present Institute building. In addition to finding a proper location it will particularly be necessary to consider the increased place requirements as well as to face future developments with regard to equipment and instrumentation.

Also the recent past has been a very prosperous time for our Institute exemplified by raising significant and prestigious third-party funds as well as by publishing our findings from scientific projects in high-ranked journals. Recently, W.F.Pickl was highly successful in representing the Austrian Society for Allergology and Immunology (ÖGAI) in the competition for the European Congress of Immunology, ECI 2015, proposing Vienna as Congress venue. Excellent preparation and presentation of the bid will bring more than 5000 scientists to Vienna in the year 2015. All these achievements would not have been possible without the engagement and the support of the many colleagues at the Institute and of the many collaborators from other institutions who have put forth their continuous efforts. I therefore wish to express my sincere thanks to all who have unremittingly contributed to our common enterprise and I hope that there will be many further years as pleasant and as successful as the time gone by.

Gerhard Zlabinger

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Our long-term research interest is to understand molecular mechanisms that regulate the development and function of immune cells. The research in our laboratory focuses on studies to investigate epigenetic and transcriptional control mechanisms that regulate CD8 coreceptor expression, on the identification of genes that control developmental processes such as CD4/CD8 cell fate decision during T cell differentiation, and on studies to reveal the various roles of Tec family kinases in different cell lineages of the immune system. The experimental strategies to address our research interests include biochemical and molecular approaches, retroviral-mediated gene transduction into hematopoietic stem cel-

MOLECULAR MECHANISMS OF T CELL DEVELOPMENT

We are interested to understand how the CD4/CD8 cell fate decision of double-positive (DP) thymocytes into helper or cytotoxic T cells is regulated. The transcriptional regulation of the Cd4 and Cd8 genes is tightly linked to the functional program of T cells. It is conceivable that factors that regulate CD4 and CD8 expression are also involved in directing DP thymocytes towards the helper or cytotoxic lineage, respectively. Therefore, it is important to understand how the coreceptor genes are transcriptionally regulated during T cell development and to identify cis-regulatory elements and trans-acting factors involved in their regulation. This will not only provide insights into transcriptional control mechanism in T cells, but may also lead to the identification of molecular factors which are involved in cell fate specifications during T cell development.

Ongoing studies in the laboratory aim to further characterize Cd8 cis-regulatory elements by performing (combinatorial) enhancer knockout experiments, to isolate additional Cd8 enhancer binding factors, and to study in detail the role of the BTB zinc finger transcription factors MAZR and PLZF during T cell development (Ref. 1). Moreover, we are investigating the role of members of the histone deacetylase family in T cells.

Transcriptional control of Cd8 gene expression during T cell development

Our laboratory is studying the regulation of CD8 coreceptor expression, a key molecule in the immune system for the development of the cytotoxic T cell lineage. CD8 coreceptor expression is tightly regulated during thymocyte development by the activity of at least five different cis-regulatory elements. We recently linked Cd8 enhancer function with chromatin remodeling of the adjacent genes Cd8a and Cd8b1 (Cd8) and demonstrated epigenetic control of the Cd8 gene complex (Bilic et al., 2006, *Nature Immunology*, 7, 392).

We further aim to identify Cd8 enhancer binding factors. Since important Cd8 cis-regulatory elements are expected to be evolutionary conserved, a cross-species comparison of the Cd8a and Cd8b genomic loci to search for evolutionary conserved regions (ECR) was performed. This approach revealed several ECRs ranging from 200bp to 500bp within known Cd8 enhancers, which can be used as molecular baits for the isolation of binding factors. Moreover, this approach led to the identification of a novel evolutionary conserved Cd8 enhancer element that is currently analyzed in more detail. Our results demonstrate that a combination of bioinformatic and biological approaches is a powerful tool to identify new cis-regulatory elements at complex regulated gene loci.

Molecular analysis of the zinc finger transcription factor MAZR

We have recently identified that the BTB domain-containing zinc finger protein MAZR is an important regulator of CD8 expression. MAZR binds to Cd8 enhancers and forced expression of MAZR during T cell development induced variegated CD8 expression, most likely due to co-recruitment of repressor complexes (Bilic et al., 2006, *Nature Immunology*). This indicates that MAZR regulates chromatin remodeling at the Cd8 gene complex (see Figure 1). We recently generated MAZR-deficient mice to learn more about the role of MAZR in T cells and other cells of the hematopoietic systems. Ongoing studies focus on the analysis of MAZR-deficient mice.

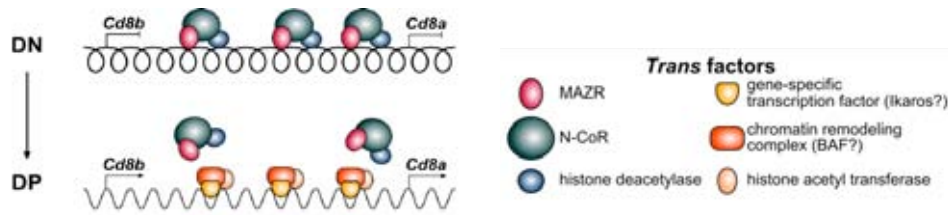


Fig. 1: Model of MAZR function. In DN thymocytes, MAZR binds to the Cd8ab gene complex, and via recruitment of nuclear corepressor (N-CoR)-containing complexes represses CD8 expression. In DP thymocytes, less MAZR is bound to the Cd8ab gene complex, and thus probably facilitating the recruitment of positive-acting chromatin remodeling complexes (model after Bilic et al., 2006, Nature Immunology, 7, 392).

In addition, to understand the molecular mechanisms of how MAZR regulates the expression of CD8, we are aiming to identify MAZR interacting factor using tandem affinity purification strategies and mass spectroscopy approaches.

The role of histone deacetylases in T cell development and function

Reversible changes in histone acetylation patterns have been shown to accompany many important processes in T cells ranging from VDJ recombination during T cell development to the induction of cytokine expression during Th1/Th2 effector differentiation. Modification of core histones by lysine acetylation is controlled by histone acetyltransferases and histone deacetylases, which are considered as transcriptional co-activators and co-repressors, respectively. Eighteen histone deacetylases (HDACs) have been identified in mammalian organisms, however dissecting individual roles for each member of the HDAC family in specific cell lineages and tissues remains a major scientific challenge. In a close collaboration with the research group of Christian Seiser (Max F. Perutz Laboratories, Vienna) we are analyzing the role of certain members of the HDAC family in T cells.

Transcriptional control of memory-phenotype T cell development by PLZF

Peripheral CD4⁺ and CD8⁺ T cell subsets have been traditionally divided into naïve CD44^{lo}CD62L⁺ and memory CD44^{hi}CD62L⁻ populations, while the surface expression phenotype of the latter population also resembles recently activated T cells. However, the memory T cell subset is not a population consisting only of true antigen-specific memory cells that developed in response to a foreign antigen. Rather, the memory population contains in addition a variety of different T lymphocyte subsets, some of which acquired their memory phenotype through homeostatic proliferation, while others have immediate effector function and may play a role in the front-line defense against certain bacterial infections (“innate-like” T cells). The signaling pathways and transcriptional networks that regulate the developmental cell fate decisions between conventional and innate-like memory-phenotype T cell subsets are not well understood.

We recently observed that the BTB domain-containing transcription factor PLZF (promyelocytic leukemia zinc finger) is expressed in CD44^{hi} memory-phenotype but not in naïve CD44^{lo} CD4⁺ T cells. To investigate a potential role of PLZF in either the generation and/or function of the CD44^{hi}CD4⁺ T cell subset, we performed gain-of-function experiments and generated PLZF transgenic mice. With this experimental approach we could show that PLZF is a crucial transcriptional regulator that induces the development of CD44^{hi} memory-phenotype T cells with innate-like characteristics (Ref. 9). Ongoing studies address mechanistic aspects of how PLZF regulates the development of CD44^{hi} memory-phenotype T cells.

TEC FAMILY KINASES AND SIGNALING PATHWAYS IN IMMUNE CELLS

Members of the Tec kinase family (Bmx, Btk, Itk, Rlk and Tec) constitute the second largest family of non-receptor tyrosine kinases and are preferentially expressed in the hematopoietic system (see Figure 2). A large number of studies have shown important roles for these kinases in the lymphoid system. Furthermore, mice with combinatorial deletions of Tec family kinases revealed both unique and redundant functions in B cells (Tec, Btk) and T cells (Rlk, Itk). In ongoing studies we are investigating in more detail the role of Tec family kinases in T cells.

Although Tec family kinase members are also expressed in myeloid cells, little is known about their function in this lineage. To learn more about the role of Tec kinases in myeloid cells, we are analyzing the function of primary myeloid cell lineages such as mast cells and monocytes/macrophages lacking single and/or multiple Tec kinase family members. Our studies will contribute to a better understanding of the immunomodulatory role of Tec kinases in cells of the immune system, and may on the long-term also help to indicate strategies for potential therapeutic intervention.

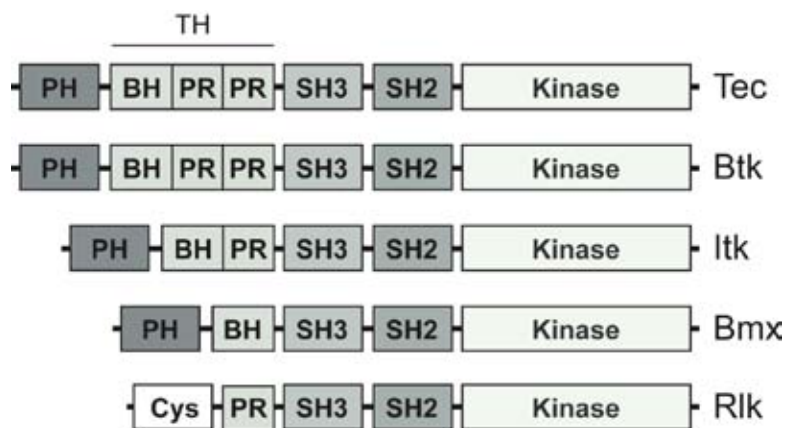


Fig. 2: Modular Structure of Tec Family Kinases. The pleckstrin homology (PH) domain located at the N-terminus of the molecule is the most characteristic feature of Tec family kinases (with the exception of Rlk). PH domains are able to bind phospholipids (or proteins) and are thereby involved in the recruitment of Tec kinases to the membrane. The Tec homology (TH) domain is formed by a Btk homology (BH) motif and by one or two proline-rich (PR) regions, and has been implicated in the auto-regulation of Tec kinases. The TH domain is followed by Src homology (SH) domains SH3 and SH2. The N-terminal palmitoylated cysteine-rich sequence of Rlk mediates membrane anchoring (drawing is taken from Ref. 12).

The role of Tec family kinases in T cells

Itk and the GDP/GTP guanine exchange factor Vav1 act in similar T cell activation pathways. Both molecules interact with members of the Cbl family of E3 ubiquitin ligases, and signaling defects in Vav1^{-/-} T cells are rescued upon deletion of Cbl-b. We investigated the relation between Itk and Cbl-b or Vav1 by generating Itk/Cbl-b and Itk/Vav1 double-deficient mice. We could show that Itk and Vav1 act mechanistically similar in peripheral T cells, since the defects in Itk^{-/-} T cells, like in Vav1^{-/-} T cells, are rescued if cells are released from the negative regulation mediated by Cbl-b. Moreover, our studies revealed that the combined activity of Itk and Vav1 is required for proper T cell development and the generation of the peripheral T cell pool (Ref. 8).

Moreover, in collaboration with Edvard Smith (Karolinska Institute, Sweden) we recently characterized the transcriptome of Itk-deficient T-cells, including CD4⁺ and CD8⁺ subsets, using Affymetrix microarrays, and provided a general overview about the global transcriptional changes in the absence of Itk (Ref. 11). Currently, differentially expressed genes are analyzed.

The role of Tec family kinases in myeloid cells

We identified important processes in myeloid cells that are regulated by Tec kinases. We demonstrated that Tec is a crucial regulator of mast cell function, since Tec-deficient mast cells have an impaired effector function upon FcεRI stimulation (Ref. 13). In addition, we showed that Tec and Btk regulate M-CSFR signaling-induced macrophage survival (Ref. 7). Currently, we investigate in detail how macrophage function is regulated by Tec family kinases.

Grants

START-Program, Ministry of Education, Science and Culture (BMBWK) „Molecular mechanisms of Lymphocyte Development and Activation“ 11/2001 – 10/2008

FWF (Austrian Science Fund) SFB F-2301 „Mechanisms of Establishment and Maintenance of Immunological Tolerance“ SFB Administration and Coordination Project 3/2005 – 8/2009

FWF (Austrian Science Fund) SFB F2305, „Signaling requirements for the induction and maintenance of T cell tolerance“ 3/2005 – 8/2009

EU Marie Curie Research and Training Network on “Chromatin Plasticity” (MRTN-CT-2006-035733) 10/2006 – 10/2010

FWF (Austrian Science Fund) P-19930-B10 “Molecular characterization of the transcriptional regulator MAZR” 8/2007 – 7/2010

FWF (Austrian Science Fund) & MUW DK-W12 „Inflammation and Immunity“, subproject 2, since 10/2007
WWTF (Vienna Science, Research and Technology Fund) (LS09-031) „Epigenetic regulation of T cell development and function“, since 10/2009

Theses**Diploma theses**

Matthias Hombauer: Molecular studies on the transcriptional regulation of the Cd8ab gene loci during T cell development (completed November 2008)

Beatrice Grabner: The regulation of T cell effector differentiation by PLZF (since September 2008)

PhD Theses

Julia Raberger: Studies on the role of the tyrosine kinase Itk in T cell development and function (completed March 2009)

Shinya Sakaguchi: Loss of function analysis of the BTB zinc finger protein MAZR (completed March 2009)

Hammad Hassan: Molecular mechanism of Cd8 enhancer function (since July 2006)
 Wolf Henning Gebhardt: Identification of MAZR binding factors (since April 2007)
 Derya Köprülü : The role of Tec family kinases in macrophages (since September 2007)
 Anastasia Abramova: The role of MAZR in myeloid cells (since October 2007)
 Roland Tschismarov: Regulation of T cell effector function (since November 2008)
 Matthias Hombauer: The role of MAZR in peripheral T cells (since January 2009)

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T cell activation is the result of a sustained antigen-specific interaction of a T lymphocyte with a professional antigen-presenting cell (pAPC) in a secondary lymphatic tissue. The detailed description of the molecular and functional events within the immunological synapse formed by T cells and pAPC is one of the keys to the better understanding of adaptive immune responses and their modulation. Model systems in which the immunological synapse (Fig. 1) can be rebuilt with receptors relevant for human diseases shall enable us to study the pathophysiology of allergies, autoimmune and infectious diseases as well as cancer in greater detail and may thus lead to novel strategies for their cure.

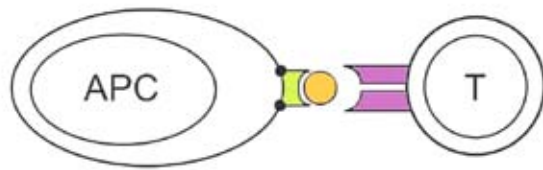


Fig. 1: The immunological synapse:
HLA molecules (green) on antigen presenting cells (APC) present antigen-derived peptides (orange) to the TCR (violet) on T cells

Direct stimulation of T lymphocytes by virus-like particles decorated with immune receptors of choice

Inducible virus-like particles (VLP), decorated with T cell ligands of choice are directly immunogenic for purified T lymphocytes. In the past we found that lipid rafts are the keys to inducible VLP production and the selective enrichment on VLP of immunologically relevant molecules (Derdak et al., PNAS 2006, 103:13144). At present we are interested to test our system for its capability to modulate allergen-specific T lymphocytes within the FWF (Austrian Science Funds)-funded special research project (SFB) "Molecular and immunological strategies for prevention, diagnosis and treatment of Type I allergies".

Modulation of allergen-specific T-lymphocyte function by virus-like particles decorated with HLA class II molecules

TH2-lymphocytes play an important role in the induction and maintenance phase of type I allergy. Modulation of the responses of TH2-lymphocytes by novel forms of antigen-presenting platforms may help shape the immune response to allergen and palliate allergic diseases. Along those lines we have presented HLA class II/allergen-peptide complexes on virus-like particles (VLP) and have evaluated their potential to modulate allergen-specific T-cell responses. VLP that express the immunodominant T-cell epitope Art v 125-34 of the major mugwort pollen allergen in the context of HLA-DR1 and costimulatory molecules were produced by transfection of 293 cells. The effect of VLP on IL-2 promoter activity, proliferation and cytokine production of allergen-specific T-cells derived from mugwort pollen-allergic and non-allergic donors was determined. Flow cytometric analyses showed that HLA class II molecules, invariant chain(Ii)::Art v 1-fusion proteins and costimulatory molecules were expressed on 293 cells. Biochemical analyses confirmed that these molecules were efficiently targeted to VLP. The engineered VLP activated Art v 1-specific T-cells in a costimulation-dependent manner. VLP lacking costimulators induced T-cell unresponsiveness, which was overcome by addition of exogenous IL-2. Costimulation could be provided by CD80, CD86 or CD58 and induced distinct cytokine profiles in allergen-specific T-cells. Unlike the other costimulatory molecules, CD58 induced IL-10/IFN-g-secreting T-cells. In conclusion, VLP represent a novel, modular, acellular antigen-presenting system able to modulate the responses of allergen-specific T-cells in a costimulator-dependent fashion. Allergen-specific VLP show promise as tools for specific immunotherapy of allergic diseases. (Leb et al. J Allergy Clin Immunol, 2009, 124:121). These studies were performed in close collaboration with Drs. Barbara Bohle and Beatrice Jahn-Schmid, Institute of Pathophysiology, Vienna.

Furthermore, we are currently exploiting particle-bound cytokines (Kuong et al., J Virol 2007, 81:8666) as modulators to re-polarize allergen-specific helper T cells (Allergy-SFB) and as novel adjuvants in order to create 'better viral vaccines' within a 'bridge-project' funded by the Austrian Forschungsförderungsgesellschaft (FFG) in co-operation with Biomay AG.

Molecular and functional analysis of antigen receptors of allergen-specific helper T lymphocytes

Although TCR gene usage by allergen-specific T lymphocytes has been studied in considerable detail in the past, molecular or functional analyses of cloned and ectopically expressed human allergen-specific TCR remained scarce so far. In a very fruitful co-operation with Doz. B. Bohle and Dr. B. Jahn-Schmid, Institute for Pathophysiology, Medical University of Vienna, we have identified and functionally characterized a mugwort-specific TCR recently (Leb et al. J Allergy Clin Immunol, 2008, 121:64). At the moment we are about to clone and characterize TCRs specific for Bet v 1, the major birch pollen allergen. We are the first group world-wide to present detailed molecular and functional analyses of human allergen-specific TCRs. Expressible allergen-specific TCRs may contribute to a better definition of the 'allergen-specific synapse' and thus the processes leading to allergic diseases and their cure. These studies are performed within the Christian Doppler Laboratory "Immunomodulation".

Fluorescent virus-like particles as novel platform to identify immune-receptor/ligand interactions on leukocyte subsets

Virus-like particles (VLP) represent a versatile platform for the display and transport of diverse biologically active immunomodulatory molecules. In this study we explored whether VLP labeled in vivo by recombinant fluorescent proteins such as GFP of *Aequorea victoria* could be used to visualize specific immune-receptor/ligand interactions. To date, translational fusions of viral proteins with GFP have been broadly used to elucidate infectious pathways of viruses. Since lipid-rafts are the meeting point for GPI-anchored surface molecules and viral core proteins we hypothesized that fluorescent proteins linked to lipid-raft targeted viral core proteins might accumulate in sufficiently high abundance to generate fluorescently labeled VLP that could be used to track the fate and interactions of VLP in different settings. We examined whether fluorescent VLP can be used to visualize immune-receptor/ligand interactions. We demonstrated that co-expression of GFP-tagged MoMLV matrix protein (MA), MoMLV gag-pol and IL-2::GPI generates IL-2 decorated MA::GFP+VLP, which can specifically identify normal and malignant IL-2 receptor (IL-2R) positive lymphocytes by flow cytometry. Apart from other cytokines or transmembrane molecules MA::GFP+VLP were successfully decorated with the heterotrimeric IL-2R, allowing identification of IL-2+ target cells. Thus, fluorescent VLP with membrane-targeted fluorescent proteins are useful tools for assessing mono- and multi-subunit immune-receptor/ligand interactions (Kuong et al. submitted). The project was performed in collaboration with Dr. Clemens Scheinecker and Ruth Byrne, Dept. of Rheumatology, MUV, Vienna.

Two newly diagnosed HLA class II deficient patients identified by rapid vector-based complementation reveal discoordinate invariant chain expression levels

Primary immunodeficiencies (PID) reveal the 'molecular Achilles' heels' of human immunity. Detailed analysis of PID extend our knowledge of pivotal immunological processes, leads to novel diagnostic algorithms and shortens time-to-diagnosis. We have a special interest in the detailed analysis of immunodeficiencies concerning constituents of the immunological synapse. Along those lines, the clinical/immunological phenotypes of two unrelated combined immunodeficiency (CID) patients from Austria were determined. Leukocyte subpopulations of patients, their parents and healthy controls were analyzed by flow cytometry. Patient-derived EBV transformed B cell lines were established and complemented by candidate cDNAs. Suspected mutations were confirmed by DNA sequencing. Phenotyping revealed a lack of constitutive HLA class II expression on antigen presenting cells of both patients, compatible with MHCII deficiency. Rapid vector-based complementation of patients' B cells identified HLA class II transactivator(CIITA)-deficiency in patient VIP1 and restriction factor X(RFX)AP-

deficiency in patient VIP2. CIITA-deficiency was caused by a homozygous p.Glu381X mutation. RFXAP-deficiency resulted from a homozygous p.Ser123ThrfsX15 mutation, not described in the Middle European population so far. Of note, HLA class II associated invariant chain (Ii) expression levels were significantly reduced in VIP1 and three additional EBV transformed B cell lines of CIITA deficient patients but normal in VIP2 EBV transformed B cells. In addition, peripheral blood B cells of VIP1' parents showed significantly reduced HLA-DR and -DP expression levels compared to healthy controls. In summary, analysis of patients' intracellular Ii and parents surface HLA class II expression levels might help to identify CIITA-deficient patients already during initial phenotyping. These studies were performed in close collaboration with Drs. Susanne Matthes and Markus Seidl from the St. Anna Childrens' Hospital, Vienna (Schmetterer et al. submitted).

Immature CD21- B lymphocyte numbers predict the response to extracorporeal photopheresis in patients with chronic graft-versus-host disease

Chronic graft-versus-host-disease (cGVHD) is a major complication of allogeneic hematopoietic stem cell transplantation (HSCT) and a leading cause of nonrelapse mortality resulting from profound immunodeficiency. Extracorporeal photopheresis (ECP) achieves high response rates in patients with steroid-refractory chronic graft-versus-host disease (cGVHD) after allogeneic hematopoietic cell transplantation. However, no biomarkers for response prediction and monitoring of patient outcome are available. In close cooperation with the research group of Dr. Hildegard Greinix, Department of Internal Medicine I, MUV, Vienna, we have performed a prospective study on 49 patients receiving ECP for moderate (n=25) and severe (n=24) cGVHD. Evaluations consisted of clinical parameters (cGVHD severity and treatment response) and analyses of peripheral blood (PB) leukocyte subpopulations. Complete response to 6 months of ECP treatment significantly correlated with lower numbers of immature CD19+/CD21- PB-B cells prior to start of ECP compared to ECP nonresponders (8% vs. 22%, p=0.02). Serial analyses of B cell subsets revealed a significant decrease of immature CD19+/CD21- PB-B cells in ECP-responders (13.7% to 6.8%, p=0.022) but not in ECP-nonresponders. After 6 months ECP-responders had significantly lower immature CD19+/CD21- PB-B cells compared to ECP-nonresponders (5% vs. 25%, p<0.001). Twelve and 21 months after start of ECP patients with resolved but not those with persistent active cGVHD demonstrated a normalization of immature CD19+/CD21- PB-B cell numbers (p>0.001). In conclusion, assessment of immature CD19+/CD21- PB-B cell numbers in patients with cGVHD allows prediction of response to ECP and could serve as biomarker for measuring disease activity of cGVHD (Kuzmina et al., Blood, 2009, 114:744)

Grants

- FWF (Austrian Science Fund), Winfried F. Pickl, „Further development of anergosomes for anergisation of allergen-specific T lymphocytes: The role of membrane bound cytokines during T cell activation' "SFB-Molecular and immunological strategies for prevention, diagnosis and treatment of Type I allergies" 2/2008 - 1/2012
- FFG (Österreichische Forschungsförderungsgesellschaft) #812079, Winfried F. Pickl „Membrane anchored growth factors as novel adjuvants for virus vaccines' bridge-project" 11/2006 - 11/2010
- CDG (Christian Doppler Society), Winfried F. Pickl „Molecular cloning and functional characterization of allergen-specific TCRs", subproject in 'Christian Doppler Laboratory for Immunomodulation' 12/2007 - 12/2014

Thesis

Diploma theses

Manta, C.: Fluorosomes: Fluorescent virus-like particles for detection of receptor/ligand interactions. 2008-2009 (completed).

PhD theses

Leb, V. M., MSc.: Influencing the allergen-specific immune response at the level of antigen presentation: Anergosomes. 2003-2009 (completed)

Kueng, H. J., MSc.: Modulation of immune responses with virus-like particles decorated with cytokines and/or integral membrane proteins. 2005-current (ongoing)

Schmetterer, K. G., MSc.: Characterization of molecular mechanisms involved in the modulation of leukocytes. 2006-current (ongoing)

Neunkirchner, A., MSc.: Functional evaluation of molecules within the ESCORT pathway concerning their contribution to vesicle secretion. 2006-current (ongoing)

Haiderer, D., MSc.: Development of better viral vaccines using membrane-bound lipid modified cytokines as natural adjuvants. 2006-current (ongoing)

Awards

Karl Landsteiner Award 2007 of the Austrian Society for Allergology and Immunology to Winfried F. PICKL for the publication: Direct stimulation of T lymphocytes by immunosomes: virus-like particles decorated with T cell receptor/CD3 ligands plus costimulatory molecules. *Proc Natl Acad Sci U S A* 2006, 103:13144.

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Division of Immune Receptors and T Cell Activation

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We are interested in the contribution of cell surface molecules (positive and negative costimulatory molecules) on human T cells and antigen-presenting cells (APC) in immune responses. For our studies we have developed experimental systems that make it possible to efficiently analyse the contribution of individual ligands to T cell activation processes. In addition we are trying to identify novel receptors that regulate the activation of T cells by APC. To achieve this goal we rely on our experience in the generation, expression and screening of cDNA expression libraries. Another focus of our research is the identification of cell surface antigens that are recognized by sera derived from cancer patients, transplant recipients or from stem cell transplanted individuals. For these projects we are developing efficient methodologies to screen retroviral expression libraries with polyclonal antibody preparations like human sera.

The role of individual costimulatory molecules in the activation of human T cells

A large number of positive and negative costimulatory molecules govern the interaction of antigen-presenting cells with T cells (Figure 1a). The manipulation of such pathways offers attractive avenues to enhance immunity to pathogens but also to attenuate unwanted responses in autoimmune conditions or transplantation. While the role of most costimulatory molecules has been extensively studied in animal models, functional data on the effects of several of these molecules on human T cells are much more limited. It is evident that a complete understanding of the role of human costimulatory molecules is a prerequisite to identify good therapeutic targets.

We are trying to learn about the contribution of individual receptor-ligand pairs to T cell activation processes and also to identify novel molecules that play a role in this context. We are studying the consequences of costimulatory signals on primary human T cells by assessing classical parameters like proliferation, cytokine secretion and activation-marker regulation. In addition, we are using genome-wide gene expression analysis and biochemical assays on intracellular signalling processes to get a more complete picture on the function of costimulatory signals during the activation of human T cells.

For our studies we have developed a novel system of T cell stimulators. This system is based on the murine Bw5147 cell line that was engineered to express a membrane-bound anti-human CD3 single chain antibody. By ligating their T cell receptor complex, this antibody gives "Signal 1" to human T cells. Upon expression of costimulatory molecules on our stimulator cells, their role in T cell activation processes can readily be studied (Figure 1b). We find this system to be an excellent tool to study the role of individual costimulatory molecules in their natural conformation but detached from the complex surface of an APC that harbours a plethora of costimulators.

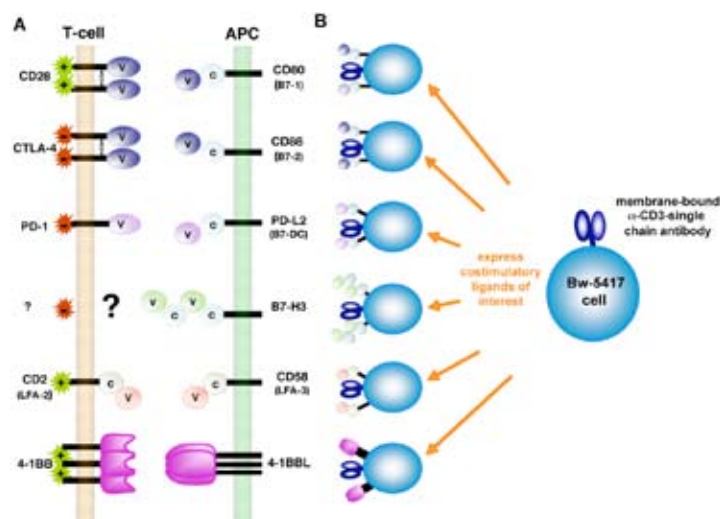


Fig. 1:

A) Selected costimulatory ligands that regulate T cell activation processes by interacting with their activating or inhibitory receptors on T cells.

B) Our system of T cell stimulators is a straightforward and versatile system to study the contribution of individual costimulatory ligands to the activation of human T cells.

Although our current studies focus on members of the B7- and the TNF-superfamilies we have generated a large set of T cell stimulator cell lines expressing additional human costimulatory molecules. Thus we are currently in the unique position to test and compare virtually all human molecules implicated in T cell costimulatory processes in one experimental system. We have recently used this system to analyze the T cell stimulatory capacity of TNF family members. In this study we show that under conditions where 4-1BBL, OX40L, CD70 and GITRL readily costimulated human T cells, CD30L and LIGHT consistently failed to contribute to T cell activation processes, indicating that these molecules might be functionally distinct from the costimulatory members of this family (Kober et al. 2009).

We are currently investigating the interplay between T cell costimulatory signals and immunomodulatory or immunosuppressive drugs. Our preliminary results indicate that the quality and strength of the costimulatory signals greatly affects the capacity of such drugs to inhibit T cell responses (Figure 2). We believe that a better understanding of the interplay between immunosuppressive drugs and costimulatory signals might eventually lead to improved therapeutic strategies to downmodulate unwanted T cell responses.

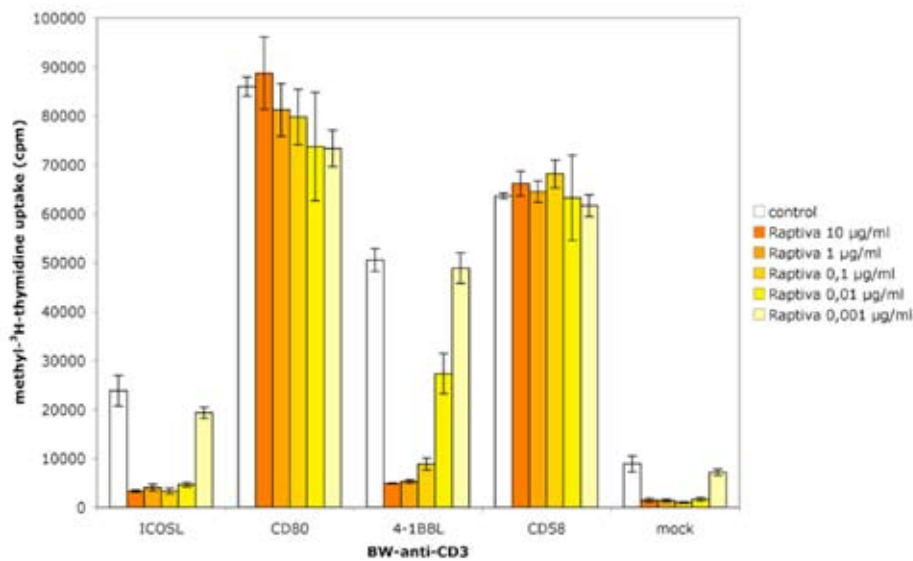


Fig. 2: Strong costimulatory signals exerted by CD58 and CD80 completely abrogate the ability of the therapeutic CD11a-Antagonist Raptiva (Efalizumab) to inhibit the activation of human T cells. Primary human T cells were activated with human T cell stimulator cells expressing costimulatory ligands in the presence of indicated concentrations of Raptiva.

Loss of the costimulatory receptor CD28 is an important phenotypic correlate for senescent T lymphocytes in humans. Such cells are functionally impaired and contribute to immune dysfunction in elderly and chronically infected individuals. Since CD28 negative T cells are deficient in the primary costimulatory pathway, insufficient activating signals might contribute to the functional senescence in this cell population. We are therefore analysing whether signals from alternative costimulatory molecules are able to revert the dysfunctional state of CD28 negative T cells derived from elderly individuals. This project is carried out in close collaboration with the group of Prof. Grubeck-Loebenstein, Institute of Biomedical Aging Research of the ÖAW, Innsbruck, Austria.

In addition we also generate and use immunoglobulin fusion proteins representing the extracellular domains of costimulatory molecules to study their functional role as well as the interaction of costimulatory ligands with their receptors. There is considerable controversy regarding the role of the B7-family member B7-H3 during the activation of T cells since inhibitory as well as activating functions have been ascribed to this molecule. Using fusion proteins representing the extracellular domains of the B7-family member B7-H3, we recently have been able to show that B7-H3 consistently and potently inhibits T cell activation. Furthermore by performing extensive binding experiments with fusion proteins representing B7-H3 and TREML-2, we have been able to show that TREML-2, which was recently claimed to serve as a costimulatory B7-H3 receptor does not interact with B7-H3 (Leitner et al.2009). Thus B7-H3 is an orphan ligand and we are using our B7-H3-immunoglobulin fusion proteins in conjunction with retroviral expression cloning to identify receptors, which mediate the potent T cell inhibitory effects of B7-H3.

Identification and characterization of surface antigens that elicit antibody responses in tumour patients or in individuals transplanted with solid organs or hematopoietic stem cells.

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative treatment for hematological and oncological diseases. Immune response to residual malignant cells (Graft-versus-leukemia; GvL) can contribute to the eradication of the tumours in the transplant recipients. However, graft-versus-host disease (GvHD) is a frequent complication of HSCT, contributing to morbidity and mortality. An important mechanism underlying both effects is the recognition of antigens by the donor derived immune cells. To date most efforts to search for molecules recognized following allogeneic HSCT have focused on the identification of antigens recognized by T cells. However antibodies directed to membrane resident antigens might also play an important role since they might directly eliminate cells by antibody- or complement dependent cytotoxicity. However, efficient methodologies to define such antigens have not been described to date.

Using retroviral expression cloning we have recently identified the surface molecule immunoglobulin transcript 5 (ILT-5) as a polymorphic antigen that is able to elicit potent immune responses in over 5 % of HSCT recipients (Pfistershammer et al. 2009). We demonstrated that ILT5 antibodies efficiently kill ILT5 bearing cells and furthermore observed a very strong increase in the ILT5 antibody titres following leukemic relapse (Figure 3). These results represent the first description of potent allogeneic antibody responses to a non-MHC surface molecule following HSCT and indicate that humoral immune responses might contribute to GvL and GvHD responses.

Our results provide evidence that allogeneic surface antigens that are recognized in HSCT recipients can be identified using eukaryotic expression cloning. In close collaboration with the group of Prof. Greinix at the Bone Marrow Transplantation Unit of the MUW and other centres where HSCT is performed, we have established a large collection of sera from HSCT patients that we plan to use for the identification of additional antigens. Beneficial anti-tumour immunity as well as deleterious immune responses against allogeneic solid organ grafts are of significant clinical relevance. Consequently they have been subjects of intense research during the last decades. In these studies the focus has been on intracellular antigens as well. In close collaboration with Dr. Pfistershammer, who is now at the Department of Dermatology, we have initiated studies to identify non-HLA surface antigens recognized by tumour patients and recipients of renal grafts. Also in these studies we rely on our extensive experience in the screening of eukaryotic cDNA expression libraries with patients' sera. For these projects we collaborate with clinicians who provide us with patients' sera and clinical information.

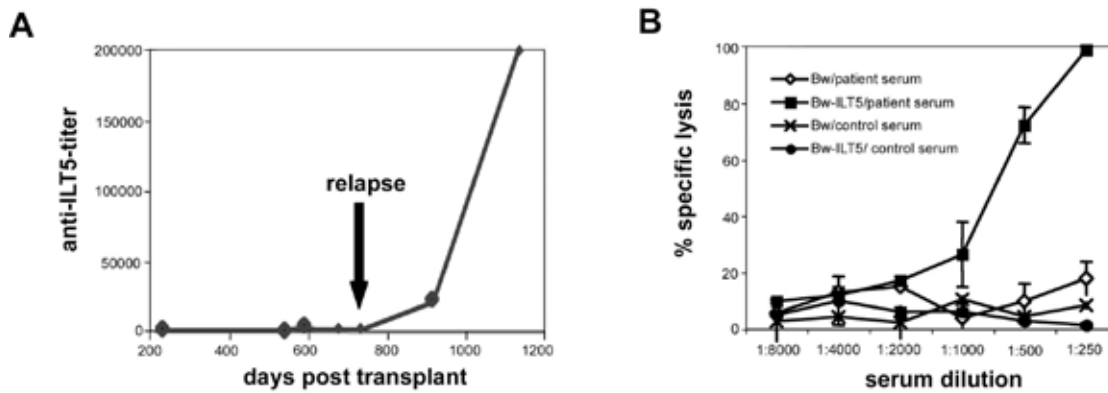


Fig. 3: Humoral immune responses to the polymorphic immunoglobulin-like transcript 5 (ILT5) in hematopoietic stem cell transplant recipients. A) ILT5-specific antibodies strongly increase in a patient following relapse from AML. B) ILT5-reactive sera efficiently kill ILT5 bearing cells by complement-dependent cytotoxicity.

Anti-thymocyte globulins (ATGs) are polyclonal antibody preparations that are widely used as very effective immunosuppressive agents. Recent research has demonstrated that ATGs can exert potent and broad immunomodulatory functions by interacting with different types of T cells including regulatory T cells and with dendritic cells. Currently there is limited information regarding the cellular targets of ATG, which are responsible for their broad immunomodulatory and immunosuppressive effects. By screening an eukaryotic expression library derived from human dendritic cells with ATGs we have been able to identify several DC antigens that are strongly recognized by ATG antibodies. We will employ eukaryotic expression libraries derived from freshly prepared and in vitro activated human PBMC to identify the ATG specificities that are directed to surface molecules expressed on these cells.

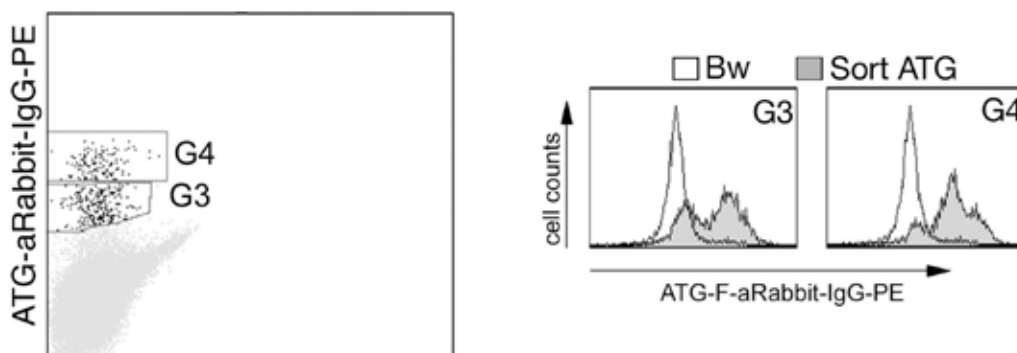


Fig. 4 Screening of a retroviral cDNA-expression library derived from human Dendritic cells with ATGs. Left panel: A cell pool representing the cDNA library was incubated with ATG and cells expressing ATG antigens (Gates 3 and 4) were isolated by FACS. Right panels: The isolated cell pools react with ATG.

Grants

- OeNB (Österreichische Nationalbank) "Responsiveness of CD28-negative T cells to alternative costimulatory signals" 1.1.2008-31.05.2010.
- ÖAW (Austrian Academy of Sciences) Doc Forte fellowship "Identification of tumor antigens using antibodies induced by DC-based vaccines" 1.1.2009 - 31.12.2010 (to Judith Leitner)
- EKFS (Else Kröner Fresenius Stiftung) "Defining the major specificities in ATGs" 1.9.2009-29.02.2012
Bürgermeisterfonds 09051 "Interaktion von Antithymozytenglobulin (ATG) mit dendritischen Zellen" 2009-2011

Theses**Diploma Theses**

- Ramona Woitek: Costimulatory molecules and exogenous cytokines in the long-term expansion of human T-cells, 01.03.2006 - 1.10.2007
- Werner Kuschei: Effects of TNF and CD11a antagonists on T cell costimulatory pathways. Since 1.10.2008

Master Thesis

- Karin Drobits: The interplay between immunosuppressive drug and costimulatory signals in the activation of human T cells. Since 01.08.2009.

PhD Thesis

- Judith Leitner: Identification of tumor antigens using antibodies induced by DC-based vaccines. Since 01.08.2006

Awards

- Judith Leitner: Doc Forte Fellowship awarded 11.2008

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1. Flicker, S., P.Steinberger, P.B.Eibensteiner, S.Lebecque, D.Kraft, P.Valenta. Molecular characterization of a human immunoglobulin G4 antibody specific for the major birch pollen allergen, Bet v 1. *Clin.Exp.Allergy* 38:365-373 (2007)
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Division of Immune Regulation

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Otto Majdic, PhD, Assistant Professor

Present Members

Maria Seyerl, PhD Student
Sabrina Zeiner, Diploma Student
Stefan Hopf, Diploma Student
Petra Cejka, Technician
Claus Wenhardt, FACS-Operator



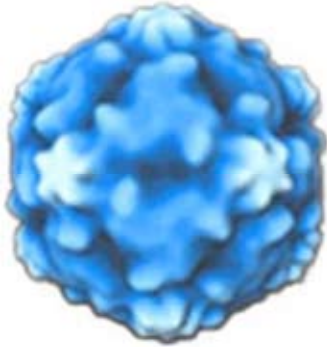
Past Members

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Olga Oskolkova, PostDoc
Nina Gundacker, PhD Student
Christoph Jindra, PhD Student
Catharina Schrauf, PhD Student
Gerlinde Ofner, Biomed. Scientist

The central research interest of our group is a better understanding of regulatory mechanisms between the innate and adaptive immune system. Our group has longstanding experience in the molecular and functional analysis of cells of the human phagocyte lineage, in particular dendritic cells (DCs). It is our long-term goal to learn how phagocyte development and function is regulated by exogenous (pathogen) and endogenous (inflammatory) danger signals and to characterize novel and functional relevant receptors on DCs and T cells via which adaptive immunity can be tuned. In order to find such regulatory mechanisms we utilize commensal pathogens as pathfinders. Commensal microbes such as human rhinoviruses or the fungus *Candida albicans* are typically harmless for most healthy individuals. Thus, the strategies used by the host to limit infectivity are necessarily efficient and, in return, such microbes have developed their own elaborate tactics to circumvent these defense mechanisms. By decoding these tricks we want to find new routes to modulate immune responses in humans.

Human Rhinoviruses (HRV)

HRV are the major cause of the common cold, one of the most frequent infectious diseases in humans. Though HRV infections of the upper respiratory tract are usually rather harmless there is increasing evidence that HRV sets the stage for more dangerous pathogens, elicits asthmatic exacerbations, severe diseases in the lower respiratory tract and even autoimmunity. The pathogenic



mechanisms of HRV infections leading to such complications are still poorly understood. It is a common strategy of pathogens to manipulate our immune system in order to evade an efficient immune response. A major characteristic of HRV is a high degree of species specificity. Thus, analyzing the potential immune evasion mechanisms used by HRV will be helpful for a better understanding of the pathogenesis of the common cold and the human immune system as well.

Dendritic cells (DCs) utilize pattern recognition receptors (PRRs) to sense invading viruses and triggering of these receptors induces a maturation program. HRV belong to the family of Picornaviridae, which have a single-stranded coding RNA genome. Since HRV does not replicate in DCs, we used genomic RNA from HRV in this study to analyze the impact of natural occurring single-stranded viral RNA (ssv-RNA) on DC function. We found that transfection of human monocyte-derived DCs with ssv-RNA induced type-I IFN production but failed to activate the NF- κ B pathway in DCs. In line with this observation, the upregulation of typical maturation markers such as CD83 or the production of the proinflammatory cytokines IL-12p40, IL-6 and TNF α was not detectable. Most importantly, the T cell stimulatory capacity of ssv-RNA-treated DCs was not enhanced and remained at the level of immature DCs. Taken together, our results demonstrate that ssv-RNA efficiently activates the innate defence arm of DCs while it is insufficient to activate the stimulatory capacity of DCs for the adaptive defence responses.

Moreover, we could demonstrate that DCs activated with HRV-14 (R-DCs) induce a new type of regulatory T cells. IL-35, a heterodimer of EBV-induced gene 3 (EBI3) and the p35 subunit of IL-12, has been recently identified as an inhibitory cytokine produced by natural regulatory T cells in mice but not in humans. We observed that dendritic cells (DCs) activated by human rhinoviruses (R-DCs) induce IL-35 production and release, as well as suppressor function in CD4⁺ and CD8⁺ T cells from human peripheral blood but not in naïve T cells from cord blood. Induction of IL-35⁺ T cells by R-DCs was Foxp3-independent but blocking of B7-H1 (CD274) and sialoadhesin (CD169) on R-DCs with mAbs against both receptors prevented the induction of IL-35. Thus, a combinatorial signal delivered from DCs to T cells via B7-H1 and sialoadhesin is critical for the induction of human IL-35⁺ regulatory T cells. These results demonstrate a novel pathway and its components for the induction of immune-inhibitory T cells.

Fungi

Fungi, such as *Candida albicans*, are a major cause of morbidity and mortality in immunocompromised hosts, but are harmless commensal microbes for most healthy individuals. Thus, the strategies used by the host to limit fungal infectivity are necessarily efficient and, in return, fungi have developed their own elaborate tactics to circumvent these defense mechanisms. Activation of the phagocyte network involving neutrophils, macrophages, monocytes and DC is fundamental for fungal clearance. Yet, recent studies also support the idea that phagocytes and factors derived from these innate immune cells such as IL-10 are pivotal in controlling adaptive immune responses against fungi.

CD45 is the prototypic transmembrane protein tyrosine phosphatase (PTP), which is expressed on all nucleated hematopoietic cells and plays a central role in the integration of environmental signals into immune cell responses. We have recently discovered an alternative function for the intracellular domain of CD45, which is abused by fungi. We discovered that CD45 is sequentially cleaved by serine/metalloproteinases and γ -secretases during activation of human monocytes and granulocytes by fungal stimuli or PMA but not by other microbial stimuli. Proteolytic processing of CD45 occurred upon activation of monocytes or granulocytes but not of T cells, B cells or DCs and resulted in a 95 kDa fragment of the cytoplasmic tail of CD45 (ct-CD45). Ct-CD45 was released from monocytes and granulocytes upon activation-induced cell death. Binding studies with ct-CD45 revealed a counter-receptor on preactivated T cells. Moreover, T cell proliferation induced by DCs or CD3 antibodies was inhibited in the presence of ct-CD45 (summarized in Fig. 1). Taken together, the results of our study demonstrate that fragments of the intracellular domain of CD45 from human phagocytes can function as intercellular regulators of T cell activation.

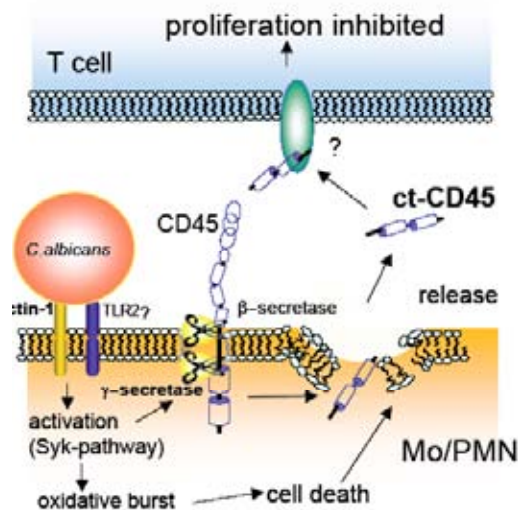


Fig. 1: Induction and release of soluble ct-CD45 from phagocytes upon stimulation with fungi.

Oxidized Phospholipids

Danger signals arising from acute infection and inflammation are recognized as potent stimuli, which up-regulate the T cell stimulatory capacity of DC. In contrast to that, little is known about regulatory signals for DC. We have recently demonstrated that oxidized phospholipids (ox-PLs), which are generated during infections, apoptosis and tissue damage, interfere with DC activation preventing their maturation.

We observed that ox-PLs, dysregulate the differentiation of DCs. DCs generated in the presence of ox-PLs (Ox-DCs) upregulated the typical DC marker DC-SIGN, but did not express CD1a, CD1b and CD1c. Strikingly, Ox-DCs had a substantially diminished T-cell stimulating capacity after stimulation with TLR-ligands. TLR-ligand-induced production of IL-12 was also strongly diminished, whereas induction of CD83 was not altered. We found that ox-PLs strongly inhibit inflammatory stimuli-induced phosphorylation of histone H3, a key step of IL-12 production. Taken together, ox-PLs present during differentiation yielded DCs with a reduced capacity to become immunostimulatory mature DCs, in part by blocking histone modifications required for full activation of DCs. Therefore, inflammation-derived ox-PLs control DC function by epigenetic mechanisms.

Ox-PLs have also a direct impact on T cell activation and function. We could demonstrate that ox-PLs strongly inhibit proliferation of purified human T cells induced with anti-CD3/CD28 or anti-CD3/CD63 mAbs, whereas proliferation of naïve T cells from human cord blood was not affected by ox-PLs. Unoxidized phospholipids showed no such effect. Inhibition of T cell proliferation by ox-PLs was not due to cell death. Moreover, T cell proliferation triggered by PMA/ionomycin activation was not diminished by ox-PLs. T cells activated in the presence of ox-PLs produced and released low amounts of IFN- γ and IL-2, whereas IL-4 was only slightly diminished. Ox-PLs prevented the expression of de novo synthesized activation markers (CD25, MHC-II) but not expression of CD63 or CD69. We further observed that T cells stimulated in the presence of ox-PLs are poor cytotoxic T cells. Most importantly, T cells activated in the presence of ox-PLs failed to proliferate in response to restimulation. This hypo-proliferative state was accompanied with an up-regulation of Egr-3 and Cbl-b. Taken together, our results demonstrate that ox-PLs are potent and specific regulators of T cell activation and function (summarized in Figure 2).

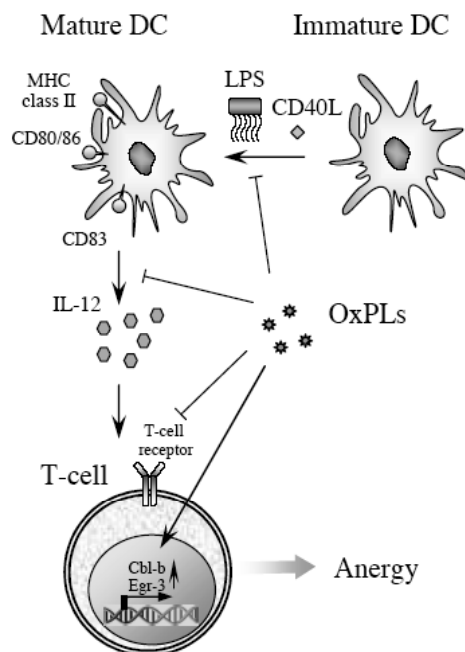


Fig. 2: Impact of oxidized phospholipids on DCs and T cells

We have recently also found, that ox-PLs inhibit the respiratory burst of neutrophils as well as monocytes stimulated with PMA as well as FMLP. In contrast, upregulation of CD11b, a classical activation marker of phagocytes, was not affected. On the other hand, unoxidized PAPC induced the production of oxidative burst, and, when given in combination with FMLP, it synergistically enhanced it. Thus, the opposing roles of PL and ox-PL suggest that PL are positive or negative regulators of phagocyte activation, depending on their oxidation status.

We have recently identified a novel receptor on human T-regs for oxidation products. Scavenger receptor on endothelial cells I (Srec-I) was characterized, using a retroviral expression cloning approach. Srec-I binds oxidized low-density lipoprotein (oxLDL), an endogenous danger signal. Since only a portion of CD4⁺ CD25⁺ T cells expresses this cell surface receptor, Srec-I could be a novel subpopulation marker on regulatory T cells. Most importantly engagement of Srec-I with our mAb 2-98 enhanced the inhibitory function of regulatory T cells significantly. Such a functional effect has so far not been described for other surface molecules on regulatory T cells.

However, our anti-Srec-I mAb 2-98 is an IgM which complicates the analysis of the functional role of Srec-I on natural CD4+CD25+ T-regs, since it is difficult to produce Fab-fragments from IgM mAbs. Therefore, we have generated novel mAbs directed against Srec-I to perform more detailed functional investigations. All new mAbs specifically reacted with Srec-I-expressing transfectants but not with untransfected cells and showed the expected reactivity profile with endothelial cells and leukocytes. In functional assays 2 mAbs (IgG1) were able to enhance the suppressive function of Tregs like mAb 2-98 although they are directed against distinct epitopes on Srec-I. With these new mAbs we are now able to continue the investigation on the molecular mechanism involved in the regulation of T-reg function via Srec-I.

Grants

WWTF (Vienna Science, Research and Technology Fund) LS200 - Johannes Stöckl: "Modulation of DC function by oxidized phospholipids"
 FWF (Austrian Science Fund) SFB F23 Vision Fund - Johannes Stöckl: "Anti-Immunity made by DC"
 FWF (Austrian Science Fund) P20266-B13 - Johannes Stöckl: "Functional role of SREC-I on regulatory T cells"

Theses

Diploma Theses

Maria Seyerl: Regulation of T cell activation by oxidized phospholipids (completed)
 Catharina Schrauf: Molecular analysis of pattern recognition receptors involved in sensing of human rhinoviruses by DC (completed)
 Stefan Hopf: Molecular and functional characterization of the ct-CD45 receptor on human T cells (ongoing)
 Sabrina Zeiner: The functional role of human IL-35 on immune cells (ongoing)

PhD Theses

Nina Gundacker: Effects of oxidized phospholipids on dendritic cell-membrane protein expression (completed)
 Maria Seyerl: Characterization of human IL-35+ regulatory T cells (ongoing)

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14. Pfistershammer, K., A.Lawitschka, C.Klauser, J.Leitner, R.Weigl, M.H.Heemskerk, W.F.Pickl, O.Majdic, G.A.Böhmig, G.F.Fischer, H.T.Greinix, P.Steinberger. Allogeneic disparities in immunoglobulin-like transcript 5 (ILT5) induce potent antibody responses in hematopoietic stem cell transplant recipients. *Blood*, in press (2009)
15. Schrauf, C., S. Kirchberger, O. Majdic, M. Seyerl, G. Zlabinger, K. Stuhlmeier, M. Sachet, J. Seipelt, J. Stöckl. The ssRNA genome of human rhinovirus induces a type-I IFN response but fails to induce maturation in human monocyte-derived dendritic cells. *J. Immunol* in press (2009)

Division of Molecular Biology of Hematopoietic Stem Cells and Dendritic Cells

Head: Herbert Strobl, MD, Associate Professor



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 Rene Köffel, PhD, PostDoc
 Susanne Richter, MD/PhD Student
 Jennifer Jurkin, PhD Student
 Thomas Bauer, PhD Student
 Gregor Eisenwort, PhD Student
 Nighat Yasmin, PhD Student
 Christina Mühlbacher, Diploma Student
 Doris Kneidinger, Diploma Student

Darina Waltenberger, Mag., Biomed.
 Scientist
 Bernhard Gesslbauer, Mag., Technician

Past Members

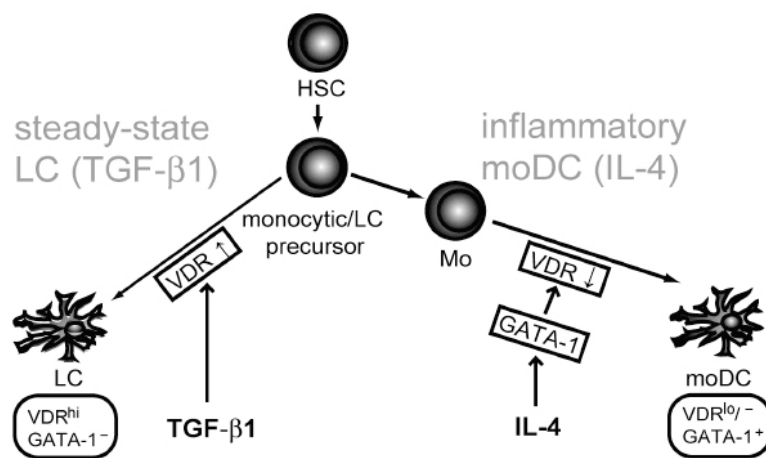
Sabine Taschner-Mandl, PhD, PostDoc, till
 3/2008
 Florian Göbel, PhD Student, PhD, till 4/2008
 Anastasia Meshcheryakova, Diploma
 Student till 4/2009

Hematopoietic stem cells differentiate to lineage-committed progenitor cells, which in turn give rise to various leukocyte subtypes. These differentiation processes are regulated by a complex interplay between synergistic and antagonistic transcription factors in response to extra-cellular signals. In most of our projects, we use in vitro differentiation models of human CD34+ cord blood hematopoietic progenitor cells and monocytes to study molecular mechanisms underlying myelopoiesis and dendritic cell (DC) subset differentiation. We use retroviral and lentiviral gene delivery systems and flow cytometry to dissect transcriptional mechanisms underlying lineage commitment and differentiation. We are particularly interested in understanding how different subsets of human DCs develop from hematopoietic stem cells and monocytes. Epidermal/mucosal Langerhans cells (LCs) and inflammatorytype DCs represent two myeloid DC subsets, which differ in many phenotypic and functional characteristics. A better understanding of how these DC subsets develop and function is critical for both our basic understanding of immune system function and for the creation of novel immunotherapy procedures. In a second research area we analyzed molecular mechanisms underlying cell plasticity of myeloid cells. In one project we studied granulopoietic monocyte differentiation in response to inflammatory signals. In another project, we asked how epithelial genes are regulated during the life cycle of LCs.

1. Molecular mechanisms of human DC subset differentiation

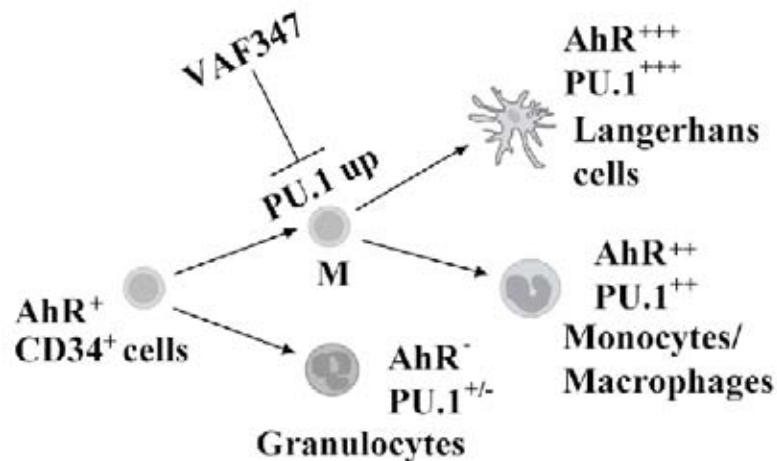
Two major pathways of human myeloid DC subset differentiation have been delineated in previous studies. LCs reside in epithelia at steady-state, whereas monocytes can provide DCs on demand in response to inflammatory signals. Both DC subset pathways arise from shared CD14⁺ monocyte precursors, which in turn develop from myeloid committed progenitor cells. The underlying hematopoietic mechanisms of myeloid DC subset differentiation still remain poorly defined. We identified a functional role of the transcription factors GATA-1, VDR and AhR in these processes.

Inverse role of GATA-1 and VDR in myeloid subset differentiation.



Using a microarray screen, we found that the vitamin D3 receptor (VDR) is induced by TGFβ1 during LC lineage commitment of hematopoietic progenitor cells. Moreover, we found that VDR exerts a positive role during LC generation. In contrast, VDR is repressed during IL-4-dependent monocyte-derived DC (moDC) differentiation. Using a functional retroviral cDNA library screen, we identified GATA-1 as a repressor of VDR in U937 monocytic cells. Studies of primary human DC subsets subsequently revealed that GATA-1 is induced by IL-4 in moDCs, but can not be detected in LCs. Moreover, we found that forced inducible expression of GATA-1 mimics IL-4 in re-directing moDC differentiation, and vice versa GATA-1 knockdown arrests moDC differentiation at the monocyte stage. Furthermore, ectopic GATA-1 expression stabilizes the moDC phenotype under monocyte promoting conditions in the presence of vitamin-D3 (VD3). In summary, human myeloid DC subset differentiation is inversely regulated by GATA-1 and VDR. GATA-1 mediates the repression of VDR and enables IL-4-dependent moDC differentiation. Conversely, VDR is induced downstream of TGF-β1 and is functionally involved in promoting LC differentiation.

Ligation of the aryl-hydrocarbon receptor (AhR) ligand impairs in vitro generation of human monocytes and Langerhans-type DCs.

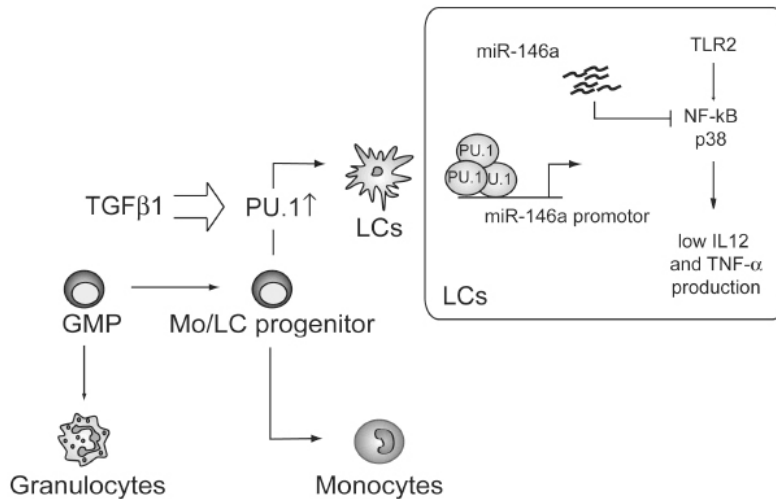


The transcription factor aryl hydrocarbon receptor (AhR) represents a promising therapeutic target in allergy and autoimmunity. AhR signalling induced by the newly described ligand VAF347 inhibits allergic lung inflammation as well as suppresses pancreatic islet allograft rejection. These effects are likely mediated via alterations in dendritic cell (DC) function. Moreover, VAF347 induces tolerogenic DCs. LCs are immediate targets of exogenous AhR ligands at epithelial surfaces; how they respond to AhR ligands remained undefined. We studied AhR expression and function in human LCs and myelopoietic cell subsets using a lineage differentiation and gene transduction model of human CD34⁺ hematopoietic progenitors. We found that AhR is highly regulated during myeloid subset differentiation. LCs expressed highest AhR levels followed by monocytes. Conversely, neutrophil granulocytes lacked AhR expression. We evaluated several AhR ligands including the newly identified compound VAF347, which we obtained through a collaboration with Novartis. We observed that various AhR ligands arrested the differentiation of monocytes and LCs at an early precursor cell stage, whereas progenitor cell expansion or granulopoiesis remained unimpaired AhR expression was co-regulated with the transcription factor PU.1 during myeloid subset differentiation. VAF347 inhibited PU.1 induction during initial monocytic differentiation, and ectopic PU.1 restored monocyte and LC generation in the presence of this compound. AhR ligands failed to interfere with cytokine receptor signalling during LC differentiation and failed to impair LC activation/maturation. VAF347-mediated antiproliferative effect on precursors undergoing LC lineage differentiation occurred in a clinically applicable serum-free culture model and was not accompanied by apoptosis induction. In conclusion, AhR agonist signalling interferes with transcriptional processes leading to monocyte/DC lineage commitment of human myeloid progenitor cells. These observations will now provide a basis for testing AhR ligand-modified DC subsets for their capacity to induce tolerogenic immune responses.

2. Studies on Langerhans-type dendritic cell function

Our previous studies showed that the cytokine TGF- β 1 induces LC differentiation from CD34+ human hematopoietic progenitor cells. Based on these observations, large numbers of human LCs can now be generated. We performed gene array profiling of LC precursor cells to identify novel molecules induced during LC differentiation (TGF- β 1-induced genes). This screen led to the identification of several interesting cell surface molecules and transcription factors that we are currently analyzing. Furthermore, we compared LCs and monocytederived DCs for the expression of microRNAs and focused on one first interesting candidate.

miR-146a is differentially expressed by myeloid DC subsets and desensitizes cells to TLR2-dependent activation



Using microarray profiling we identified microRNA (miR)-146a to be constitutively expressed at higher levels in LCs compared to intDCs. Moreover, miR-146a levels were low in monocyte and non-detectable in neutrophil granulocytes. Constitutive high miR-146a expression in LCs is induced by the transcription factor PU.1 in response to TGF- β 1, a key micro-environmental signal for epidermal LC differentiation. We identified miR-146a as a regulator of monocyte and DC activation but not myeloid/DC subset differentiation. Specifically, ectopic miR-146a in monocytes and intDCs interfered with TLR2 downstream signaling and cytokine production, without affecting phenotypic DC maturation. Inversely, silencing of miR-146a in LCs enhanced TLR2-dependent NF- κ B signaling. We therefore conclude that high constitutive miR-146a levels in epithelial LCs are induced by micro-environmental signals and may render these cells less susceptible to commensal bacterial TLR2 triggers at body surfaces. (Jurkin, J. et al., submitted).

3. Molecular mechanisms of cell plasticity within the myelopoietic cell system

Usually, mature myeloid cells are considered end-stage differentiated cells. However, under certain conditions these cells are still capable of expressing genes affiliated to other developmental pathways. These processes might play important roles in autoimmune / inflammatory diseases and during steady-state immune homeostasis. In two projects we studied (1) granulopoietic monocyte differentiation in response to inflammatory signals and (2) the regulation of epithelial genes during the life cycle of Lcs.

Transcriptional control of “transdifferentiation” of granulopoietic cells to monocytes and osteoclasts.

How intracellular signalling cascades downstream of cytokine signals orchestrate transcription factors (TFs) to induce defined myeloid sub-lineage differentiation programs is so far poorly understood. We found that the direct upstream kinase of p38MAPK, MKK6, is more abundantly expressed by monocytes (Mo) than neutrophil granulocytes (G). Moreover, conditional induction of dominant-active (d.a.) MKK6 in G-CSF-dependent G reprograms them to Mo. D.a.MKK6-dependent G to Mo lineage conversion is mediated by p38-mediated proteasomal degradation of C/EBP α , and induction of c-Jun, followed by the upregulation of MafB/KLF4 and repression of Gfi-1. G to Mo differentiation required only low ectopic d.a.MKK6 expression levels as well as short-term (6 h) d.a.MKK6 expression. Moreover d.a.MKK6 rendered Mo or myeloid cell lines highly immunostimulatory. Since phospho-MKK6 expression marks Mo in rheumatoid arthritis (RA) joints we analyzed RA. Neutrophils from GCSF-mobilized but not normal blood differentiated to Mo in a murine RA model. Moreover, RA-associated pro-inflammatory cytokines enabled RANKL-dependent osteoclastogenesis from G-CSF mobilized human neutrophils via Mo intermediates in vitro. These data identified a critical role of MKK6/p38 as a trigger of emergency monocytopoiesis from left-shifted G under inflammatory situations.

The role of developmental concepts during epidermal Langerhans cell differentiation.

The expression of epithelial genes in LCs is a unique feature among hematopoietic cells. One crucial question is therefore, how hematopoietic LCs adopt these epithelial features. In a genome-wide analysis, we could identify the cytokine TGF- β 1 as an inducer of multiple epithelial genes during LC differentiation, including a restricted set of epithelia-associated transcription factors. We verified that LCs express a whole set of novel epithelial genes such as Cytokeratins (CK8,18). This indicates that LCs display substantial common features with epithelial cells, allowing them to integrate and interact within epithelial tissue. We therefore postulate that transcription factors involved in epithelial differentiation might also play a role in LCs. We currently address the involvement of candidate transcription factors identified by microarray in the induction of epithelial genes in LCs downstream of TGF- β 1. To perform functional studies, we use a state-of-art retroviral approach coupled with a Tet-inducible system, allowing to simultaneously express up to three genes in LC precursors and to induce gene-expression at specific time-points. This work will contribute to a better understanding of cellular mechanisms underlying LC function.

Grants

- FWF (Austrian Science Fund) SFB-2304 „TGF- β 1-polarized epithelial Langerhans cells - a candidate tolerogenic DC subset.“ 5.10.2004-30.8.2009
- FWF (Austrian Science Fund) P19425 „Transcriptional networks in human dendritic cell subset differentiation“ 1.2.2007-30.1.2010
- OeNB (Österreichische Nationalbank) Jubiläumsfonds #12111. “Signal integration by epithelial Langerhans cells” 1.7.2006-30.6.2008
- FWF (Austrian Science Fund) PhD Program Immunity and Inflammation. International PhD program, Deputy Director. 1.1.2007-31.12.2009
- FWF (Austrian Science Fund) Lise-Meitner Stipendium M1096-B13 to Dr. Sabine Witzel (now Konradi). Approved: 6.10.2008 (2 years)

Thesis

PhD Theses

- Florian Göbel: Molecular analysis of differentiation and life cycle of dendritic cells, completed 2008
- Susanne Richter: Regulation of myeloid subset differentiation: - Signals directing pDC development - Role of AhR ligation during myeloid cell differentiation and maturation. Started: August 2005
- Jennifer Jurkin: Transcriptional control of LC differentiation and activation. Started: February 2007
- Thomas Bauer: Novel surface receptors and their role in Langerhans cell differentiation and activation. Started: August 2007
- Nighat Yasmin: Regulation of epithelial gene expression during Langerhans cell differentiation. Started: February 2008
- Gregor Eisenwort: TACSTD2 – a novel molecule in Langerhans cell biology. Started: Sept. 2008

Diploma Thesis

- Anastasia Meshcheryakova: Molecular Analysis of p38MAPK Signal Integration in Granulocytes. Started: Nov. 2007
- Christina Mühlbacher: Transcription Factors in Myeloid Development – the Role of Krüppel-like factor 4 (KLF4). Started: January 2008
- Doris Kneidinger: Functional and molecular analysis of different transcription factors (Ahr, VDR and GATA-1) in dendritic cell development. Started: February, 2008

Awards

- Sabine Witzel: Lise-Meitner Scholarship from the Austrian Science Fund for her project „Regulation of epithelial properties in Langerhans cells“

Publications 2007-2009

- Jörgl, A., B.Platzer, S.Taschner, L.X.Heinz, B.Höcher, P.M.Reisner, F.Göbel, H.Strobl. Human Langerhans cell activation triggered in vitro by conditionally expressed MKK6 is counter-regulated by the downstream effector RelB. *Blood* 109:185-193 (2007)
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- Strehl, S., K.Nebral, M.König, J.Harbott, H.Strobl, R.Ratei, S.Struski, B.Bieloraj, M.Lessard, M.Zimmermann, O.A.Haas, S.Izraeli. ETV6-NCOA2: a novel fusion gene in acute leukemia associated with coexpression of T-lymphoid and myeloid markers and frequent NOTCH1 mutations. *Clinical Cancer Research* 14:977-983 (2008)
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- Platzer, B., S.Richter, S., D.Kneidinger, D.Waltenbegger, M.Woitschläger, H.Strobl. Aryl hydrocarbon receptor activation inhibits in vitro differentiation of human monocytes and Langerhans dendritic cells. *J.Immunol.*, 183 (1): 66-74 (2009)

Division of Clinical Experimental Immunology

Head: Gerhard Zlabinger, MD, Professor of Clinical Experimental Immunology



Current Members

Johannes Hofer, PhD Student
Markus Hölzl, PhD Student
Johannes Kovarik, PhD Student
Miriam Gärtner, Diploma Student
Philip Matzinger, Diploma Student
Alessandra Mathe, Biomed. Scientist

Margarethe Merio, Biomed. Scientist

Saro König, Biomed. Scientist
Jasmin Tancos, Biomed. Scientist

Past Members

Cornelia Stöckl, Biomed. Scientist (2007)
Irene Popow, Diploma Student (2008)
Angela Trausnitz, Biomed. Scientist (2009)

It is a central concern in modern immunology to recognise how the immune system succeeds to simultaneously defeat potentially harmful invaders and to spare host tissue. Both the knowledge about triggering appropriate reactions in case of imminent threat for the host and the insight into the highly complex regulatory mechanisms operating to sustain the integrity of an individual are essential to foster the development of effective strategies for interference in case of disturbed or unwanted immune reactivity as operative in allergy, autoimmunity, malignancy or transplantation. A longstanding interest of my research group concentrates on the issue of naturally occurring ways of immunomodulation as particularly operating in local immunity and on clinical conditions in consequence of inappropriate immune reactivity as observed after transplantation or in autoimmune diseases. Identification of immunological deregulation as early as possible and the understanding of the ongoing processes are pivotal for early diagnosis and the development as well as implementation of purposeful interference strategies.

Mediators of Local Immunity. Defense against urinary tract infection has evolved to be multilayered but is far from being well-understood. A major line of defense is constituted by the steady urinary flow, causing a wash-out of bacteria from the urinary tract. To resist, bacteria express a variety of adhesive surface structures that facilitate microbial interaction with bladder or kidney cells. On the other hand soluble host proteins are able to coat pathogens and by that mechanism prevent their attachment to uroepithelial receptors so that bacteria can be eliminated by urinary flow more effectively. If pathogens overcome these physical defense mechanisms, as a first cellular reaction of the host, shedding of superficial uroepithelial cells is initiated, representing a powerful mechanism to get rid of invading microbes. In the next phase, recruitment of immunocompetent cells is essential for the clearance of bacteria. Neutrophils are attracted by chemotactic signals and may cross the uroepithelium, which manifests clinically as leukocyturia. While the innate immune system fights the establishment of UTI, recognition of pathogens by dendritic cells (DCs) induces a typical maturation program in resident DCs including upregulation of costimulatory and MHC molecules, production of cytokines and their migration to adjacent lymph nodes. At this site, naive, antigen-specific T cells are primed resulting in the induction of cellular and humoral immunity. The production of pathogen-specific antibodies leads to the establishment of protective immunity and therefore helps also to avoid recurrence of UTI.

Tamm Horsfall Protein (THP) is a heavily glycosylated protein, which is exclusively expressed in the thick ascending limb of the Henle's loop in the kidney in amounts of 30 – 50 mg/day and therefore comprises the most abundant protein in the urine. THP is normally expressed only at the luminal surface of renal tubular epithelial cells and excreted to the urine. Earlier studies demonstrated an active role of THP in the pathogenesis of interstitial nephritis, since intravenous challenge of animals with THP resulted in the induction of a tubulointerstitial inflammatory response and microscopic scarring localized to the distal nephron segments. THP binds type 1 fimbriated *Escherichia coli* and therefore constitutes a soluble receptor, which competitively inhibits bacteria to adhere to highly mannosylated uroplakin Ia and Ib receptors, present on the urothelial surface. Regarding its proinflammatory activity, it was shown that administration of THP to human monocytes induced the expression of TNF and tissue factor, while neutrophils increase chemotaxis and phagocytosis in response to THP. These findings are also in line with previous studies showing that intravenous challenge with THP or autologous urine results in rapid induction of THP specific antibodies, indicating THP might be beneficial for the host by immediately activating innate and adaptive immune responses.

Since the urinary tract is devoid of specialized barriers, local defense mechanisms may preferentially rely on soluble anti-microbial defense molecules, like THP (see Figure). Considering the properties of THP, this protein is thought to be an essential defense molecule against UTI for at least two reasons:

i) THP binds type 1 fimbriated *Escherichia coli* and therefore constitutes a soluble receptor, which competitively inhibits bacteria to adhere to highly mannosylated uroplakin Ia and Ib receptors, present on the urothelial surface. Furthermore, recent data unequivocally demonstrated that THP is pivotal to combat bacterial infection *in vivo*. THP^{-/-} mice were shown to be profoundly hampered to combat colonization of the bladder tissue when infected with uropathogenic type-1 fimbriated *E. coli*. In addition, it has been demonstrated that the absence of THP predisposes the host to severe urinary tract infection. These findings indicate that the effect of THP might be attributed to the blockade of bacterial adhesion, leading to impaired colonization.

ii) Additionally to this physical - competitive action, which takes place in the lumen of the urinary tract, we have recently identified Tamm Horsfall Protein (THP) as an endogenous molecule that potently mediates immunomodulation. Because of its restricted expression the activity of THP can be expected to exclusively take place in the genitourinary tract. We found that THP is a strong activator of human DCs and initiates the upregulation of costimulatory molecule and MHC expression, inducing de novo cytokine production and optimal T cell stimulation. Interestingly, THP was shown to induce DC maturation via activating a TLR4 dependent cell signaling machinery including activation of IRAK, Akt, p38, ERK1/2 and NF- κ B. Moreover, we have demonstrated that intravenous challenge with THP rapidly induces the production of THP-specific antibodies and systemic TNF- α release, which was completely absent in TLR4 -/- or MyD88 -/- mice, but not in TLR2 -/- or TLR9 -/- mice.

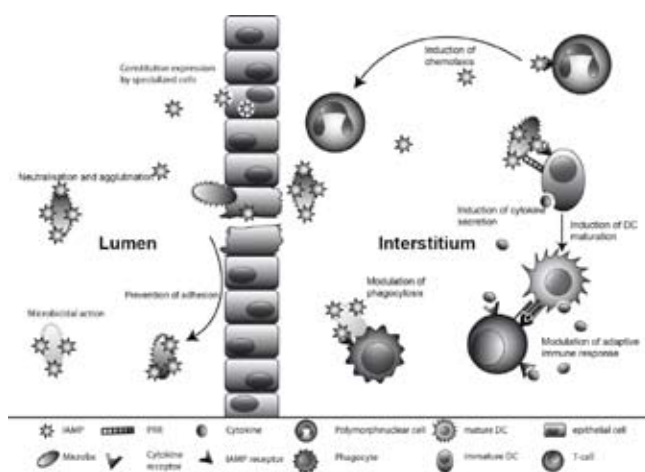


Fig. 1: THP acts as an antimicrobial protein in the lumen of the urinary tract. However, when translocated to the interstitium during urinary tract infection, it mediates immunomodulatory signal

Recent research, showed that TLR4, MD2 or CD14 are not the binding receptors for THP. Employing fluorescence activated cell sorting (FACS) we sorted THP binding BW murine lymphoma cells, expressing proteins of a DC cDNA library. Thereby we were able to identify scavenger receptors, as for example scavenger receptor on endothelial cells I (SRECI), scavenger receptor AI (SR-AI) and scavenger receptor BII (SR-BII) as binding partners for THP. Currently, we are evaluating the physiological impact of these THP binding molecules.

Hallmarks of Antibody-mediated rejection. In clinical transplantation, up to now, diagnostic and therapeutic efforts have mainly focused on T-cell-mediated immunity. However, over the last fifteen years there has been an increase in evidence for a critical role of B cell immunity and antibody-mediated effector mechanisms on allograft loss. Although in the early days of transplantation humoral immunity was recognised to be causal for hyperacute graft rejection due to preformed donor-specific antibodies, only recently the important contribution of alloantibodies to more “common” rejection types could be established, e.g. acute or chronic rejection. Pivotal for the progress in the field have been the definition of acute Antibody-Mediated Rejection (AMR) as a novel clinical entity, the description of capillary deposition of C4 complement split product C4d as marker of AMR, and the establishment of efficient “anti-humoral” treatment, most importantly apheresis.

The first two diagnostic criteria of AMR, histo-morphology and capillary C4d-deposition, are now well defined and have recently been standardised. However, despite the diagnostic value of biopsy-findings, which today are the basis for rejection therapy, histologic diagnosis in most cases comes rather late.

The indication for graft biopsy is given by a clinically manifest deterioration of organ function (e.g. rising serum creatinine concentration), which can either be a sign of an acute, potentially reversible process (e.g. acute AMR) or that of a chronic, often irreversible organ damage (e.g. chronic rejection).

For the evaluation of circulating donor specific antibodies, numerous techniques are available, including cell- and solid phase-based assays. These tests are particularly important for the assessment of the risk for acute AMR prior to transplantation. For this purpose cytotoxic crossmatch test is performed routinely before transplantation, which is suitable to detect the majority of deleterious allospecific antibodies of the recipient. Novel methods for the detection of HLA antibodies employ HLA-coated microbeads. The great advantage of these bead based methods over the use of test lymphocytes is that of employing standardised reagents which allow to perform repeated tests under the same experimental conditions, e.g. monitoring of patients post-transplantation. There are a number of hypotheses on the causes for false negative results in serologic tests, e.g. absorption of donor-specific antibodies by the graft, apheresis therapy or alloantibody concentrations simply below the detection threshold of the applied test. Since it would be of great benefit if the reactivity of donor specific B cells (i.e. alloantibody production) could be accurately assessed prior to transplantation as well as for monitoring purposes in the post transplant phase we have started to implement a test procedure for ex vivo testing of alloantibody production. Appropriate stimulation of B-cells reveals the release of particular alloantibody specificities both in healthy individuals and in sensitized transplant patients. Follow-up studies in transplant patients will demonstrate the clinical relevance of such testing.

Molecular mechanisms of butyrate action. *n*-Butyrate, a short chain fatty acid (SCFA) produced by bacterial fermentation in the intestine proved to be an important factor in maintaining mucosal homeostasis in the gastrointestinal tract. Beside its cell regulating functions, *n*-butyrate exerts potent effects on a variety of colonic mucosal functions, thereby reinforcing colonic defence barrier and decreasing oxidative stress. Furthermore, it has been shown to inhibit inflammation as well as carcinogenesis (2,3). Prominent molecular mechanisms of *n*-butyrate action are inhibition of nuclear factor kappa B transactivation and of histone deacetylation.

In previous studies our group has been engaged in investigating the effect of this SCFA on different immune cell populations and elucidating molecular pathways particularly in T-cells. With regard to the molecular way of action only limited information is available how this SCFA brings about its anti-inflammatory effects. In addition to its well-known effect as an HDAC inhibitor, which however appears not to be able to explain these effects at the molecular level the only common finding of numerous studies is inhibition of NF- κ B transactivation. Also in this regard the actual level of interference/mode of action has so far not been elucidated. A classical read-out of *n*-butyrate interference with innate immunity is maturation of dendritic cells, which we utilize in order to elucidate the molecular mechanisms in more detail. This process has been demonstrated to be efficiently inhibited by *n*-butyrate. In addition to the inhibition of transcription factor activation, modulation of cyclooxygenase and PPARgamma pathways also seem to be central mechanisms in downregulating inflammatory reactions. Here it is hypothesized that the SCFA *n*-butyrate because of its gene-regulating capacity might have an influence on that crucial pathways as well. It therefore is crucial to analyse the signalling events along the NF- κ B pathway induced by TLR2 or TLR4 ligation in great detail as well as to study the involvement of the cyclooxygenase and the PPARgamma pathway in the inhibition of DC maturation by *n*-butyrate at length. Results of this study are expected to deepen the understanding of immune regulatory capacity by this endogenous immunomodulator and could help to develop new therapeutic strategies in inflammatory conditions.

In IBD (inflammatory bowel disease) patients the mucosa characteristically shows a higher grade of local inflammation. Among the various theories implicated in the pathogenesis of IBD genetic and environmental factors as well as an imbalance of inflammatory responses including both hypo- and hyporesponsiveness to commensal bacteria have been put forward but this issue remains far from being elucidated. IBD patients seem to have an imbalance in this milieu of their mucosal environment. So it might be hypothesized that such patients do not respond appropriately to the anti-inflammatory action of such immunomodulating agents. Thus local n-butyrate concentrations might not be sufficient in IBD patients either because these patients do not have adequate n-butyrate levels or immune cells might respond in a different manner in diseased than in healthy individuals. A deficiency of n-butyrate can be excluded by findings showing that there is no significant difference in n-butyrate concentration between healthy individuals and IBD patients. It, therefore, might be hypothesized that n-butyrate cannot act appropriately in Crohn's Disease (CD) and Ulcerative Colitis (UC) patients, which should be reflected by the fact that P MNC of IBD patients were less sensitive to the inhibitory action of n-butyrate. To study potential differences between patients with IBD and healthy individuals a pilot study is under way enrolling patients with Crohn's Disease (CD) and Ulcerative Colitis (UC) as well as healthy individuals. In order to test for a feature inherent to immune cells, it is evaluated whether cytokine production by peripheral blood mononuclear cells (PBMNC) induced by TLR2 or TLR4 ligation can be inhibited with differing efficacy by n-butyrate.

Grants

FWF (Austrian Science Fund) P20508-B11 „Identification of functional receptors for the Tamm Horsfall Glycoprotein on Dendritic cells“ (2007- 2010) Else Kröner Gedächtnisstiftung A88/07 “Immunological Hallmarks of Humoral rejection“ (2008 – 2010) OeNB (Österr. Nationalbank Jubiläumsfonds) 12977 „Sensitivity to n-butyrate in patients with inflammatory bowel disease“ (2008 – 2009)
 WWTF (Vienna Science, Research and Technology Fund) – University Infrastructure Programme 2009 „Multiparameter Analysis in Immune cells“

Theses

Diploma Theses

Irene Popov: Einfluß von ATG Präparationen auf die B-Zell Funktion. (completed 2008)
 Philip Matzinger: Modulation of CD69 expression by the bacterial metabolite n-butyrate (ongoing)
 Miriam Gärtner: Flow cytometric assessment of leukocyte alpha-galactosidase A activity in Anderson Fabry patients (ongoing)

PhD Theses

Johannes Hofer: Impact of antibody-mediated effector mechanisms on the evolution of allo responsiveness (ongoing)
 Markus Hölz: Identification of functional receptors for the Tamm Horsfall glycoprotein on dendritic cells (ongoing)
 Johannes Kovarik: Molecular mechanisms of n-butyrate action (ongoing)

Diploma Theses for Biomedical Scientists

Jasmin Tancos: Nachweis der Sensibilisierung nach Therapie mit humanisierten, monoklonalen Antikörpern gegen VEGF bei Patienten mit altersbezogener Makuladegeneration (completed 2009)
 Veronika Seidl: Fluorimetrischer Nachweis der a-Galactosidase A Aktivität in Leukozyten (completed 2008)

Publications 2007-2009

1. Geyeregger R, M.Zeyda, W.Bauer, E.Kriehuber, M.D.Saemann, G.J.Zlabinger, D.Maurer, T.M.Stulnig. Liver X receptors regulate dendritic cell phenotype and function through blocked induction of the actin bundling protein fascin. *Blood* 109:4288-4295 (2007)
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5. Stefanova T., N.Nikolova, A Michailova, I.Mitov, I.Iancov, G.J.Zlabinger, H.Neychev. Enhanced resistance to Salmonella enterica serovar Typhimurium infection in mice after coumarin treatment. *Microbes Infect.* 9:7-14 (2007)
6. Zeyda M., D. Farmer, J.Todoric, O.Aszman, M.Speiser, G.Gyori, G.J.Zlabinger, T.M.Stulnig. Human adipose tissue macrophages are of an anti-inflammatory phenotype but capable of excessive pro-inflammatory mediator production. *Int.J.Obes.* 31:1420-1428 (2007)
7. Zeyda M., R.Geyeregger, M.Poglitsch, T. Weichhart, G. Zlabinger, S.Koyasu, W.H.Horl, T.M.Stulnig, B.Watschinger, M.D.Saemann. Impairment of T cell interactions with antigen-presenting cells by immunosuppressive drugs reveals involvement of calcineurin and NF-kappaB in immunological synapse formation. *J Leukoc Biol.* 81(1):319-27 (2007)
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9. Huber, J., F.W.Kiefer, M.Zeyda, B.Ludvik, G.R.Silberhumer, G.Prager, G.J.Zlabinger, T.M.Stulnig. CC chemokine and CC chemokine receptor profiles in visceral and subcutaneous adipose tissue are altered in human obesity. *J.Clin.Endocrin.Metab.*93:3215-3221 (2008)
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16. Muhammad A., H.B.Schiller, F.Forster, P.Eckerstorfer, R.Geyeregger, V.Leksa, G.J.Zlabinger, M.Sibilia, A.Sonnleitner, W.Paster, H.Stockinger. Sequential cooperation of CD2 and CD48 in the buildup of the early TCR signalosome. *J.Immunol.* 182:7672-7680 (2009)
17. Regelsberger, G., R.Höftberger, W.F.Pickl, G.J.Zlabinger, U.Körmöczy, U.Salzer-Muhar, D.Luckner, O.A.Bodamer, J.A.Mayr, W.H.Muss, H.Budka, H.Bernheimer. Danon disease: Case report and detection of new mutation. *J.Inherit.Metab.Dis.* in press (2009)
18. Schrauf, C., S. Kirchberger, O. Majdic, M. Seyerl, G. Zlabinger, K. Stuhlmeier, M. Sacht, J. Seipelt, J. Stöckl. The ssRNA genome of human rhinovirus induces a type-I IFN response but fails to induce maturation in human monocyte-derived dendritic cells. *J.Immunol* in press (2009)

TEACHING

Both teaching and continuing professional development are essential milestones to spark interest in a particular discipline and to impart the necessary skills so that individuals acquire the competence to be able to deal properly with the problems imposed during their professional life.

Among the multiple teaching activities the primary task of the Institute of Immunology is to instruct students both about the fundamentals in immunology at the beginning of their studies and to confront them also with more specialized subject matters of the discipline during advanced stages or postgraduate studies. Students are supervised during their diploma work as well as when preparing their thesis, which enables them to get proper insight into the field and into the actual challenges in research and science. Through participation in competitive PhD programs funded by the Austrian Science Fund we attract highly motivated and outstanding students also from abroad to perform their studies at our institution. In addition to the training activities in studies like medicine or natural sciences members of the institute are engaged in teaching courses for other biomedical studies at universities of applied sciences as well.

Besides the engagement in actual teaching and education members of the Institute are also active in the advancement of study programmes at the Medical University by coordinating and refining selected modules of the study of medicine. Furthermore, attempts are made in preparing novel devices to provide teaching material in a modern and attractive way like web-based interactive learning tools.

Regarding professional education our Institute is engaged in training physicians for specialization in immunology, which is a distinct speciality among the laboratory medicine specialities. In this respect it is also a concern to us that there is continuous advancement and thus we substantially contribute to the establishment of regulations for training as well as for subject-specific examinations. These activities are also directed to set up training programmes at the European level and to prepare the ground for harmonization of education.

Within the Institute particular emphasis is laid on continued education of staff members by in-house as well as off-the job training and personal development training measures.

PROGRESS REPORTS and JOURNAL CLUBS

All scientists of the six divisions of the Institute of Immunology present their work in progress or interesting new articles to their colleagues on a regular weekly basis. These obligatory in-house seminars consist of a 20 minutes presentation followed by discussion. The often very intensive discussions are chaired by colleagues. After their presentations the speakers are invited to take part in our special "Improve your presentations skills" programme. Veronika Maierhofer, who has undergone a special education in Communication and Presentation Techniques is offering a very personal feedback regarding these skills. This service is now going in its fifth year and very much appreciated, especially by younger scientists.

DIAGNOSTICS

The activities of the Institute of Immunology in research and teaching are centered around aspects of human medicine and thus it is a matter of importance to transfer our expertise to subjects with immediate relevance to patients. Therefore, one main interest of our institution is the provision of appropriate, state-of-the-art test systems for the diagnosis of immune-mediated diseases. This effort also includes the establishment of new assay procedures as well as the further refinement of existing ones. In this regard it is also a great concern to contribute to the further development of immunodiagnostic procedures by the integration of new methodological approaches. These processes are fostered by our in depth interest and knowledge acquired in basic research projects.

The Institute offers diagnostic procedures for diseases, which are the consequence of disturbed or missing function of the human immune system. Such clinical conditions include diseases caused by the malignant transformation of cells of the immune system (leukemia/lymphoma), clinical states with diminished or lacking function of the immune system (immunodeficiencies) or disorders associated with immune reactivity directed against components of the own body (autoimmune disorders/transplantation immunology). The establishment at our institution of valuable binding reagents (monoclonal antibodies) for the flow cytometric analysis of malignant cell types has critically contributed to international standards for the classification of these malignancies. The majority of specimen obtained are from peripheral blood and bone marrow, which are processed by a whole-blood staining procedure developed in house. Our ongoing engagement in international standardization circles, such as ELN (European Leukemia Network Program) and EGIL (European Group for the Immunological Characterization of Leukemias) guarantees the high quality of this diagnostic segment. In the field of immunodeficiencies as well as in autoimmune diseases the Institute disposes a longstanding experience, which allows us to perform these diagnostic procedures at a high quality level. Since July 2008 the diagnostic laboratory is certified according to the ISO9001:2008 standard, which represents another milestone to ensure the highest quality of services for our customers.

In addition to providing our diagnostic service to hospitals as well as office based practitioners and specialists it is also a great concern to us to contribute to the further improvement of standardization as well as implementation of quality control measures in immunologic testing procedures.



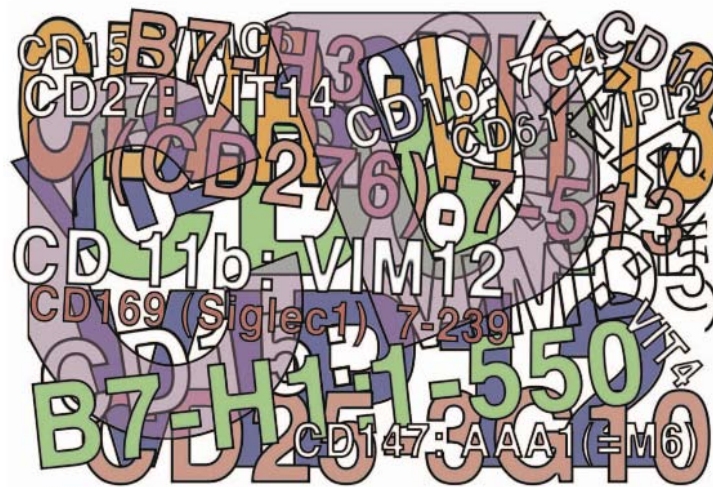
ANIMAL HOUSE

Research groups at the Institute of Immunology use also transgenic and “knockout” mouse models for the identification of basic processes regulating immune cell function and for the development of therapeutic strategies to manipulate the immune response. The technical procedures for the generation of (conditional) knockout mice are established at the Institute, and several knockout mice (e.g. MAZR-deficient mice) were recently generated.



HYBRIDOMA GENERATION UNIT

At the Institute of Immunology different scientific and diagnostic groups use monoclonal antibodies from our hybridoma cell lines (more than 300 hybridomas generated since 1978) as well as hybridoma cell lines available from reference cell culture collections which needs a large scale manufacture of antibodies. So our services include antibody production by in vitro cultures, antibody purification, antibody conjugation and fragmentation.



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LIFE IN AND OUTSIDE THE INSTITUTE



40 Years' Party at the Society of Physicians



J.Kovarik, M.Hözl, I.Popov, V.Leb, J.Hofer



A.Trausnitz, U.Körmöczy



Opening of the Christian Doppler Lab (W.F.Pickl, B.Bohle, Staatssekretärin Ch.Marek)



„New“ Professors W.Ellmeier (Immunobiology)
G.Zlabinger (Clinical Experimental Immunology)



Conferral of Doctorates
G.Zlabinger capped and gowned



Congratulations Judith Leitner!



B.Bohle and W.F.Pickl
ECI 2015 goes to Vienna



Scientific Report Presentation
A.Amramova and M.Hombauer

LIFE IN AND OUTSIDE THE INSTITUTE



Are beards contagious?



C.Wenhardt: ensuring junior staff



Luning Shao(going back to China) and C.Schrauf (leaving for Hannover)



S.Kirchberger leaving for Oxford



Immunology close to nature



Paper accepted! Pilgrimage to Mariazell
C.Schrauf, O.Majdic, P.Cejka, J.Stöckl, M.Seyerl



Barbecue in the Institute's Garden



