

Division of Cellular Immunology and Immuno Hematology

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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.

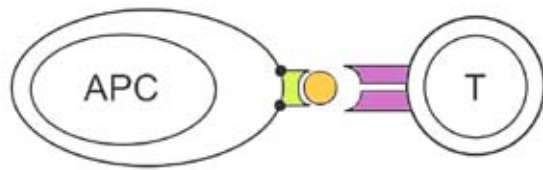


Fig. 1: The immunological synapse:
HLA molecules (green) on antigen presenting cells (APC) present antigen-derived peptides (orange) to the TCR (violet) on T cells

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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