Österreichische Gesellschaft für
Allergologie und Immunologie
Austrian Society for Allergology and Immunology

ÖGAI Symposium 2015
50 Years of B-Lymphocytes

Delineation of the Thymic and Bursal Lymphoid Systems in the Chicken.
Cooper, MD, Peterson RD, Good RA
Pediatric Res.Lab. of the Variety Club Heart Hospital, Univ. of Minnesota, Minn., USA
Nature, 1965, 205:143-6

December 11-12, 2015
College of Physicians in Vienna, Billrothhaus
1090 Vienna, Frankgasse 8

PROGRAMME
ABSTRACTS

Welcome

Dear guests, dear colleagues and friends,

We cordially welcome you to the symposium '50 Years of B-Lymphocytes' which is organized by the Austrian Society for Allergology and Immunology, ÖGAI, in Vienna, from December 11-12, 2015. With this symposium we are celebrating the discovery of B-lymphocytes, which play a key role in the defense against pathogens, tumors, and environmental factors potentially harming human beings. The understanding of B lymphocytes and antibodies produced by these cells is also crucial for the understanding of the large variety of diseases such as allergies, autoimmune diseases, immunodeficiencies and other immune-mediated diseases.

The symposium will take place in the historical building of the College of Physicians in Vienna, the Billroth-Haus. The program consists of plenary lectures, given by outstanding international scientists, selected oral presentations by members of the Austrian Society for Allergology and Immunology, ÖGAI, and five poster sessions, which offer early career scientists to present their cutting edge research.

On the occasion of this meeting the Austrian Society for Allergology and Immunology is proud to award the Clemens von Pirquet medal to Prof. Gabriel Pauli from Strasbourg, France, and the Karl Landsteiner Medal to Prof. Lorenzo Moretta from Rome, Italy.

Since the symposium will take place during the pre-Christmas time our guests and the participants of the symposium will have time to experience the beautiful sceneries of the Christmas markets in Vienna as well as cultural and culinary high-lights of Vienna.

We cordially thank the generous active participation of the organizations and companies who have contributed to the successful organization of this symposium.

We trust that you will experience a wonderful meeting full of exciting science and collegiality. On behalf of the local organizing committee I wish you a wonderful meeting!

Winfried F. Pickl
President of the Austrian Society for Allergology and Immunology
(For the Local Organizing Committee)

ÖGAI

The Austrian Society for Allergology and Immunology represents Austrian scientists and practitioners interested in the physiology and pathophysiology of the immune response as well as the phenotypic expression, diagnosis and therapy of all diseases involving the immune system

The principal aims of ÖGAI are to foster the understanding of the functioning of the immune system in general as well as the consequences of its aberrations. Thus reducing the burden of immunologically-mediated diseases on the individual and also the society, and to advance the treatment and prevention of these disorders. Hence, ÖGAI promotes basic and applied research advances and their translation into the clinical practice

ÖGAI supports excellence in education and training in the fields of allergology and immunology and further provides and also encourages the spread of specific information on the vital importance of the immune system and its disorders, such as allergy, autoimmunity, immunodeficiencies, to the lay, legal and professional public.

Gesellschaft der Ärzte

The Gesellschaft der Ärzte (College of Physicians in Vienna) is a registered association and was founded in 1837 and is the medical society with the richest historical tradition in Austria. Its headquarter, the Billroth-Haus in the centre of Vienna, is certainly one of the most beautiful monuments of medicine history in Europe.

The main task is continuing education and the presentation of new scientific findings. For that purpose the society organises scientific events, runs a library, grants their members access to online journals and databases and offers them a large choice of videos for medical education. Also Landsteiner and Pirquet had given lectures in these rooms on their groundbreaking discoveries of blood groups and the definition of allergy.

LOCAL ORGANIZING COMMITTEE

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EXHIBITION

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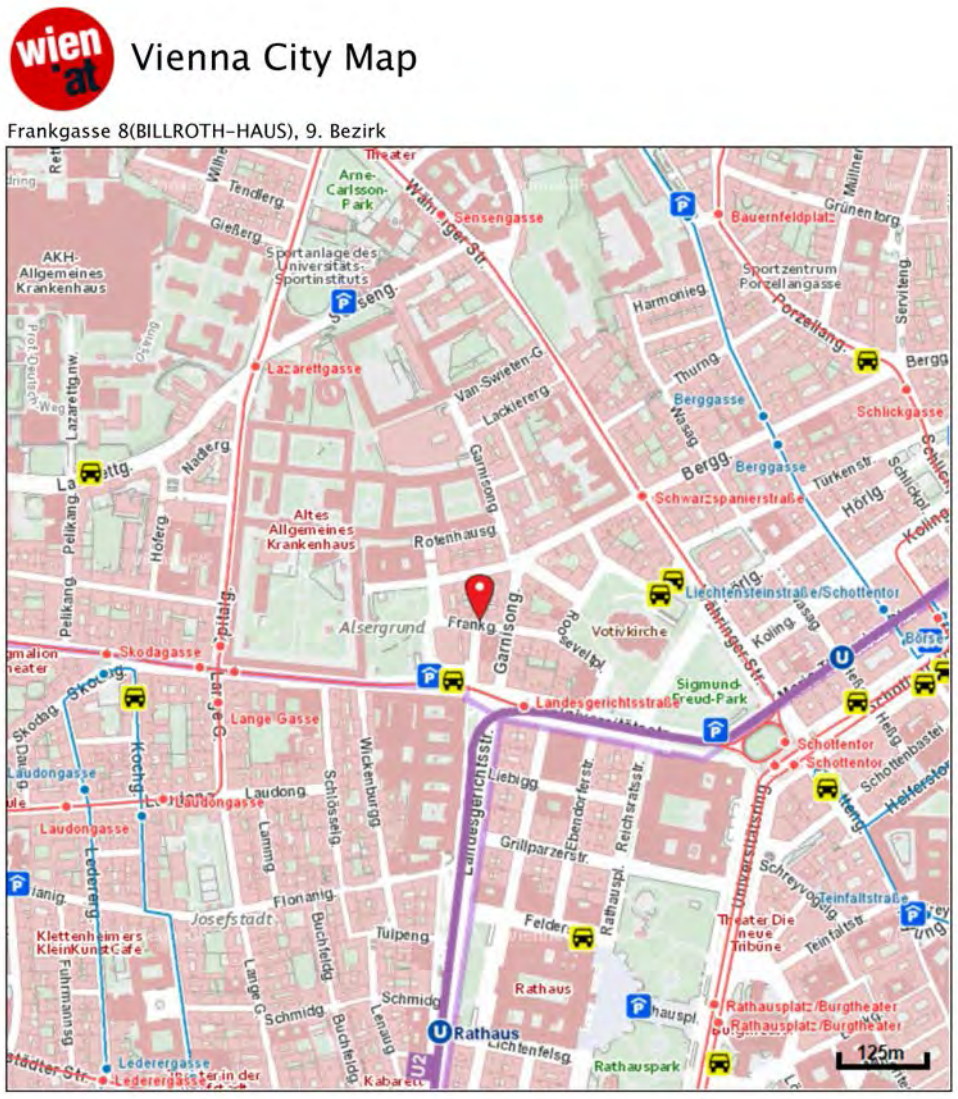

Public transportation

Subway U2 - Station Rathaus or Schottentor,
Tram line 43 or 44 (Universitätsstraße)

By car

- Garage Otto Wagner Platz:
Open 24h, whole day 37 €, per hour 3,70 €, 18-24h 6 €
- Parking spots in the near surroundings:
Whole district short term parking zone from Monday to Friday from 09.00 - 22.00, parking duration: 2 hours, weekend free.

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SCIENTIFIC PROGRAMME - ÖGAI SYMPOSIUM 2015
50 YEARS OF B-LYMPHOCYTES

Friday, December 11, 2015

08:00 - 08:45	**Registration**
08:45 - 09:00	**Opening** **Winfried F. Pickl,** President ÖGAI
09:00 – 09:45	**SESSION I** *Chairs: Lorenzo Moretta (Rome), Meinrad Busslinger (Vienna)* **B-Lymphocyte Ontogeny** **Max D. Cooper**, The Emory Vaccine Center, Atlanta, GA, USA
09:45 – 10:30	**Early B-Lymphocyte Development** **Fritz Melchers**, Max Planck Institut für Infektionsbiologie, Berlin, Germany
10:30 – 11:00	Coffee Break
11:00 – 11:45	**SESSION II** *Chairs: Rudolf Valenta (Vienna), Hannes Stockinger (Vienna)* **Plasma Cell Development** **Andreas Radbruch**, Rheumaforschungszentrum, Berlin, Germany
11:45 – 12:30	**Regulatory B-Lymphocytes** **Claudia Mauri**, University College of London, London, UK
12:30 – 13:15	**B-Lymphocytes in Skin Diseases** **Georg Stingl**, DIAID, Department of Dermatology, Medical University of Vienna, Vienna, Austria
13:15 – 14:15	Lunch Break
14:15 – 15:15	**SESSION III** **Oral Presentations of Selected Abstracts 1** *Chairs: Sabine Flicker (Vienna), Winfried F. Pickl (Vienna)*

OI_1 Are house dust mites (HDM) potential carriers of bacteria responsible for the induction of sensitization to microbial "allergens"?

Dzoro, S.[1], Mittermann, I[1], Nehr, M.[2], Hirschl, A.[2], Wikberg, G.[3], Johansson, C.[4], Lundeberg,L.[3], Scheynius, A.[4], Valenta, R.[1]

[1]Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria, [2]Division of Clinical Microbiology, Clinical Institute of Laboratory Medicine, Medical University of Vienna, Vienna, Austria, [3]Dermatology and Venereology Unit, Karolinska University Hospital, Stockholm, Sweden, [4]Translational Immunology Unit, Department of Medicine, Solna, Karolinska Institute and Karolinska University Hospital, Stockholm, Sweden

OI_2 Rise in total IgE levels upon omalizumab treatment is not caused by activation of IgE$^+$ memory B cells

Eckl-Dorna, J.[1], Fröschl, R.[1], Lupinek, C.[1], Kiss, R.[1], Marth, K.[1], Campana, R.[1], Blatt, K.[1], Valent, P.[1], Selb, R.M.[1], Mayer, A.[1], Gangl, K.[1], Steiner, I.[1], Ziegelmayer, P.[2],, Gevaert, P.[2], Valenta, R.[1], Niederberger, V.[1]

[1]Medical University of Vienna, Waehringer Guertel 18-20, Vienna, Austria [2]

Allergiezentrum Wien West, Huetteldorfer Straße 46, Vienna, Austria [3] Upper Airway Research Laboratory (URL), Ghent University Hospital, Sint-Pietersnieuwstraat 25, Ghent, Belgium

OI_3 Induction of functional MHC-specific IgE in murine allotransplantation

Farkas, A.M.[1], Baranyi, U.[1], Unger, L.[1], Schwarz, C.[1] , Mahr, B.[1], Hock, K.[1], Pilat, N.[1], Valenta, R.[2] and Wekerle, T.[1]

[1]Section of Transplantation Immunology, Dept. of Surgery, Medical Univ. of Vienna, Vienna, Austria, [2]Div. of Immunopathology, Dept. of Pathophysiology and Allergy Research, Center of Physiology and Pathophysiology, Medical University of Vienna, Austria

OI_4 Effects of allergy and SIT treatment on humoral and cellular immune responses to routine vaccination with TBE vaccine

Garner-Spitzer E.[1], Hofer M.[1], Seidl-Friedrich C.[1], Jarisch R.[2], Kinaciyan T.[3], Kundi M.[4], Wiedermann U.[1]

[1]Institute for Specific Prophylaxis &Tropical Medicine, Center for Pathophysiology, Infectiology and Immunology; Medical University of Vienna, [2]Allergiezentrum Floridsdorf, Vienna, [3]Department of Dermatology, DIAID, Medical University of Vienna, [4]Institute for Public Health, Medical University of Vienna

OI_5 Shielding of the major mugwort pollen allergen Art v 1 inside of virus-like nanoparticles makes it invisible for B-lymphocytes *in vivo*

Kratzer B.,[1] and Pickl W. F.[1,2]

[1]Institute of Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, [2]Christian Doppler Laboratory for Immunomodulation, Vienna, Austria

OI_6 The environment alters allergenicity of ragweed pollen

Liu, S.[1], Debiasi, M.[1], Anea, C. B.[1], Karrer, G.[2], Bellaire, A.[3], Chaturvedi, P.[4], Weckwerth, W.[4], Epstein, M. M.[1]

[1] Department of Dermatology, DIAID, Medical University of Vienna, Vienna, Austria, [2] University of Natural Resources and Applied Life Sciences, Vienna, Austria, [3] Department of Botany and Biodiversity Research, University of Vienna, Vienna, Austria, [4] Department of Molecular Systems Biology, University of Vienna, Vienna, Austria.

OI_7 Differential fold-stability during endolysosomal maturation determines immunogenicity and allergenicity of the major birch pollen allergen

Machado, Y.[1], Freier, R[1], Scheiblhofer, S.[1], Thalhamer, T.[1], Mayr, M.[1], Briza, P.[1], Grutsch, S.[2], Ahammer, L.[2], Fuchs, J. E.[3], Wallnoefer, H. G.[3], Isakovic, A.[1], Kohlbauer, V.[1], Hinterholzer, A.[1], Steiner, M.[1], Danzer, M.[4], Horejs-Hoeck, J.[1], Ferreira, F.[1], Liedl, K. R.[3], Tollinger, M.[2], Lackner, P.[1], Johnson, C. M.[5], Brandstetter, H.[1], Thalhamer, J.[1], Weiss, R.[1]

[1] University of Salzburg, Department of Molecular Biology, 5020 Salzburg, Austria, [2] University of Innsbruck, Center of Molecular Biosciences & Institute of Organic Chemistry, 6020 Innsbruck, Austria, [3] University of Innsbruck, Center of Molecular Biosciences & Institute of General, Inorganic and Theoretical Chemistry, 6020 Innsbruck, Austria, [4] Austrian Red Cross, Blood Transfusion Service for Upper Austria, 4020 Linz, Austria, [5] MRC Laboratory of Molecular Biology, Cambridge CB2 0QH, UK

15:20 – 16:20	**ÖGAI Award Ceremony and ÖGAI Medal Award Ceremony**

16:20 – 17:25 **SESSION IV**

Oral Presentations of Selected Abstracts 2

Chairs: Adelheid Elbe-Bürger (Vienna), Wilfried Ellmeier (Vienna)

OII_1 Multifunctional role of the transcription factor Blimp1 in coordinating plasma cell differentiation

Minnich, M.[1], Tagoh, H.[1], Bönelt, P.[1], Axelsson, E.[1], Fischer, M.[1], Cebolla B.[1], Tarakhovsky, A.[2], Nutt, SL.[3,4], Jaritz, M.[1] and Busslinger, M.[1]

[1] Research Institute of Molecular Pathology (IMP), Vienna Biocenter (VBC), Dr. Bohr-Gasse 7, A-1030 Vienna, Austria, [2] Laboratory of Lymphocyte Signaling. The Rockefeller University, New York, USA, [3] The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia, [4] Department of Medical Biology, The University of Melbourne, Parkville, Victoria, Australia

OII_2 Mapping of human rhinovirus-specific antibody responses using high resolution microarray

Niespodziana, K.[1], Stenberg-Hammar, K.[2,3], Cabauatan, C. R.[1], Napora-Wijata, K.[1], Vacal P.L.[1], Gallerano, D.[1], Lupinek, C.[1], Ebner, D.[4], Schlederer, T.[4], Harwanegg, C.[4], Melén, E.[5,6], Söderhäll, C.[7], van Hage, M.[8], Hedlin, G.[2,3], and Valenta, R.[1]

[1]Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria [2] Astrid Lindgren Children´s Hospital, Karolinska University Hospital, Stockholm, Sweden [3] Department of Women´s and Children´s Health, Karolinska Institutet, Stockholm, Sweden [4] Phadia Austria GmbH, Part of Thermo Fisher Scientific ImmunoDiagnostics, Vienna, Austria [5]Institute of Environmental Medicine, Karolinska Institutet, and [6]Sachs´Children´s Hospital, Södersjukhuset, Stockholm, Sweden [7] Department of Biosciences and Nutrition, and Center for Innovative Medicine (CIMED), Karolinska Institutet, Stockholm, Sweden [8] Clinical Immunology and Allergy Unit, Department of Medicine, Solna, Karolinska Institutet and University Hospital, Stockholm, Sweden

OII_3 The effects of Btk inhibitors on IgE receptor-mediated signal transduction and activation of mast cells and basophils

Smiljkovic, D.[1], Blatt, K.[1], Stefanzl, G.[1], Dorofeeva, Y.[2], Focke-Tejkl, M.[2], Valenta, R.[2], Valent, P.[1,3]

[1]Department of Internal Medicine I, Division of Hematology & Hemostaseology, Medical University of Vienna, Austria; [2] Division of Immunopathology, Department of Pathophysiology, Center for Pathophysiology, Immunology and Infectiology, Medical University of Vienna, Austria, [3] Ludwig Boltzmann Cluster Oncology, Medical University of Vienna, Austria

OII_4 Immunization with Immune complexes modulates the fine-specificity of antibody responses to a flavivirus antigen

Tsouchnikas G.[1], Zlatkovic J.[1], Jarmer J.[1], Strauss J.[1], Vratskihk O.[1], Kundi M.[2], Stiasny K.[1], Heinz F.X.[1]

[1]Department of Virology, Medical University of Vienna, Vienna, Austria [2] Institute of Environmental Health, Medical University of Vienna, Vienna, Austria

OII_5 Development of the recombinant *Blomia tropicalis* allergen Blo t 2 for immune-diagnosis

Urrego J.[1,2], Hofer H.[1], Aglaz L.[1], Pinheiro C.[2], Briza P.[1], Wallner M.[1], Alcantâra-Neves, N.[2], Ferreira F.[1]

[1]University of Salzburg, Salzburg, Austria, [2]Instituto de Ciências da Saúde, Universidade Federal da Bahia, Salvador, Bahia, Brazil.

OII_6 Interaction of human B cells and melanoma cells to induce therapy resistance

Wagner, S.N. [1], Somasundaram, R. [2], Zhang, G. [2], Perego, M. [2], Fukunaga-Kalabis, M. [2], Garg, K. [1], Maurer, M.[1], Herlyn, M. [2]

[1]DIAID, Dept. of Dermatology, Medical University of Vienna, Austria, [2]The Wistar

Institute, Philadelphia, PA, USA

OII_7 **Analyzing the cross-reactivity of Amb a 1, the major allergen of short ragweed**

Wolf M.[1], Hauser M.[1], Pichler U.[1], Twaroch T.[2], Gadermaier G.[1], Ebner C.[3], Yokoi H.[4], Takai T.[5], Didierlaurent A.[6], Mari A.[7], Briza P.[1], Behrendt H.[8], Neubauer A.[2], Stolz F.[2], Ferreira F.[1], **Wallner M.[1]**

[1]University of Salzburg, Salzburg, Austria, [2] Biomay AG, Vienna, Austria, [3] Allergieambulatorium am Reumanplatz, Vienna, Austria, [4] Kyorin University, School of Medicine, Tokyo, Japan ,[5] Juntendo University, Graduate School of Medicine, Tokyo, Japan , [6] Stallergenes S.A., Antony, France, [7] Associated Centers for Molecular Allergology, Rome, Italy, [8] ZAUM, Center for Allergy and Environment, Munich, Germany

17:30 – 19:00 **POSTER VIEWING, Coffee, Wine and Cheese**

Poster Session I_Molecular Allergology

Chairs: Susanne Vrtala (Vienna), Petra Zieglmayer (Vienna)

PI_1 ***Dorofeeva, Y.*** Cloning, expression in insect cells and immunological characterization of Par j 2.0101, a major allergen of *Parietaria judaica* pollen

PI_2 ***Gattinger, P.*** Towards the characterization of the allergenic activity of carbohydrate-reactive IgE

PI_3 ***Gepp, B.*** A chimeric protein containing the C-terminus of Bet v 1 is a potent inducer of basophil degranulation

PI_4 ***Humeniuk, P.*** Seed-specific allergens associated with severe symptoms of celery allergic patients

PI_5 ***Kodydek, M.*** Mapping IgE epitopes of food allergens which cross-react with the major birch pollen allergen, Bet v 1

PI_6 ***Kurtaj, A.*** The immune response against the timothy grass pollen allergen Phl p 5 in non-allergic humans

PI_7 ***Najafi, N.*** Epitope presentation on allergens is critical for allergenic activity

PI_8 ***Resch, Y.*** Der p 5, Der p 7, Der p 21 and Der p 23 show high allergenic activity in HDM-allergic patients

PI_9 ***Rodriguez Dominguez, A.*** Development of sandwich ELISAs for the quantification of clinically relevant house dust mite allergens

PI_10 ***Roulias, A.*** Enhancing recombinant production yield of Bet v 1 through codon usage harmonization

PI_11 ***Tscheppe, A.*** Production of a recombinant hypoallergenic variant of the major peanut allergen Ara h 2 in the baculovirus insect cell system

PI_12 ***Wieser-Pahr, S.*** Characterization of gamma gliadins as major allergens in IgE-mediated wheat food allergy

PI_13 ***Zulehner, N.*** Dau c 1, the Bet v 1-homolog in carrot, bears sensitizing activity: evidence at the T cell level

Poster Session II_Mechanisms in Allergy

Chairs: Birgit Linhart (Vienna), Margarete Focke-Tejkl (Vienna)

PII_1 ***Bosnjak, B.*** Mouse lung-specific initiation of allergic asthma ignorome

PII_2 ***Campana, R.*** Epicutaneous allergen application induces allergen-specific IgG and T cell responses but not boosts of IgE production

PII_3 ***Candia, M.R.*** Establishment of a cellular, fluorescent-based, peptide binding assay for the selection of altered peptide ligands (APL) of immunodominant peptides of major pollen allergens

PII_4 ***Flicker, S.*** Isolation and characterization of an IgG-derived ScFv specific for the major birch pollen allergen Bet v 1 from a healthy donor immunized with hypoallergenic Bet v 1 fragments: High affinity binding despite germline configuration - challenging the principle of affinity maturation

PII_5 ***Huang, H.J.*** Towards a non-allergenic peptide mix containing the T cell epitopes of the clinically most relevant house dust mite allergens for tolerance induction

PII_6 ***Kalic, T.*** Major allergens from fish and peanut interact with plasma membranes of intestinal and bronchial epithelial cells and induce differential gene expression of cytokines

PII_7 ***Moñino Romero, S.*** Possible implications of soluble Fc-epsilon RI presence in different populations

PII_8 ***Moñino Romero, S.*** Modulatory capacities of soluble Fc-epsilon RI in the IgE-mediated immune response

PII_9 ***Palladino, C.*** The impact of peanut lipids on Ara h 1-induced immune responses in MoDCs

PII_10 ***Polak, D.*** Neutrophils are potential APC in IgE-mediated allergy

PII_11 ***Ponce, M.*** Assessing basophil activation pathways via flow cytometry in the context of food allergy

PII_12 ***Samadi, N.*** Phenotyping of allergen-reactive $CD8^+$ T cells in type I allergy

PII_13 ***Sanchez Acosta, G.*** The role of Phl p 5 specific IgG antibodies for allergen presentation

PII_14 ***Vizzardelli, C.*** Humanized mice as *in vivo* model for therapy of IgE-mediated allergy

Poster Session III_Treatments in Allergy

Chairs: Barbara Bohle (Vienna), Richard Weiss (Salzburg)

PIII_1 ***Asam, C.*** Are birch pollen AIT induced blocking antibodies protective for cross-reactive allergens?

PIII_2 ***Chen, K.W.*** Dissection of specific IgG responses with recombinant house dust mite allergens reveals that poor effect of specific immunotherapy is due to failure of blocking antibody induction towards certain allergens

PIII_3 ***Cornelius, C.*** Hepatitis B-specific immune responses in grass pollen allergic patients immunized with the preS-based grass pollen allergy vaccine BM32

PIII_4 ***Focke-Tejkl, M.*** Immunotherapy of allergic patients with the B cell epitope-based recombinant grass pollen allergy vaccine BM32 induces allergen-specific IgG antibodies which inhibit immediate allergic inflammation and allergen-specific T cell responses

PIII_5 ***Freidl, R.*** Oral tolerance induction to the major fish allergen parvalbumin in a mouse model of fish allergy

PIII_6 ***Hofer, H.*** Novel drug design for birch pollen and associated food allergies

PIII_7 ***Kazemi, S.*** A novel glutarimide derivative XC8 suppresses acute experimental allergic asthma in BALB/c mice

PIII_8 ***Kitzmüller, C.*** Fusion proteins of the TLR5 ligand flagellin and the major birch pollen allergen Bet v 1 show intrinsic adjuvanticity

PIII_9 ***Niespodziana, K.*** Rhinovirus infections rather than allergen exposure trigger wheezing attacks in preschool children

PIII_10 ***Reithofer, M.*** Characterization of NET responses to adjuvants used in allergy vaccines

Poster Session IV_Immune cells

Chairs: Beatrice Jahn-Schmid (Vienna), Johannes Huppa (Vienna)

PIV_1 ***Anderseen, L.*** Molecular analysis of MAZR function in $CD4^+$ T cells

PIV_2 ***Bonelli, M.*** Abatacept (CTLA-4Ig) treatment reduces T cell apoptosis and regulatory T cell suppression in patients with rheumatoid arthritis (RA), (presented by Scheinecker, C.)

PIV_3 ***Boucheron N.*** Modulation of TH17 responses by the protein tyrosine kinase Tec

PIV_4 ***Didara, Z.*** STAT1-S727 - the license to kill

PIV_5 ***Edlinger, L.*** The role of STAT5A and STAT5B in natural killer cells

PIV_6	***Hainberger, D.*** MAZR and Runx factors synergistically repress ThPOK during $CD8^+$ T cell lineage development
PIV_7	***Kraller, M.*** Molecular imaging of the antigen recognition dynamics in $CD8^+$ cytotoxic T-cells
PIV_8	***Mayer, K.*** The role of AMP-activated protein kinase in T helper cell differentiation
PIV_9	***Müller, L.*** Nuclear receptor corepressor 1 (NCoR1) in T cell development and homeostasis
PIV_10	***Mungenast, F.*** Characterization of B-cell subsets in follicular structures: from classical germinal centers to ectopic follicles at tumor site
PIV_11	***Preglej, F.*** The role of HDAC2 in T cells
PIV_12	***Schatzlmaier, P.*** Rapid multiplex analysis of lipid raft components with single cell resolution
PIV_13	***Tauber, P.,*** The novel mTOR inhibitor AZD8055 shuts-off T helper 1 and 2 but not interleukin-2 secretion early upon activation of allergen-specific T cells
PIV_14	***Tauber, P.*** The 3-phosphoinositide-dependent kinase-1 targeting drug BX795 promotes interleukin-2 but shuts off T helper 1 and 2 cytokine secretion upon activation of allergen-specific T cells
PIV_15	***Watzenböck, M.*** The role of B-cells and humoral immunity in the lung

Poster Session V_Inflammation and Clinical Immunology

Chairs: Clemens Scheinecker (Vienna), Gerhard Zlabinger (Vienna)

PV_1	***Brezinsek, H.P.*** Diminished CD19-expression on newly produced $IgD^+/CD27^-$ B cells after B cell depletion is associated with clinical response in rheumatoid arthritis and scleroderma
PV_2	***Changi, K.*** Testing biocompatibility of thermo-sensitive elastin-like recombinamer (ELR) biogels for bone repair and regeneration *in viv*o in BALB/c mice
PV_3	***Freire, P.*** IgE autoreactivity in Bullous Pemphigoid
PV_4	***Gudipati, V.*** Molecular insights into chimeric antigen receptors (CARs) targeted against leukemias: A major breakthrough in adoptive immunotherapy
PV_5	***Höftberger, R.*** Autoimmune encephalomyelitis in humans: what can we learn about B-cells in multiple sclerosis?
PV_6	***Holcmann, M.*** Investigating the role of EGFR in myeloid cells in inflammatory diseases
PV_7	***Novoszel, P.*** Role of the AP-1 protein c-Jun in Imiquimod mediated tumor clearance
PV_8	***Stulnig, G.*** The role of plasmacytoid dendritic cells in Imiquimod mediated skin inflammation and melanoma clearance in mice
PV_9	***Tajpara, P.*** Examining virus-recognizing receptors in Langerhans cells following human skin barrier disruption and stimulation with synthetic RNA
PV_10	***Unger, L.*** The effect of anti-CD40L mAb induction therapy on graft survival in a CTLA4Ig-based murine heart transplantation model
PV_11	***Waltl, E.*** Comparison of five different damaging models of the respiratory epithelium
PV_12	***Zeka, B.*** Discovery of mimotopes for pathogenic antiaquaporin 4 autoantibodies in neuromyelitis optica via phage display
PV_13	***Zeka, B.*** AQP4-specific T cells and NMO IgG orchestrate NMO-like lesions in Lewis rat

Saturday, December 12, 2015

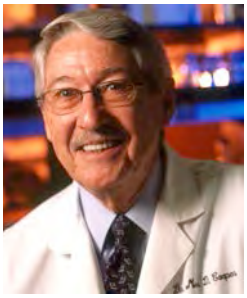
the Saturday Program will traditionally be held in German language, with exceptions

08:00 - 09:00	**Meet the Professor (F. Melchers, A. Radbruch, G. Stingl)**
09:00 – 09:45	**ERÖFFNUNG** **Petra Zieglmayer,** Vorsitzende Komitee Klinische Allergologie **SESSION V** *Chairs: Zsolt Szepfalusi (Vienna), Felix Wantke (Vienna)* **Die Rolle des Nasensekretes bei der allergischen Rhinitis** **Valentin Tomazic**, HNO Universitätsklinik, Medizinische Universität Graz, Graz, Austria
09:45 – 10:30	**Was gibt es Neues in der Insektengiftallergie?** **Christoph Schrautzer**, Universitätsklinik für Dermatologie, Medizinische Univertsität Graz, Graz, Austria
10:30 – 11:15	**NSAR-Intoleranz in der klinischen Praxis** **Tilman Keck**, Krankenhaus der Elisabethinen, Graz, Austria
11:15 – 11:45	Coffee Break
11:45 – 12:30	**SESSION VI** *Chairs: Verena Niederberger (Vienna), Thomas Hawranek (Salzburg)* **Aktuelle Entwicklungen in der Allergen-spezifischen Immuntherapie** **Gabrielle Pauli,** Department of Pulmology, Strasbourg University Hospital, Strasbourg, France
12:30 – 13:15	**IgE-Depletion zur Behandlung schwerer Allergiesymptome** **Christian Lupinek**, Institut für Pathophysiologie und Allergieforschung, Medizinische Universität Wien, Wien, Austria
13:15 – 14:00	**Transkutane Vakzinierung und Hyposensibilisierung mittels einer neuen Laser-Mikroporationsplattform** **Richard Weiss**, Abteilung für Molekulare Biologie, Universität Salzburg, Salzburg, Austria
14:00	**Closing Ceremony,** Small Lunch, Awards of Best Orall Presentation Prizes and Best Poster Prizes

The Austrian Medical Chamber has accredited the ÖGAI Symposium '50-Years of B-Lymphocytes' with a maxiumum of 12 DFP credits (12 Diplomfortbildungspunkte).

SPEAKERS' BIOSKETCHES

MAX D. COOPER



Max D. Cooper, M.D., is a Georgia Research Alliance Eminent Scholar, Professor of Pathology and Laboratory Medicine and member of the Vaccine Center at the Emory University School of Medicine. Cooper obtained his medical degree and pediatric residency training at Tulane University Medical School. While at the University of Minnesota from 1963-1967 he worked with Robert Good to establish the dual nature of the immune system. With UAB graduate student Paul Kincade, he discovered isotype switching by IgM^+ B cells. Dale Bockman and Cooper described the lymphoid follicle-associated epithelial "M" cells in the intestine and their transcytotic function. While on sabbatical at University College London in 1974, he worked with Martin Raff and John Owen to define the fetal liver and bone marrow origin of B cells and pre-B cells. His laboratory currently studies the evolution of adaptive immunity and explores the use of lamprey monoclonal antibodies for diagnosis and therapy of infectious diseases and lymphoid malignancies. Cooper is a former president of the American Association of Immunologists, the Clinical Immunology Society and the Kunkel Society. He is a member of the U.S. National Academy of Sciences, the Academy of Medicine and the American Academy of Arts and Sciences. Honors include the Society for Experimental Biology and Medicine Founder's Award (1966), Sandoz Prize in Immunology (1990), American College of Physicians Science Award (1994), American Association of Immunologists (AAI) Lifetime Achievement Award (2000), AAI-Dana Foundation Award in Human Immunology Research (2006), Avery-Landsteiner Prize (2008) and the Robert Koch Prize (2010).

FRITZ MELCHERS

Fritz Melchers graduated at the Institute of Genetics of the University of Cologne, then continued his career as Fulbright Travel Scholar at the Salk Institute for Biological Sciences in La Jolla, CA, USA, in 1965 and gained further international experience as Visiting Scientist at the Weizmann Institute in Rehovot and at the Stanford University. Since 1970 he was Member of the Basel Institute for Immunology, where he held the position as director from 1981 – 2001. From there he moved to Berlin for the position of a Senior Research Group Leader at the Max Planck Institute for Infection Biology, Berlin. He is Max Planck Fellow and a founding and honorary member of the German Society for Immunology e.V. He is particularly noted for his groundbreaking research, which was critical for our understanding of the development and the maturation of antibody-secreting B lymphocytes. His presentation will recount some of the exciting scientific discoveries clarifying the functions of antibody-producing B lymphocytes in the immune system.

"Fifty years ago Norbert Hilschmann, then at the Rockefeller Institute, discovered that antibodies have variable region domains to bind antigen, and constant region domains to carry out effector functions. Ten years later, Susumu Tonegawa and his colleagues discovered the genetic basis of the variability of antibodies – at the Basel Institute for Immunology. At the same time, Georges Köhler – a "son" of the Basel Institute - and Cesar Milstein invented the revolutionizing method to make monoclonal antibodies. Yet

another 10 years later, with Nobuo Sakaguchi, we found, again at the Basel Institute, the surrogate light chain – guiding B cell development. A personal recollection of the history of these discoveries should be helpful to understand, how we do science: guided by history, philosophy and serendipity."

ANDREAS RADBRUCH

A biologist by education, Andreas Radbruch did his PhD at the Genetics Institute, Cologne University. In 1996, Andreas Radbruch became Director of the German Rheumatism Research Centre Berlin, a Leibniz Institute, and in 1998, Professor of Rheumatology at the Charité Medical School of the Humboldt University of Berlin. Andreas Radbruch has been President of the German Society for Rheumatology, the German Society for Immunology and is President of the International Society for Advancement of Cytometry (ISAC). He is editorial chair of the European Journal of Immunology. He was awarded the Carol Nachman Prize and an advanced research grant of the European Research Council and was elected a Fellow of the American Institute for Medical and Biological Engineering (AIMBE). He is recipient of the Avery Landsteiner Award. In 2015, he became Spokesman of Section C of the Leibniz Association. In 2016, he will be President Elect of the European Federation of Immunological Societies (EFIS). Andreas Radbruch has (co-)authored over 250 original publications on immunological memory, antibody class switching, T and B lymphocyte differentiation, cytometry and cell sorting. His research group described the organization of memory plasma cells and memory T helper lymphocytes in bone marrow, and identified memory plasma cells secreting pathogenic antibodies as novel target in chronic immune-mediated diseases. He demonstrated that antibody class switch recombination in activated B lymphocytes is targeted to distinct switch regions by transcription. He advanced our understanding of Th1 and Th2 cytokine memory, its imprinting and plasticity. He has identified critical molecular adaptations of Th effector memory cells to chronic inflammation and developed the MACS technology and the cytometric secretion assay.

CLAUDIA MAURI

Claudia Mauri received her PhD (equivalent) magna cum laude degree in 1992 from the University La Sapienza in Rome. She worked as post-doctoral fellow at The Kennedy Institute of Rheumatology. She moved to the University College London in 2002 where she has established her group. She is Professor of Immunology and the new Champion for Women. Her research interest is to understand the mechanisms driving autoimmunity with a particular interest in understanding B cell regulation in experimental models of rheumatic disease and in patients with systemic lupus erythematous (SLE) and rheumatoid arthritis (RA).

GEORG STINGL

Georg Stingl received his MD degree from the University of Vienna and his clinical and research training at the Deptepartments of Dermatology and Immunology of this institution. After postdoctoral years at the Dermatology Branch (1977-1978) and the Laboratory of Immunology (1985-1986) at the National Institutes of Health, Bethesda, MD, U.S.A., he became Staff Member at the University Departments of Dermatology in Innsbruck and, later on, in Vienna. In 1992, he was appointed Full Professor and Chairman of the Division of Immunology, Allergy and Infectious Diseases at the Department of Dermatology of Vienna's Medical University. Dr. Stingl is best known for his groundbreaking work on cutaneous immunology. He discovered the immuno-phenotype and –function of epidermal Langerhans cells as well as a novel population of intraepidermal T lymphocytes in rodent skin. In addition, he made major contributions to our understanding of the immunopathogenesis of various types of skin disorders, including atopic dermatitis, psoriasis and chronic urticaria. His presentation will address some of the most exciting findings about the role of antibody-producing B lymphocytes in the development of inflammatory and neoplastic processes affecting the skin.

VALENTIN TOMAZIC

Born in 1982, Dr. Tomazic graduated from the Medical University Graz in 2008. After being research fellow with Prof. H. Stammberger from November 2008 until May 2009, he became resident at the ENT-University Hospital Graz, Department of General ORL Head and Neck Surgery, where he is active since May 2009. PhD-student since 2011, „Habilitation" for Otorhinolaryngology in 2012. Member of ERS and EAACI. Vice-president of the junior member board of the ERS, assistant professor at the Medical University of Graz since December 2014. Representative of the ENT-section in the JMA of EAACI since March 2015. Member of the organizing committee of the Graz International and National FESS courses, Endovienna 2012 and Austrian National Congress for ENT 2013. Member of the Graz Interdisciplinary Unit for Rhinoneurosurgery. Research interests include rhinology, endoscopic sinus and skull base surgery, oncology, allergy and immunology.

His presentation will deal with the nasal mucus proteome and its alteration in allergic rhinitis patients compared to healthy controls. Of note, the nasal mucus acting as defense barrier shows perennial inflammatory responses and reduced defense mechanisms in allergic individuals at the protein level.

CHRISTOPH SCHRAUTZER

Dr. Christoph Schrautzer, University Clinic for Dermatology and Venerology, Graz. Current research interests in insect venom allergy and birch pollen allergy. Managing bee and wasp venom allergy in daily routine always rises questions about interpretation of asymptomatic sensitization, onset of protective action of specific immunotherapy and added value of component resolved diagnosis. New evidence in these and other relevant research topics will be presented and discussed.

TILMAN KECK

ENT specialist, graduated from the Medical University Ulm, since 2009 head of the Krankenhaus der Elisabethinen in Graz, Department of General ORL head, neck and plastic face surgery. Research interests in nasal function, cell culture of nasal mucosa, intolerance reactions against antiphlogistics, nasal polyps in cystic fibrosis

Hypersensitivity reactions against nonsteroidal antirheumatic agents (NSAR) and salicylic acid in association with *polyposis nasi* and intrinsic asthma bronchiale are termed Morbus Vidal, Samter Trias or AERD (Aspirin-Exacerbated Respiratory Disease). This pseudo-allergy is evident in about 10 % of all asthmatic patients and causes severe, delimitating and sometimes life-threatening anaphylactic episodes. The pathogenetic mechanism is a deviation in the arachidonic acid metabolism. Disease verification can be achieved by titrated oral salicylic acid challenge tests. Other applications forms (intravenous, intranasal, intrabronchial) are also suitable. Adaptive desensitization as treatment of choice and its value compared to surgical sinus intervention will be discussed.

GABRIELLE PAULI – SEE ALSO CLEMENS VON PIRQUET AWARDEE

Gabrielle Pauli studied medicine at the faculty of medicine of Strasbourg University. She qualified as medical doctor in 1967 and was appointed assistant at the Department of Chest Diseases. She specialized in asthma and allergy. Her research was first aimed at the role of mites in allergic asthma and she performed the first bronchial challenge tests with mite allergens. She also demonstrated the plurality of allergens in house dust using *in vivo* and *in vitro* tests, initiated immunotherapy with mite extracts and testing eviction methods. She obtained for her work the „Environment and Health Prize" delivered by the French Academy of Medicine in 1993 and the Gold Medal of the Foundation for Research in Allergy in 1995. As a pneumologist, she took a thirty-year long interest in occupational asthma and trained a team of practitioners, nurses and technicians, which made her department a pole of reference. She cofounded the occupational asthma registry in France and issued the first French book on occupational asthma (first edition 1999, second edition 2012). From 1995 onward, her research has been aimed at molecular allergens and thanks to exceptional collaborations with

European researchers in the same field, she was one of the first clinicians to test recombinant molecules in patients. Together with two other groups, she initiated the first trial of immunotherapy with a recombinant allergen showing that the concept led to satisfactory results. In 2011 the European Academy of Allergy and Clinical Immunology gave her the Clemens von Pirquet Award for her research, which found expression in more than 300 publications, general reviews and book chapters. Gabrielle Pauli is *professor emeritus*, former Head of the Pneumology Department of the Strasbourg University Hospital, former President of the French Society of Allergology, former President of the French Society of Pneumology and a member of the Collegium Internationale Allergologicum.

Immunotherapy (IT) is a well-documented treatment of allergic rhinitis and asthma. The major limitation is the risk of anaphylactic side effects. The documentation of clinical efficacy is based on crude allergenic extracts sometimes containing varying amounts of individual allergens including allergens to which the patient may not be sensitized. The introduction of recombinant allergens offer a possibility to use well-defined molecules with consistent pharmaceutical quality defined in mass units. The proof-of-concept of the clinical efficacy of recombinant allergens IT was demonstrated by two studies for birch and grass pollens. The goal of current IT research is to allow strong stimulation of the immune system, while bypassing potential adverse reactions, in order to increase efficacy and safety. Different approaches based on vaccination with recombinant allergen derivatives will be reviewed: use of synthetic peptides, recombinant allergen derivatives, different hypoallergenic molecules, combination of recombinant allergens to virus-like particles, or to immunomodulatory substances. Many of these new vaccines hold promise but only a few of them have been investigated in clinical trials which will be the gold standard for evaluation of safety an d efficacy in allergic patients.

CHRISTIAN LUPINEK

Dr. Cjristian Lupinek graduated as M.D. from the Medical University of Vienna, and performed his thesis and post-gradual education at the Medical University of Vienna, Department of Pathophysiology and Allergy Research.

Depletion of IgE antibodies by plasma exchange or immune adsorption enables to successfully treat patients with IgE associated diseases who are currently excluded from standard therapies due to extremely elevated total IgE levels or broad range sensitizations. Specific and efficient removal of IgE from plasma is provided by a newly developed immune absorber.

RICHARD WEISS

Dr. Richard Weiss has been working on the generation of novel types of vaccines for more than 15 years. After initially developing protective vaccines against infectious diseases such as Lyme disease and malaria, Dr. Weiss has subsequently focused on prophylactic and therapeutic DNA and RNA vaccination approaches for allergic diseases and has been a PostDoc at the Christian Doppler Laboratory for Allergy Diagnosis and Therapy at the University of Salzburg where he established himself as an independent scientist. He has supervised numerous Master and PhD students in the course of Austrian Science Fund (FWF) and industry funded projects as well as within the FWF funded PhD program "immunity in Cancer and Allergy". In recent years, he has focused on skin based vaccination strategies and its applicability for specific immunotherapy, generation of DC-targeting nanoparticles for cutaneous vaccination, and the influence of protein fold-stability on immunogenicity and allergenicity. Since 2012 he is holding a permanent position as Associate Professor at the Department of Molecular Biology in Salzburg.

During recent years, the skin has been shown to have several advantages for allergen-specific immunotherapy. In a mouse model he substituted allergen extracts or recombinant allergens with nanoparticle formulations, in which the recombinant allergen was bound covalently to carbohydrates such as mannan, and then administered them by means of microporation through the uppermost skin layer. This combination of a laser-based microporation platform (P.L.E.A.S.E. technology, Pantec Biosolutions) and an optimized allergen formulation represents a very promising approach for a highly efficient form of immunotherapy that is pain-free and without side effects.

The Austrian Society for Allergology and Immunology, ÖGAI, is proud to announce its Awardees of the Karl Landsteiner and Clemens von Pirquet Medals 2015

The award ceremony will take place on Friday, December 11, 2015 at 3:20 pm
Laudators: Rudolf Valenta and Hannes Stockinger

AWARDEE OF THE CLEMENS VON PIRQUET MEDAL OF THE AUSTRIAN SOCIETY FOR ALLERGOLOGY AND IMMUNOLOGY 2015

GABRIELLE PAULI

Prof. Gabrielle Pauli
Department of Pneumology
Strasbourg University Hospitals,
Strasbourg, France

According to the statutes of ÖGAI *'awardees of the Clemens von Pirquet Medal are internationally renowned scientists, who have made major contribution to the advancement of allergy research. Moreover, awardees have a close contact to the Austrian Society for Allergology and Immunology, ÖGAI, and/or its members'*
Former awardees: 1988: Robin R.R. Coombs, Cambridge, UK; 1990: Alain de Weck, Bern, Schweiz; 1994: Alec Sehon, Winnipeg, Canada; 2001: Allen P. Kaplan, Charleston, SC, USA; 2002: Gunnar Johansson, Stockholm, Schweden; 2002: Sergio Romagnani, Florenz, Italien; 2003: Dietrich Kraft, Vienna, Austria; 2006: Radvan Urbanek, Vienna, Austria; 2009: William Paul, Bethesda, MD, USA; 2010: Thomas Platts-Mills, Charlottesville, VA, USA; 2012: Reinhart Jarisch, Vienna, Austria; 2014: Jean Bousquet, Montpellier, France.

AWARDEE OF LANDSTEINER MEDAL OF THE AUSTRIAN SOCIETY FOR ALLERGOLOGY AND IMMUNOLOGY 2015

LORENZO MORETTA

Prof. Lorenzo Moretta
Department of Immunology
Bambino Gesù Hospital
Rome, Italy

According to the statutes of ÖGAI *'awardees of the Karl Landsteiner Medal are internationally renowned scientists, who have made major contribution to the advancement of immunology research. Moreover, awardees have a close contact to the Austrian Society for Allergology and Immunology, ÖGAI, and/or its members'*
Former awardees: 2004: Georg Wick, Innsbruck, Austria; 2008: Ronald N.Germain, Bethesda, MD, USA; 2009: Georg Stingl, Vienna, Austria; 2010: Josef Smolen, Vienna, Austria; 2014: Stephen Galli, Stanford, USA.

SESSION III
ORAL PRESENTATIONS OF SELECTED ABSTRACTS 1

Abstract Title	Are house dust mites (HDM) potential carriers of bacteria responsible for the induction of sensitization to microbial "allergens"?
Authors Family name, initials	Sheron Dzoro[1], Irene Mittermann[1], Marion Nehr[2], Alexander Hirschl[2] , Gustav Wikberg[3], Catharina Johansson[4], Lena Lundeberg[3], Annika Scheynius[4], Rudolf Valenta[1]
Affiliation	[1]Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria [2]Division of Clinical Microbiology, Clinical Institute of Laboratory Medicine, Medical University of Vienna, Vienna, Austria [3]Dermatology and Venereology Unit, Karolinska University Hospital, Stockholm, Sweden [4]Translational Immunology Unit, Department of Medicine, Solna, Karolinska Institute and Karolinska University Hospital, Stockholm, Sweden
Presenter	Dzoro. S

Introduction: Up to 25% of atopic dermatitis (AD) patients have been shown to display IgE reactivity against various antigens from *Staphylococcus aureus* and *Escherichia coli*. A genomic analysis has recently described *S. aureus* and *E. coli* species as the second and third most abundant bacteria within the microbiome of house dust mites (HDM). We therefore investigated the role of mites as carriers for IgE sensitization to microbial elements in AD patients.
Materials and Methods: We analysed sera from AD patients (n=179) for IgE reactivity to *S. aureus* and *E. coli* by immunoblotting, and to a comprehensive panel of HDM allergens by chip analysis. Rabbit antibodies specific for *S. aureus* and *E. coli* antigens were tested for reactivity to HDM extracts.
Results: Rabbit antibodies raised against bacterial antigens reacted with HDM extracts, demonstrating the presence of microbial antigens in HDM. However, the patient sera tested by inhibition experiments, shows no evidence for IgE cross-reactivity between *S. aureus* and *E. coli.* Importantly, we found no association between IgE sensitization to HDM and sensitization to *S. aureus* or *E. coli*, in the patients analysed, suggesting that there is a genuine IgE sensitization against bacterial components in these patients, a finding that is unusual because *S. aureus* and *E. coli* are rather known to induce Th1 immune responses and tolerance, respectively.
Conclusions: Our results indicate that IgE sensitizations against *S. aureus* and *E. coli* antigens in AD patients are genuine and not due to HDM which carry these microbes.

OI_1

Abstract Title	Rise in total IgE levels upon omalizumab treatment is not caused by activation of IgE^+ memory B cells
Authors Family name, initials	Eckl-Dorna, J.[1], Fröschl, R.[1], Lupinek, C.[1], Kiss, R.[1], Marth, K.[1], Campana, R.[1], Blatt, K.[1], Valent, P.[1], Selb, R.M.[1], Mayer, A.[1], Gangl, K.[1], Steiner, I.[1], Ziegelmayer, P. [2],, Gevaert, P.[2], Valenta, R.[1], Niederberger, V.[1]
Affiliation	[1]Medical University of Vienna, Waehringer Guertel 18-20, Vienna, Austria [2] Allergiezentrum Wien West, Huetteldorfer Straße 46, Vienna, Austria [3] Upper Airway Research Laboratory (URL), Ghent University Hospital, Sint-Pietersnieuwstraat 25, Ghent, Belgium
Presenter	Eckl-Dorna, J.

Omalizumab targets free IgE and inhibits its binding to mast cells. Interestingly during omalizumab therapy an increase in total serum IgE levels has been observed. In this study we investigated whether the latter is caused by a prolonged half-life of IgE upon complex formation with omalizumab or by enhanced IgE production of IgE^+ memory B cells upon crosslinking of their B cell receptor with omalizumab.

Total and allergen-specific serum IgE were determined in patients before and after subcutaneous treatment with omalizumab (n=15) or placebo (n=5).Omalizumab treated patients showed a 2-6 fold increase of total IgE and a polyclonal rise in specific IgE. To investigate whether this rise could be due to enhanced IgE production by IgE^+ memory B cells, we intranasally challenged patients (5/group) with omalizumab, placebo or Bet v 1 and measured total and allergen-specific IgE before and 8 weeks after the challenge. Intranasal omalizumab did not induce a change in total or allergen-specific serum IgE. A rise of Bet v 1-specific serum IgE was observed in Bet v 1 challenged patients as previously reported. Furthermore we tested the effect of omalizumab on IgE production by B cells *in vitro*. Omalizumab did not increase IL-4 and anti-CD40 induced IgE production in culture.

In summary, we observed no effect of omalizumab on IgE production. Thus the total IgE increase is likely to be by complex formation of omalizumab with IgE in the blood, thereby prolonging its half-life.

Supported by FWF (grant no. F4613)

OI_2

Abstract Title	Induction of functional MHC-specific IgE in murine allotransplantation
Authors Family name, initials	Farkas, A.M.[1], Baranyi, U.[1], Unger, L.[1], Schwarz, C.[1] , Mahr, B.[1], Hock, K.[1], Pilat, N.[1], Valenta, R.[2] and Wekerle, T.[1]
Affiliation	[1]Section of Transplantation Immunology, Dept. of Surgery, Medical Univ. of Vienna, Vienna, Austria; [2]Div. of Immunopathology, Dept. of Pathophysiology and Allergy Research, Center of Physiology and Pathophysiology, Medical University of Vienna, Austria
Presenter	Farkas, A.M.

Background: The presence of DSA is an adverse marker in most, but not all settings of allotransplantation. Additionally DSA-isotypes are insufficiently understood. In particular it is unknown if IgE is induced, which is capable of mediating unique effector mechanisms. Recently we developed a non-MHC antigen-mismatched transgenic mouse model (expressing the antigen Phl p 5 ubiquitously on the cell-surface), in which we found high levels of mismatch-specific IgE after rejection of heart and skin grafts. Here, we studied if donor-specific IgE is induced in an MHC-mismatched mouse model.

Methods: Skin or hearts of Balb/c (H-2^d) or C3H (H-2^k) mice was grafted in an allo-setting onto naïve B6 (H-2^b) or C3H or Balb/c mice. Serum-samples were taken pre and post transplantation at several time-points to analyze H-$2D^{d\ or\ k}$-, H-$2K^{d\ or\ k}$-, and I-$E^{d\ or\ k}$-specific IgE levels were measured via ELISA by using recombinant MHC monomers provided by the NIH tetramer facility. Additionally serum-samples were used for an *in vitro* basophil degranulation assay to assess if IgE is functional.

Results: This novel ELISA-approach revealed an induction of MHC-I-specific IgE detectable upon rejection of MHC-mismatched skin and heart grafts in all strain combinations tested. MHC-II-specific IgE was only detected specific for I-E^d. Additionally we were able to detect basophil degranulation upon cross-linking with recombinant MHC in the in vitro RBL-assay.
Conclusion: These data demonstrate that MHC-specific IgE develops upon allograft rejection. Additionally IgE is functional on the effector cell level *in vitro*.

OI_3

Abstract Title	Effects of allergy and SIT treatment on humoral and cellular immune responses to routine vaccination with TBE vaccine
Authors Family name, initials	Garner-Spitzer E.[1], Hofer M.[1], Seidl-Friedrich C.[1], Jarisch R.[2], Kinaciyan T.[3], Kundi M.[4], Wiedermann U.[1]
Affiliation	[1]Institute for Specific Prophylaxis &Tropical Medicine, Center for Pathophysiology, Infectiology and Immunology; Medical University of Vienna [2]Allergiezentrum Floridsdorf, Vienna [3]Department of Dermatology, DIAID, Medical University of Vienna [4]Institute for Public Health, Medical University of Vienna
Presenter	Garner-Spitzer E.

Type I allergies have increased drastically and afflict up to 30% of western populations. Allergic sensitization results in Th2-biased immune-responses and specific immunotherapy (SIT) leads to immuno-modulation via IL-10/TGF-ß and shift to Th1-profile (IFN-g).

In a clinical trial we investigate whether allergy alters responsiveness to routine vaccines, such as tick-borne encephalitis (TBE) vaccine. Allergic patients with or without SIT treatment and healthy controls received a booster vaccination against TBE. Immune-responses were evaluated via specific Ab-titers, cytokine production in PBMC and quantification of naïve/memory/regulatory subsets of B- and T-lymphocytes.

Our results show that humoral responses (NT titers) to booster vaccination were not reduced in allergics +/-SIT. Additionally TBE-IgG avidity and subclasses are currently determined. Analyses of in-vitro cytokine production confirmed a Th2 biased profile in allergics +/-SIT. Lymphocyte characterization in allergics +SIT showed expanded CD4, CD4-memory and central-memory (CM) subsets and enhanced FOXP3+ T-regs; in CD8 T-cells CM and EMRA subset were increased. Allergics without SIT feature less early vs. more late-differentiated CD4-memory T-cells. Alterations of B-cell subsets in allergics +SIT include expanded B memory subsets (IgD+/-, IgM+), decreased immature transitional B-cells and increased plasmablasts, which further increase post booster in all groups.
The results of this study will complete our understanding of vaccine responsiveness in these patient groups and so far indicate that allergy and SIT treatment do not impair humoral immune responses to booster vaccination, yet changes in the TBE-specific antibody subclasses seem to occur along with considerable effects on the cellular level.

OI_4

Abstract Title	Shielding of the major mugwort pollen allergen Art v 1 inside of virus-like nanoparticles makes it invisible for B-lymphocytes *in vivo.*
Authors Family name, initials	Kratzer B.,[1] and Pickl W. F.[1,2]
Affiliation	[1] Institute of Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna [2] Christian Doppler Laboratory for Immunomodulation, Vienna, Austria
Presenter	Kratzer B

Virus-like nanoparticles (VNP) are safe vaccine platforms, consisting of virus capsid proteins and a lipid envelope but lacking viral genome. Proteins of interest can be targeted to VNP by either C-terminally fusing them to a GPI anchor acceptor sequence – leading to surface expression – or, alternatively, by N-terminally fusing them to the viral matrix protein p15Gag – leading to their 'shielded' expression inside of VNP. Allergen-specific immunotherapy requires the repetitive delivery of potentially anaphylactogenic proteins to patients in order to achieve desired immunomodulatory effects. Consequently, safe containment strategies for unmodified allergens seem to be desirable. Therefore, VNP expressing Art v 1 either on the surface or shielded inside particles were analyzed for their potential to activate T and B lymphocytes or effector cells along with mugwort extract *in vitro* and *in vivo*. Degranulation of RBL cells sensitized with Art v 1-specific IgE only occurred upon exposure to VNP expressing surface exposed but not shielded allergen. In contrast, Art v 1 protein derived from both particles was well-presented to allergen-specific T cells, with shielded allergen exhibiting a 2.6±1.6-fold better stimulatory capacity compared to surface expressed allergen. Upon intranasal application into wildtype or Art v 1-specific 'allergy mice' VNP expressing surface exposed allergen induced significant titers of allergen-specific IgE, IgG1 and IgG2a, while VNP expressing shielded allergen entirely failed to do so. In summary, shielding of allergens inside of VNP might represent a safe and versatile alternative for *in vivo* delivery of potentially anaphylactogenic proteins, while preserving their T cell stimulatory (modulatory?) capacity.

Supported by the Austrian Science Fund (FWF) SFB-F4609, DK-W01248FW, Christian Doppler-Research Association and Biomay AG.

OI_5

Abstract Title	The environment alters allergenicity of ragweed pollen
Authors Family name, initials	Liu, S.[1], Debiasi, M.[1], Anea, C. B.[1], Karrer, G.[2], Bellaire, A.[3], Chaturvedi, P.[4], Weckwerth, W.[4], Epstein, M. M.[1]
Affiliation	[1] Department of Dermatology, DIAID, Medical University of Vienna, Vienna, Austria, [2] University of Natural Resources and Applied Life Sciences, Vienna, Austria, [3] Department of Botany and Biodiversity Research, University of Vienna, Vienna, Austria, [4] Department of Molecular Systems Biology, University of Vienna, Vienna, Austria.
Presenter	Liu, S.

Introduction: Ambrosia artemisiifolia, commonly known as ragweed, is a highly invasive plant with pollen that causes severe allergy. We sought to establish an experimental mouse model of ragweed pollen-induced allergic disease and to determine whether the environment alters pollen allergenicity.

Materials and methods: Ragweed (Ambrosia artemisiifolia) pollen (Allergon, Sweden and ALK-Abelló, Denmark), pollen samples collected from urban and rural areas of Austria and pollen treated under different environmental conditions such as changes in pH, temperature and pollutants were used to induce allergic asthma in 6-8 week old female BALB/c mice. We administered treated and untreated ragweed pollen (0.1-100 µg) suspended in 50 µl PBS intranasally 6 times over a 3-week period and 72h after the last pollen challenge, evaluated the mice for lung and airway inflammation, mucus secretion and serum ragweed-specific IgG1.

Results: Ragweed pollen induced dose-dependent allergic airway and lung inflammation, mucus hypersecretion, and IgG1 antibody production. We observed differences in the severity of disease dependent upon the area that the pollen was harvested from with urban pollen inducing more severe allergic disease compared with rural pollen. Furthermore, when we mimicked environmental conditions, such as acid rain, heat waves and pollution by treating pollen with high temperatures, low pH and certain pollutants, e.g. ozone, we also observed changes in disease severity.
Conclusion: Taken together, our data demonstrate that inhalation of environmentally-treated pollen and pollen obtained from distinct environments alter ragweed pollen allergenicity in this mouse model.

This research was supported by EC-FP7-ATOPICA project, grant no. 282687.

OI_6

Abstract Title	Differential fold-stability during endolysosomal maturation determines immunogenicity and allergenicity of the major birch pollen allergen
Authors Family name, initials	Machado, Y.[1], Freier, R[1], Scheiblhofer, S.[1], Thalhamer, T.[1], Mayr, M.[1], Briza, P.[1], Grutsch, S.[2], Ahammer, L.[2], Fuchs, J. E.[3], Wallnoefer, H. G.[3], Isakovic, A.[1], Kohlbauer, V.[1], Hinterholzer, A.[1], Steiner, M.[1], Danzer, M.[4], Horejs-Hoeck, J.[1], Ferreira, F.[1], Liedl, K. R.[3], Tollinger, M.[2], Lackner, P.[1], Johnson, C. M.[5], Brandstetter, H.[1], Thalhamer, J.[1], Weiss, R.[1]
Affiliation	[1] University of Salzburg, Department of Molecular Biology, 5020 Salzburg, Austria [2] University of Innsbruck, Center of Molecular Biosciences & Institute of Organic Chemistry, 6020 Innsbruck, Austria [3] University of Innsbruck, Center of Molecular Biosciences & Institute of General, Inorganic and Theoretical Chemistry, 6020 Innsbruck, Austria [4] Austrian Red Cross, Blood Transfusion Service for Upper Austria, 4020 Linz, Austria [5] MRC Laboratory of Molecular Biology, Cambridge CB2 0QH, UK
Presenter	Machado, Y.

Bet v 1.0101, the major allergen of birch pollen is known as a strong primary sensitizer. However, the mechanisms underlying its allergy promoting capacity remain to be clarified. In the present work, we studied the effect of fold-stability on immunogenicity and allergenicity of Bet v 1. Four, fold-stabilized Bet v 1 variants were generated based on in silico calculations and screening. Crystal structures of the mutants were obtained. Thermal and chemical stability as well as molecular flexibility of the molecules was monitored. . Antigen processing and presentation was studied in human monocyte-derived dendritic cells. Stability to proteolysis and pH-induced unfolding was determined in vitro. Immunogenicity was studied in vivo using BALB/c mice. Antibody titers, basophil activation, and T cell polarization were assessed. By in silico mutation and screening, four mutants of the prototype pollen allergen Bet v 1, with predicted increased structural stability, were generated. The mutants displayed an increased thermal and chemical stability, and a stepwise rigidification of their backbone. In contrast to wild type Bet v 1, the variants induced an allergy-promoting T helper 2 type immune response upon adjuvant-free immunization of mice, which was increased by the number of mutations. Interestingly, the immunodominant T cell-activating epitope was more efficiently presented by monocyte-derived dendritic cells pulsed with the mutated proteins. Whereas at pH values above 5.2, protease resistance was increased, at pH below 5.2 the mutants were efficiently processed. These data indicate that differential fold-stability along endolysosomal maturation is a crucial determinant for allergenicity of Bet v 1.

OI_7

SESSION IV
ORAL PRESENTATIONS OF SELECTED ABSTRACTS 2

Abstract Title	Multifunctional role of the transcription factor Blimp1 in coordinating plasma cell differentiation
Authors Family name, initials	Minnich, M.[1], Tagoh, H.[1], Bönelt, P.[1], Axelsson, E.[1], Fischer, M.[1], Cebolla B.[1], Tarakhovsky, A.[2], Nutt, SL.[3,4], Jaritz, M.[1] and Busslinger, M.[1]
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Presenter	Minnich, M.

Blimp1 is necessary for plasma cell generation. Here we studied its functions in plasmablast differentiation by identifying regulated Blimp1 target genes. Blimp1 promoted plasmablast migration and adhesion. It directly repressed several transcription factor genes and *Aicda*, thus silencing B cell-specific gene expression, antigen presentation and class switch recombination in plasmablasts. It directly activated genes, leading to increased expression of the plasma cell regulator IRF4 and proteins involved in immunoglobulin secretion. Blimp1 induced immunoglobulin gene transcription by controlling *Igh* and *Igk* 3′ enhancers and regulated the posttranscriptional expression switch from the membrane-bound to secreted immunoglobulin heavy-chain by activating *Ell2*. Notably, Blimp1 recruited chromatin-remodeling and histone-modifying complexes to regulate its target genes. Hence, many essential functions of plasma cells are under Blimp1 control.

OII_1

Abstract Title	Mapping of human rhinovirus-specific antibody responses using high resolution microarray
Authors Family name, initials	Niespodziana, K.[1], Stenberg-Hammar, K.[2,3], Cabauatan, C. R.[1], Napora-Wijata, K.[1], Vacal P.L.[1], Gallerano, D.[1], Lupinek, C.[1], Ebner, D.[4], Schlederer, T.[4], Harwanegg, C.[4], Melén, E.[5,6], Söderhäll, C.[7], van Hage, M.[8], Hedlin, G.[2,3], and Valenta, R.[1]
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Presenter	Niespodziana, K.

Rhinovirus (RV) infections are major triggers of acute exacerbations of asthma and chronic obstructive pulmonary disease (COPD) in both children and adults. The association of rhinovirus infections with exacerbations of respiratory disease is mainly based on the demonstration of the presence of virus at the onset of exacerbation. However, there are currently no serological tests available which would allow detecting specificities of antibody responses against RV epitopes as a result of infection. We, therefore, developed a high resolution antibody assay based on recombinant antigens and peptides from the most common RV strains. The optimized microarray contains in total 130 components and includes 48 recombinant RV proteins and 66 VP1-derived synthetic peptides. We demonstrated that extremely small sample volumes are sufficient to detect RV-specific IgG and IgA antibodies to a broad panel of micro-arrayed RV antigens. Moreover, using serum samples from 120 preschool children collected during an acute episode of wheeze and at follow-up visit after approximately 12 weeks, it was possible to discriminate between group- and partially strain-specific antibody responses in RV-infected patients and thus, to identify the most relevant and clinically important RV strains involved in triggering exacerbations of respiratory diseases. The microarray will be useful to identify the most common RV strains involved in asthma exacerbations and thus provide a rational basis for the design of a RV vaccine.

This study was supported by the European Commission's Seventh Framework programme under grant agreement N° 260895 (PreDicta) and by a research grant from Biomay AG, Vienna, Austria.

OII_2

Abstract Title	The effects of Btk inhibitors on IgE receptor-mediated signal transduction and activation of mast cells and basophils
Authors Family name, initials	Smiljkovic, D.[1], Blatt, K.[1], Stefanzl, G.[1], Dorofeeva, Y.[2], Focke-Tejkl, M.[2], Valenta, R.[2], Valent, P.[1,3]
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Presenter	Smiljkovic, D.

Mast cells (MC) and basophils (BA) are regarded as key effector players in allergic disorders. Both types of cells express high-affinity receptors for IgE (FcεRI). Activation of MC and BA through FcεRI is associated with activation of downstream signalling pathways and enhanced expression of cell-surface antigens such as CD63 or CD203c. Recently, the Bruton´s tyrosine kinase (BTK) has been identified as a new potential downstream-target in FcεRI-cross-linked MC and BA. The aim of this study was to explore the effects of various BTK blockers on IgE-mediated histamine release, phosphorylation of downstream signalling targets and upregulation of cell-surface antigens. We examined human blood BA from 3 healthy donors and 9 patients allergic to Bet v 1, Der p 2, and/or Phl p 5 as well as the human mast cell line HMC-1 by flow cytometry. In addition, histamine release experiments were performed with BA. We found that the BTK blocker Ibrutinib counteracts anti-IgE-induced and allergen-induced upregulation of CD63 and CD203c (IC50 <0.5 μM) and histamine release (IC50 values <0.025 μM) in BA. The other two Btk inhibitors tested, AVL-292 and CNX-774, were also found to suppress IgE-mediated histamine release (IC50 <0.05). Moreover, as determined by flow cytometry, all BTK blockers tested were found to inhibit phosphorylation of BTK in HMC-1 cells as well as in FcεRI-cross-linked BA. Together, our data show that Ibrutinib and other BTK inhibitors suppress anti-IgE-induced upregulation of CD63 and CD203c as well as IgE-mediated histamine release in BA at reasonable drug concentrations.

Supported by grants F4605, F4611 and by the PhD program MCCA of the Austrian Science Fund (FWF).

OII_3

Abstract Title	Immunization with Immune Complexes Modulates the Fine-Specificity of Antibody Responses to a Flavivirus Antigen
Authors Family name, initials	Tsouchnikas, G.[1], Zlatkovic, J.[1], Jarmer, J.[1], Strauss,J.[1], Vratskikh, O.[1], Kundi,M.[2], Stiasny, K.[1], Heinz, F.X.[1]
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Presenter	Georgios Tsouchnikas[1]

Immune complexes (ICs) can modulate the immune response to the antigen via several antibody feedback-mechanisms, resulting in enhancement or suppression. However, there is less information about the capacity of ICs to alter the epitope-specificity of the antibody response. Since in polyclonal sera, the specificity of antibodies to different antigenic sites can affect functional activity, variations can be relevant for an effective immune response.

The objective of this mouse immunization-study was to investigate changes in quantity and fine-specificity of antibody responses to the tick-borne encephalitis (TBE) virus envelope (E) protein administered alone or as an IC with monoclonal antibodies (mAbs) directed to its structural domains (DI, DII, DIII).
Due to the modular organization of E, the fine-specificity of polyclonal antibodies can be dissected by immunoassays using recombinant single domains or domain-combinations of E.

Our analyses revealed that immunization with the IC containing a monoclonal antibody specific for DII strongly modulated the fine-specificity of the antibody. These results could be explained mechanistically by demonstrating that this mAb dissociates the E dimer into monomers, and thus exposes new immunogenic epitopes. A different mechanism was found for a DIII-specific mAb which reduced the response to the virion by shielding its highly surface-accessible epitope.
Our results provide direct mechanistic insights into the structure-specific modulation of an immunogen by bound antibodies that lead to a change in the specificity of polyclonal antibody responses. Similar phenomena can play also a role in a natural situation in which pre-existing antibodies encounter the antigen and form ICs in vivo.

OII_4

Abstract Title	Development of the recombinant *Blomia tropicalis* allergen Blo t 2 for immune-diagnosis
Authors Family name, initials	Urrego J.[1,2], Hofer H.[1], Aglaz L.[1], Pinheiro C.[2], Briza P.[1], Wallner M.[1], Alcantâra-Neves N.[2], Ferreira F.[1]
Affiliation	[1] University of Salzburg, Salzburg, Austria [2] Instituto de Ciências da Saúde, Universidade Federal da Bahia, Salvador, Bahia, Brazil.
Presenter	Juan Urrego

Background: *Blomia tropicalis* is a species of house dust mite that is associated with allergic symptoms in tropical and subtropical regions. However, the clinical importance of group 2 allergens from this mite is still under debate. Until now only rare information of Blo t 2 is available.

Methods: The recombinant Blo t 2 sequence was codon-harmonized, transformed and expressed in the E. coli strain Shuffle T7. The protein was purified under non-denaturing conditions via anion exchange chromatography. Physico-chemical characterization was performed using circular dichroism (CD) and Fourier transform infrared spectroscopy (FTIR) as well as mass spectrometry (MS). The allergenicity was determined by immunoblot and ELISA.

Results: rBlo t 2 was expressed as soluble protein in *E. coli*. The CD spectra of rBlo t 2 displayed a broad minimum at 215 nm and a maximum at 195 nm, which is typical for proteins with a mixed α/β-fold with elevated β-sheet content. Interpretation of the FTIR spectra revealed secondary structure elements mainly in the area corresponding to β-sheets and unordered elements. According to these analyses 55 % of the protein is assembled by β-sheets. Almost 54 % (24/44) of Brazilian patients allergic to *Blomia tropicalis* reacted with rBlo t 2.
Conclusion: We successfully expressed, purified and characterized the rBlo t 2. For the first time, we confirmed that rBlo t 2 is an important allergen inducing high sensitization rates in the Brazilian population, which should be considered for diagnosis and drug development for immunotherapy.

OII_5

Abstract Title	Interaction of human B cells and melanoma cells to induce therapy resistance
Authors Family name, initials	Wagner, S.N. [1], Somasundaram, R. [2], Zhang, G. [2], Perego, M. [2], Fukunaga-Kalabis, M. [2], Garg, K. [1], Maurer, M.[1], Herlyn, M. [2]
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Presenter	Wagner, S.N.

Tumors actively recruit immune cells into their tumor microenvironment (TME). In melanoma, up to 33% of the infiltrating immune cells are of 'B cell'-lineage and yet, there is very little information on the role of these cells in melanoma cell biology.

We have collected from melanoma patients matched pairs of melanoma cells, PBMCs and EBV-transformed tumor-associated and PBMC-derived B cells. In autologous co-cultures we have performed qRT-PCR-, FACS-, and phospho-RTK array-based phenotyping of melanoma and B cells. Interference strategies included neutralizing antibodies and shRNA-based gene knock-down. Confirmatory immunostainings in human melanoma samples were evaluated by TissueFAX.

Our results suggest that B cells infiltrating human melanoma samples assume an inflammatory phenotype characterized by the increased expression of pro-inflammatory cytokines (IGF-1, IL-1, PDGF and VEGF) when compared to circulating B cells. Induction of this inflammatory phenotype is dependent on FGF-2, which is secreted by melanoma cells. B cell-derived growth factor IGF-1 is critical for induction of drug resistance of melanoma cells since it stimulates the emergence of heterogeneous subpopulations and activation of FGFR-3. Neutralization of B cell-produced IGF-1 or knockdown of FGFR-3 reverses tumor heterogeneity and restores sensitivity to kinase inhibition.

Our findings suggest an important role of B cells in the induction of therapy resistance and offer a new (combination) treatment approach.

Supported by: Vienna Science and Technology Fund (WWTF through project LS11-045 to Stephan N. Wagner)

OII_6

Abstract Title	Analyzing the cross-reactivity of Amb a 1, the major allergen of short ragweed
Authors Family name, initials	Wolf M.[1], Hauser M.[1], Pichler U.[1], Twaroch T.[2], Gadermaier G.[1], Ebner C.[3], Yokoi H.[4], Takai T.[5], Didierlaurent A.[6], Mari A.[7], Briza P.[1], Behrendt H.[8], Neubauer A.[2], Stolz F.[2], Ferreira F.[1], Wallner M.[1]
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Presenter	Wallner M.

Background: Amb a 1, the major allergen of the Asteraceae species ragweed, belongs to the pectate lyase allergen family. To date, five different Amb a 1 isoforms have been officially acknowledged. Moreover, pectate lyases have been identified in related Asteraceae weeds, as well as within trees belonging to the Cupressaceae order. Thus, we thought to investigate cross-reactivity pattern of Amb a 1 isoforms as well as related allergens from different sources.

Methods: Pectate lyase allergens from ragweed (Amb a 1), mugwort (Art v 6), cypress (Cup a 1), mountain cedar (Jun a 1), and Japanese cedar (Cry j 1) were purified from pollen extracts. Moreover, three Amb a 1 isoforms (Amb a 1.01, 02, and 03, respectively) were either purified from pollen extracts or produced as recombinant proteins in *P. pastoris*. The allergens were characterized physico-chemically and thereafter IgE binding was assayed.

Results: We used four different cohorts for cross-reactivity profiling. Each of the four cohorts showed a distinct sensitization fingerprint, which reflected the natural allergen exposure of the patients. Moreover, we analyzed the IgE binding to different Amb a 1 isoforms using sera of Amb a 1 sensitized individuals from Central Europe. Within this cohort, we found that all three tested Amb a 1 isoforms were recognized by IgE to a similar extent.
Conclusion: Our data suggests that cross-reactivity between Asteraceae and Cupressaceae allergens was limited, but we found considerable cross-reactivity within each order. Moreover, there was no significant difference in IgE reactivity of Amb a 1 isoforms.

Supported by FWF project L688, CK-CARE AG Individual Project 2009-02, Biomay AG, the Christian Doppler Research Association, and Sparkling Science SPA 05-193.

OII_7

POSTER SESSION I_Molecular Allergy

Abstract Title	Cloning, expression in Insect cells and immunological characterization of Par j 2.0101, a major allergen of *Parietaria judaica* pollen
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Presenter	Dorofeeva , Y.

Introduction: *Parietaria judaica* is one the most common pollen allergen sources in the Mediterranean area and with a long period of pollination from February to November Par j2.0101, a cysteine-rich, lipid transfer protein (LTP) with a molecular weight of 11.3 kDa is the major allergen in *Parietaria judaica* recognized by more than 80% of allergics. It shows a high cross-reactivity with *Parietaria officinalis* that is represented also in Western Asia and the Caucasus.

Using *Escherichia coli*-based expression systems it is difficult to obtain soluble and folded LTPs.

Materials and Methods: A synthetic gene, codon-optimized for insect cells coding for Par j 2 including 6xHistag at the C-terminus sites was subcloned into pTM1 vector into the BamHI/SmaI sites. This construct was transformed into *E. coli* to generate high molecular weight recombinant bacmid DNA and then transfected into insect cells to obtain recombinant baculovirus for expression.

Results: Recombinant soluble Par j 2 which was secreted into the culture supernatant was obtained by expression in baculovirus-infected insect cells. The recombinant protein reacted on sera of the allergic donors (Greece), with an anti-6XHistag antibody and cross-reacted with specific antibodies raised against LTP from peach. The protein was analyzed by gel filtration and CD.

Conclusions: By expression in baculovirus-infected insect cells it was possible to obtain soluble and folded recombinant rPar j2 for diagnosis and possibly for therapy of patients with *Parietaria* pollen allergy.

The work has been supported by the FWF-funded PhD program DKW1248-B13, MCCA

PI_1

Abstract Title	Towards the characterization of the allergenic activity of carbohydrate-reactive IgE
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Presenter	Gattinger P.

Introduction: A large number of allergens derived from insect venoms, moulds, pollen and plant-derived food are glycoproteins. Their Asparagine (N)-linked carbohydrates contain structural motifs which are not found on human glycoproteins. This N-linked glycans are part of the cross-reactive carbohydrate determinants (CCD) that can elicit IgE in about 20% of allergic patients. In order to investigate the allergenic activity of carbohydrates we have engineered N-linked glycosylation sites into the non-allergenic protein horse heart myoglobin (HHM).

Methods: Sequences coding for one or two N-glycosylation sites were engineered into the 5' end of the HHM cDNA and the proteins were expressed in baculovirus-infected High-FiveTM insect cells. A non-glycosylated version of HHM was tested for control purposes. The glycoproteins were analysed regarding fold and aggregation circular dichroism and gel filtration, respectively. IgE reactivity was assessed by ELISA and Immunoblotting.

Results: HHM-glycovariants were expressed and purified from insect cells as monomeric and folded proteins. IgE from patients with bee and/or wasp, as well as pollen sensitization showed reactivity to the HHM-glycovariants but not to non-glycosylated HHM.

Conclusion: The HHM-glycovariants can be useful as a marker for IgE-reactivity to carbohydrates and will allow to determine the allergenic activity of carbohydrate IgE epitopes.

Supported by the FWF-funded PhD program MCCA, the FWF projects P26728-B20, P23350-B11 and F4604, by the Christian Doppler Research Association, Austria and by a research grant from Thermofisher, Uppsala, Sweden.

PI_2

Abstract Title	A chimeric protein containing the C-terminus of Bet v 1 is a potent inducer of basophil degranulation
Authors Family name, initials	Gepp, B.[1], Lengger, N.[1], Kitzmüller, C.[1,2], Radauer, C.[1], Bohle, B.[1,2], Breiteneder, H.[1]
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Presenter	Gepp, B.

Background: Characterization of IgE epitopes of the major birch pollen allergen, Bet v 1, is essential for the design of hypoallergenic vaccine components. However, little is known about the biologic relevance of IgE specific for different surface areas of Bet v 1.

Methods: Four Bet v 1-specific surface areas were grafted onto the Bet v 1-related allergen Api g 1. The resulting chimeras, called Api-Bet-1 to Api-Bet-4, were expressed in *Escherichia coli* and purified. Basophil activation tests using blood of 15 birch pollen allergic donors were performed with the recombinant proteins. CD63-positive cells in the $CCR3^{+}CD123^{+}$ population were measured by flow cytometry. The concentration required to reach half maximum activation was defined as AC50.

Results: Bet v 1 exhibited the highest and Api g 1 the lowest potency to activate basophils with a median AC50 of 0.2 ng/mL and 30 ng/mL, respectively. Api-Bet-3 revealed the lowest median AC50 among the chimeras with 2.2 ng/mL compared to Api-Bet-2, Api-Bet-1 and Api-Bet-4 with AC50s of 3.5 ng/mL, 4 ng/mL, and 6 ng/mL, respectively. In 6/15 patients, Api-Bet-3 showed the highest ability to activate basophils.

Conclusion: Grafting of four Bet v 1-specific areas onto Api g 1 increased its potency to activate basophils, indicating that the whole surface area of Bet v 1 contains biologically active IgE-binding epitopes. However, Api-Bet-3 was the most potent chimera to activate basophils, indicating that important IgE-binding epitopes are located at the C-terminus.

Supported by FWF grants SFB F4608, F4610 and the Christian Doppler Laboratory for Immunomodulation.

PI_3

Abstract Title	Seed-specific allergens associated with severe symptoms of celery allergic patients
Authors Family name, initials	Humeniuk P.[1], Dubiela P.[1], Pfeifer S.[1], Aina R.[1], Bublin M.[1], Bienvenu F.[2], Pauli G.[2], Hoffmann-Sommergruber K.[1]
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Presenter	Humeniuk P.

Background: Vegetables represent a large group of plant foods which provide important nutrients but can also induce allergic reactions.. Celery seems to frequently induce severe allergic reactions and can cause fatal anaphylactic shocks. Although a number of celery allergens were identified, it seems that some allergens are still missing in the panel of proteins to be used as diagnostic tools. The identification of seed-specific allergens could improve the sensitivity of in vitro allergy diagnosis. The aim of this study is to identify allergens from celery seeds associated with severe symptoms of celery-allergic patients.

Methods: Sera from four patients with severe reactions upon celery consumption were tested in customized allergen microarrays. Celery seed proteins were extracted by standard protocol. In immunoblots using total protein extracts sera were tested for celery specific IgE antibodies.

Results: While in the microarray analysis only Api g 1 positive IgE antibodies were detected, the immunoblot analysis provided additional information. IgE binding to proteins at molecular masses of 13, 40, 50 and 90 and 100 kDa was observed.

Conclusions: We could identify additional IgE binding proteins present in celery seeds which may contribute to improved allergen specific in vitro diagnosis.

Supported by grants SFB F4603 and W1248 (Austrian Science Fund) to K. Hoffmann-Sommergruber and P. Humeniuk, respectively.

PI_4

Abstract Title	Mapping IgE epitopes of food allergens which cross-react with the major birch pollen allergen, Bet v 1
Authors Family name, initials	Kodydek, M. A. A. [1], Hoffmann-Sommergruber, K. [1], Keller, W. [2], Pavkov-Keller, T.[2], Valenta, R. [1], Focke-Tejkl, M. [1]
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Presenter	Kodydek, M. A. A.

Consumption of plant food often results in oral allergy syndrome (OAS) in birch pollen allergic patients due to cross-reactivity of IgE antibodies specific for the major birch pollen allergen, Bet v 1, with the respective food allergens. We compared epitope recognition patterns responsible for this cross-reactivity with five homologous plant food allergens. Rabbit antisera against six peptides spanning the Bet v 1 sequence were used to inhibit allergic patients (N=33) IgE binding to rBet v 1 (birch pollen), rAra h 8 (peanut), rPru av 1 (cherry), rApi g 1 (celery), rDau c 1 (carrot) and rMal d 1 (apple). These patients complained about symptoms of OAS to one or more of the food sources and had birch specific IgE levels of ≥10 kUA/l. Twenty three patients showed reactivity to rBet v 1, 19 to rPru av 1, 17 to rAra h 8, nine to rMal d 1, seven to rApi g 1 and four to rDau c 1. Inhibition results revealed different IgE epitope-containing patches on the allergens. Four major epitopes were identified in rBet v 1; two were common for rPru av 1 and rMal d 1. Allergens rAra h 8, rApi g 1 and rDau c 1 each consisted of only one IgE epitope-containing patch. Our study identifies Bet v 1 as the major IgE epitope-containing allergen which therefore appears to be the primary sensitizing molecule in patients suffering from OAS to Bet v 1-cross-reactive food allergens.

PI_5

Abstract Title	The immune response against the timothy grass pollen allergen Phl p 5 in non-allergic humans
Authors Family name, initials	Kurtaj, A.[1], Hillebrand, C.[1], Fichtinger, G.[1], Danzer, M.[2], Gabriel, C.[2], Thalhamer, T.[1], Scheiblhofer, S.[1], Thalhamer, J.[1], Weiss, R.[1]
Affiliation	[1]University of Salzburg, Molecular Biology, Salzburg, Austria [2]Red Cross Blood Transfusion Service, Linz, Austria
Presenter	Kurtaj, A.

In our present study, we examine a new hypothesis, which postulates that non-allergic individuals mount antigen-specific immune responses against rPhl p 5 and that different immune response types exist and maintain this healthy condition. Furthermore, we hypothesize that depending on the living environment, non-allergic immune responses can be different. Hence, we assessed the immune status of non-allergic people living in a farming environment and non-allergic people living in an urban environment.

After enrichment of antigen-specific memory T cells, cytokine secretion and transcription factors, allowed identification of different T helper subsets. Moreover, antigen-specific IgE, IgG1, IgG4, and IgA antibody levels in non-allergic humans were measured by ELISA.

We found three different immune response types in non-allergic donors. First, a “Th0” immune response type displaying individuals with no clear polarization and weak immune response. Next, a “Tr1” immune response type characterized by IL-10 secreting cells. Last but not least, a “Th1” immune response type showing high IFN-γ secretion. These three immune response types were found in a significantly different distribution in townspeople and farmers.

Additionally, we detected significantly higher Phl p 5-specific IgG1 titers than IgG4 titers in both non-allergic groups. Interestingly, townspeople showed significantly increased Phl p 5-specific IgG4 titers and IgG seroconversion compared to famers. In summary, it can be stated that tolerance induction is not the only mechanism to maintain a non-allergic state but rather multiple mechanisms of naturally acquired protection exist and depending on the living environment different immune response types can establish and maintain a healthy non-allergic status.

PI_6

Abstract Title	Epitope presentation on allergens is critical for allergenic activity
Authors Family name, initials	Najafi, N.[1], Hofer, G.[2], Blatt, K.[3], Selb, R.[4], Stoecklinger, A.[5], Keller, W.[2], Valent, P.[3], Niederberger, V.[4], Thalhamer, J.[5], Valenta, R.[1], Flicker, S.[1]
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Presenter	Najafi, N.

Introduction: Usually reduction of the allergenic activity of an allergen can only be achieved by reduction of its IgE reactivity by denaturation, fragmentation, mutation or structural reassembly. So far, only one example of a trimeric form of the major birch pollen allergen Bet v 1 has been reported which despite maintained IgE reactivity exhibited reduced allergenic activity.

Material and Methods: A hybrid molecule consisting of the major grass pollen allergen Phl p 5 and major birch pollen allergen Bet v 1 was expressed. IgE reactivity and basophil activation of the purified hybrid allergen were studied and compared to results obtained with the equimolar mixture of Phl p 5 and Bet v 1. Secondary and tertiary structures of the hybrid were investigated by circular dichroism (CD), size exclusion chromatography (SEC) and dynamic light scattering (DLS). The appearance of the hybrid was studied by negative stain electron microscopy.

Results: The hybrid exhibited stronger IgE reactivity than the equimolar allergen mixture but unexpectedly showed a reduced allergenic activity. SEC and DLS showed that the hybrid formed stable and soluble high molecular weight aggregates which according to CD spectra lost alpha helical fold to a large extent. The hybrid occurred in monomeric, oligomeric and polymeric appearance in solution.
Conclusion: The hybrid is the second known example of a protein which despite maintained IgE reactivity exhibited reduced allergenic activity due to aggregation and thus due to altered and/or orientation presentation of IgE epitopes.

Supported by FWF grants P23318, F4604, F4605, F4607 and F4611

PI_7

Abstract Title	Der p 5, Der p 7, Der p 21 and Der p 23 show high allergenic activity in HDM-allergic patients
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Presenter	Resch, Y.

Introduction: Der p 1 and Der p 2, the two major allergens in house dust mites (HDMs), have been extensively studied, however, little is known about their allergenic activity in comparison to other HDM allergens.

Methods: Seven important HDM allergens (Der p 1, 2, 5, 7, 10, 21 and Der p 23) were studied regarding their IgE-binding frequency, allergen-specific IgE antibody levels (ImmunoCAP) and allergenic activity in CD63-based basophil activation tests, in 30 clinically well-characterized HDM-allergic patients.

Results: Der p 1 and Der p 2 were the most frequently recognized allergens (90%) and bound the highest levels of allergen-specific IgE (mean: 8.59 kUA/L and 7.73 kUA/L respectively) in the tested patients, but they were not the most potent allergens regarding basophil activation. In fact, the newly identified major allergen Der p 23 (IgE-binding frequency: 83%) induced the strongest basophil activation, although the allergen-specific IgE levels were lower than those for Der p 1 and Der p 2 (mean: 2.61 kUA/L). Der p 5 showed the third highest IgE levels (mean: 4.18 kUA/L) and the second strongest basophil activation. The allergenic activity of Der p 21 was as high as that of Der p 2, while the basophil activation of Der p 7 and Der p 10 was lower in the patients.

Conclusion: Besides Der p 1 and Der p 2, Der p 5, Der p 7, Der p 21 and Der p 23 are important HDM allergens due to high allergenic activity and IgE recognition frequency.

This work was supported by grants F4602 and F4605 of the Austrian Science Fund (FWF), Thermofisher, Uppsala, Sweden and the Christian Doppler Research Association, Austria.

PI_8

Abstract Title	Development of sandwich ELISAs for the quantification of clinically relevant house dust mite allergens
Authors Family name, initials	Azahara Rodríguez[1], Yvonne Resch[1], Mirela Curin[1], Huey-Jy Huang[1], Srinita Banerjee[1], Rudolf Valenta[1], Susanne Vrtala[1]
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Presenter	Azahara Rodríguez[1]

House dust mite (HDM) allergy affects more than 10% of the population in industrialized countries. Beside Der p 1 and Der p 2, the HDM allergens Der p 5, Der p 7, Der p 21 and Der p 23 have been identified as the clinically most important allergens with high allergenic activity. Assays for measuring allergen concentrations in environmental samples, diagnostic and therapeutic allergen extracts are available only for Der p 1 and Der p 2.

The aim of this study was to develop sandwich ELISAs for the detection and quantification of Der p 5, Der p 7, Der p 21 and Der p 23.

Allergen-specific antibodies with defined specificities were obtained by immunizing rabbits with synthetic peptides derived from different portions of the allergens and with the complete recombinant allergens. The rabbit antisera were tested for allergen reactivity towards immobilized allergens and allergens in solution and used to build sandwich ELISAs based on capturing and detecting antisera with defined specificity. Using purified allergens for standardization will allow to quantify the natural allergens in biological samples. The sandwich ELISAs will be useful to measure and quantify the HDM allergens Der p 5, Der p 7, Der p 21 and Der p 23 in environmental samples, in allergen extracts used for challenge tests as well as in diagnostic and therapeutic allergen extracts

Supported by the FWF-funded PhD program MCCA, by the FWF projects F4605, F4602 and by a research grant from Biomay AG, Vienna, Austria.

PI_9

Abstract Title	Enhancing recombinant production yield of Bet v 1 through codon usage harmonization
Authors Family name, initials	Roulias, A.[1], Parigiani, M.A.[1], Hofer, H.[1], Asam, C.[1], Ebner, C.[2], Wallner, M.[1], Ferreira, F.[1]
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Presenter	Roulias, A.

Improving production yield and elevating quality of recombinantly produced molecules is a high priority for the biotechnological as well as for the clinical sector. This issue has been tackled from numerous angles, one of them being codon usage bias. Crucial information concerning the overall protein synthesis procedure is encoded by the codon usage frequency and suboptimal codon usage bias can significantly limit heterologous protein expression. “Codon harmonization” is a strategy that effectively minimizes codon usage disparities between native organism and heterologous host by closely matching their respective codon usage frequencies. In this study we investigated the effects of codon harmonization in the recombinant production of the allergen Bet v 1.0101.

Different batches of rBet v 1 and its harmonized version, Bet-Harm, were produced in parallel. Production yields were quantified by SDS-PAGE densitometry. All batches were phisicochemically analyzed by Circular Dichroism, Dynamic Light Scattering, Fourier Transform Infrared Spectroscopy and ANS-binding assays. Immunological properties of the rBet v 1 and Bet-Harm batches were compared by endolysosomal degradation assays, ELISA and mediator release assays.

A significant increase in protein yield and solubility was observed for Bet-Harm compared to rBet v 1. Besides, the two proteins displayed no alterations in their secondary structure elements, their behavior in solution and their ligand-binding ability. Furthermore, no differences in the proteolytic susceptibility, IgE-binding and IgE cross-linking ability of rBet v 1 and Bet-Harm were observed.

Codon harmonization is an effective approach towards increasing protein expression levels and should be considered as a potent strategy for overcoming protein production problems.

PI_10

Abstract Title	Production of a recombinant hypoallergenic variant of the major peanut allergen Ara h 2 in the baculovirus insect cell system
Authors Family name, initials	Tscheppe, A.[1],Palmberger, D.[2], Bublin, M.[1], Radauer, C.[1], Palladino, C.[1], Gepp, B.[1], Lengger, N.[1], Grabherr, R.[2], Breiteneder, H.[1]
Affiliation	[1]Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria [2]Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria
Presenter	Tscheppe, A.

Introduction: Peanut allergy is one of the most dangerous food allergies. Ara h 2 is a major peanut allergen. At present, peanut allergen-specific immunotherapy is not available for clinical use. We aimed to produce a hypoallergenic mutant (mt) Ara h 2.
Methods: An *in silico* designed mtAra h 2 (IgE-binding surface exposed loops were removed) and wild-type (wt) Ara h 2 with a hexahistidyl-tag were expressed in the baculovirus insect cell system. Following purification from supernatants, mtAra h 2 and wtAra h 2 protein expression was verified by Western blotting. After determining physicochemical characteristics, IgE-binding to purified natural (n) Ara h 2, wtAra h 2 and mtAra h 2 was tested by direct ELISA, Western blotting and inhibition ELISA.

Results: Mass spectrometry analysis confirmed the absence of post-translational modifications for the main fraction of wtAra h 2. The folding and the N-terminal amino acid sequence of wtAra h 2 corresponded to the natural protein. For mtAra h 2, mass spectrometry and N-terminal sequencing yielded a mass corresponding to the predicted size and the correct N-terminus. CD spectrometry showed a high content of alpha-helices. Immunoblots of Ara h 2-sensitized patients showed lower IgE-binding to mtAra h 2. In direct ELISA, peanut allergic patients' sera revealed a 20-50% reduced IgE-binding to mtAra h 2 compared to wtAra h 2. Inhibition ELISAs showed significantly reduced IgE-binding of mtAra h 2 compared with nAra h 2.

Conclusion: mtAra h 2 is a promising template for designing the next generation of hypoallergenic mutants.

Supported by the Austrian Science Fund doctoral program W1248-B13 (Doctoral Program Molecular, Cellular and Clinical Allergology, MCCA).

PI_11

Abstract Title	Characterization of gamma gliadins as major allergens in IgE-mediated wheat food allergy
Authors Family name, initials	Wieser, S.[1,2], Baar, A.[1,2], Srinivasan, B.[1], Papadopoulos, N.G.[3], Giavi, S.[3], Mäkelä, M.[4], Pelkonen, A.[4], Ebner, C.[5], Thalhamer, J.[6], Vrtala, S.[1,2], Valenta, R.[1]
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Presenter	Wieser, S.

Wheat is an important element of the human diet but can induce life-threatening IgE-mediated food allergy. Recombinant wheat gamma gliadins were expressed in *Escherichia coli* cells, purified to homogeneity and characterized regarding molecular, structural and immunological properties. A set of synthetic overlapping peptides spanning the gamma gliadin sequence was produced by solid phase peptide chemistry and used for mapping of IgE epitopes recognized by wheat food allergic patients. More than sixty percent of wheat food allergic patients showed IgE reactivity to the recombinant gamma gliadins, which also exhibited strong allergenic activity when tested in basophil activation assays. IgE epitope mapping experiments revealed that gamma gliadins contained major sequential IgE epitopes at their N-terminus. Recombinant gamma gliadins representing major wheat food allergens were expressed and may be used for diagnosis and immunotherapy of wheat food allergy.

Supported by EFIS-Acteria (2015 ACTERIA Doctoral Thesis Prize in Allergology), Biomay AG (Vienna, Austria), Thermofisher (Uppsala, Sweden), by the Christian Doppler Research Association (Austria) and the European Commission´s Seventh Framework program (FP7-funded EU project MeDALL).

PI_12

Abstract Title	Dauc1, the Betv1-homolog in carrot, bears sensitizing activity: evidence at the T cell level
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Presenter	Zulehner, N.

Carrot allergy may result from initial sensitization to Betv1 and subsequent immunological cross-reactivity with its homolog Dauc1. However, the major carrot allergen has also been suggested to induce food allergy independently from Betv1. Since T cells play a relevant role in the sensitization process of IgE-mediated allergy we characterized the cellular response to Dauc1 and its cross-reactivity with Betv1. Therefore, Dauc1-specific T cell lines (TCL) and clones (TCC) were established from PBMC of birch pollen-allergic patients with carrot allergy, mapped for epitope recognition, analyzed for cytokine production and stimulated with Betv1, respectively. CFSE-stained PBMC were stimulated with either Dauc1 or Betv1 and mRNA expression of GATA3 and Tbet was analyzed in sorted $CD3^+CD4^+CFSE^{low}$ cells. Dauc1 was subjected to endolysosomal degradation assay and the resulting fragments were sequenced by mass spectrometry. Among 14 distinct regions, $Dauc1_{139\text{-}153}$ was recognized by 55% of the patients. Only 5/15 (33%) of Dauc1-specific TCL and 8/20 (40%) TCC cross-reacted with Betv1. Most $Betv1^+Dauc1^+$ TCC were TH0/2-like whereas $Betv1^-Dauc1^+$ TCC were mainly Th1-like. A Th1-like response was also detected in Dauc1-reactive $CD3^+CD4^+CFSE^{low}$ cells. Dauc1 was relatively stable in endolysosomal degradation assays. Proteolytic fragments matched the T cell-activating region. In summary, Dauc1 contains a major T cell-activating region, shows limited cellular cross-reactivity with Betv1 and low susceptibility to endolysosomal degradation. All these features are characteristic for sensitizing allergens. We conclude that Dauc1 induces allergen-specific T cell responses independently from Betv1.

Supported by Austrian Science Fund, projects SFB F4610-B19 and W1212

PI_13

POSTER SESSION II_Mechanisms in Allergy

Abstract Title	Mouse lung-specific initiation of allergic asthma ignorome
Authors Family name, initials	Riba, M.[1#], Garcia Manteiga, J. M.[1#], Bošnjak, B.[2#], Cittaro, D.[1], Mikolka, P.[2], Le, C.[2], Epstein, M. M.[2§], Stupka, E.[1§]
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Presenter	Bošnjak, B.

Systems biology provides opportunities to fully understand the genes and pathways in disease pathogenesis. We used literature knowledge and unbiased multiple data meta-analysis paradigms to analyze microarray datasets across different mouse strains and acute allergic asthma models. Our combined gene-centric and pathway-centric strategies generated a stringent signature list totaling 933 genes with 41% (440) asthma-annotated genes and 59% (493) ignorome genes, not previously associated with asthma. Within the list, we identified inflammation, circadian rhythm, lung-specific insult response, stem cell proliferation domains, hubs, peripheral genes, and super-connectors that link the biological domains (*Il6, Il1ß, Cd4, Cd44, Stat1, Traf6, Rela, Cadm1, Nr3c1, Prkcd, Vwf, Erbb2)*. In conclusion, this novel bioinformatics approach will be a powerful strategy for clinical and across species data analysis that allows for the validation of experimental models and might lead to the discovery of novel mechanistic insights in asthma.

PII_1

Abstract Title	Epicutaneous allergen application induces allergen-specific IgG and T cell responses but not boosts of IgE production
Authors Family name, initials	Campana, R.[1], Moritz, K.[2], Neubauer, A.[3], Huber, H.[3], Henning, R.[3], Blatt, K.[4], Hoermann, G.[5], Brodie, T. M.[6], Kaider, A.[7], Valent, P.[4], Sallusto, F.[6], Wöhrl, S.[2], Valenta, R.[1]
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Presenter	Campana R.

Allergen-specific immunotherapy (SIT) is the only allergen-specific and disease-modifying treatment for allergy but can induce side effects and suffers from inconvenient administration protocols. We have conducted a clinical trial using rBet v 1 and two hypoallergenic rBet v 1 fragments for epicutaneous administration (i.e., atopy patch testing) in 30 adult subjects (15 ***birch pollen allergic patients suffering from AD, 5 birch pollen-related RC*** patients, ***5 allergic patients without birch pollen allergy and 5 non-allergic individuals). Blood samples were collected before and 6-8 weeks after application and used to*** compare allergen-specific IgE and IgG antibody levels, T cell and cytokine responses. ***E***picutaneous administration of rBet v 1 and rBet v 1 derivatives leads to a significant boosting of allergen-specific T cell proliferation and IgG production mainly in APT-positive birch pollen allergic patients, but not IgE production. In these patients significant increases in skin-homing CLA+ and CCR4+ T cells were observed after allergen administration. No systemic side effects were observed. Our results demonstrate that epicutaneous application of recombinant allergens boosts allergen-specific T cell and IgG antibody responses and thus may be considered as a possible route for SIT.

Supported by the Austrian Science Fund (FWF) SFB project F4605, Vienna, Austria.

PII_2

Abstract Title	Establishment of a cellular, fluorescent-based, peptide binding assay for the selection of altered peptide ligands (APL) of immunodominant peptides of major pollen allergens
Authors Family name, initials	Candia, M. R.[1], Tauber, P.[1], Neunkirchner, A.[1], Trapin, D.[1], Rosskopf, S.[1], Campana, R.[2], Steinberger, P.[1], Valenta, R.[2] and Pickl, W. F.[1]
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Presenter	Candia. M. R.

$CD4^+$ T lymphocyte activation requires T-cell antigen receptor-dependent recognition of immunogenic peptides bound to and presented by MHC-II molecules. Previous reports have shown that T-cell function can be modulated by altering the sequence of immunogenic peptides. We here used HLA-DR1$^+$ K562 cells to establish a fast, flow cytometry-based competitive binding assay for the characterization of putative APL of the immunodominant Art v $1_{23\text{-}36}$ peptide of the major mugwort pollen allergen Art v 1. Different concentrations of 25 Art v $1_{23\text{-}36}$-derived peptides along with seven MHC loading enhancers (MLE) were pre-incubated with wild-type or HLA-DR1$^+$ K562 cells 2 hours. Subsequently, incubation with biotinylated-$HA_{306\text{-}318}$ (from haemagglutinin influenza A) reference peptide was performed and its specific binding was determined with phycoerythrin-labeled streptavidin by flow cytometry. Pre-incubation with the 1-adamantaneethanol (100μM) led to a significant 5.38±0.04 fold increase in peptide binding ($p<0.05$). In the competitive assays two peptides with increased (IC50 competitor/wt ratio>1.50), eight with similar (ratio 0.50-1.50), and 15 with decreased (ratio<0.5) binding capabilities were identified. Functional evaluation in T cell proliferation and cytokine secretion assays identified two superagonists, one partial agonist and three *bona fide* antagonists. One superagonist revealed increased binding affinity, while it was similar to the wt for the partial agonist and the antagonists. In summary, a robust and fast system to determine peptide binding affinity for HLA class II molecules on entire APC has been established and is currently used to identify APL with clinical relevance.

Funded by the Austrian Science Fund (FWF): DK-W-1248-B13, SFB F4609-B19 and supported by Biomay AG and the Medical University of Vienna.

PII_3

Abstract Title	Isolation and characterization of an IgG-derived ScFv specific for the major birch pollen allergen Bet v 1 from a healthy donor immunized with hypoallergenic Bet v 1 fragments: High affinity binding despite germline configuration - challenging the principle of affinity maturation
Authors Family name, initials	Gadermaier, E.[1], Marth, K.[1], Blatt, K.[2], Lupinek, C.[1], Roder, U.[3], Focke-Tejkl, M.[1], Vrtala, S.[1], Valent, P.[2], Valenta, R.[1], Flicker, S.[1]
Affiliation	[1] Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna General Hospital, Vienna, Austria [2] Division of Hematology and Hemostaseology, Department of Internal Medicine I, Medical University of Vienna, Vienna General Hospital, Vienna, Austria [3] GE Healthcare Europe GmbH, Freiburg, Germany
Presenter	Flicker, S.

Background: Allergen-specific blocking IgG, as induced in the course of specific immunotherapy (SIT) is important for successful SIT outcome by blocking allergen-IgE interactions. The current dogma is that somatic hypermutation is important for high affinity binding of antibodies to antigens.

Methods: We have constructed a combinatorial ScFv library from lymphocytes of a healthy donor who was immunized with hypoallergenic derivatives of the major birch pollen allergen Bet v 1 to analyze Bet v 1-specific IgG antibodies induced by the immunization. Isolated ScFvs were tested for specificity and cross-reactivity to Bet v 1 and homologous pollen and food allergens and epitope mapping was performed. Possible germline ancestor genes were determined with the ImMunoGeneTics (IMGT) database and mutations were revealed. The affinity to cross-reactive allergens was determined by Surface Plasmon Resonance (SPR) measurements. The ability to inhibit patients' IgE binding to ELISA plate-bound allergens and allergen-induced basophil activation was assessed.

Results: Screening of our ScFv library led to the identification of a Bet v 1-specific ScFv (clone H3-1) directed to the C-terminus of Bet v 1, which cross-reacts with homologous allergens. Although IMGT analysis revealed that H3-1 hardly deviates from germline configuration and that diversity was mostly induced by P- and N-nucleotide insertions, H3-1 binds to Bet v 1 and its homologues with high affinity.
Conclusion: Our results demonstrate that an allergen-specific IgG antibody developed in the course of immunization and it exhibits high affinity binding without showing extensive signs of somatic hypermutation.

Supported by FWF grants F4607, P233-B11, F4605 and F4611

PII_4

Abstract Title	Towards a non-allergenic peptide mix containing the T cell epitopes of the clinically most relevant house dust mite allergens for tolerance induction
Authors Family name, initials	Huang, H-J.[1] Banerjee, S.[1], Curin, M.[1] Chen, K-W.[1] Resch, Y.[1] Campana, R.[1] Focke-Tejkl, M.[1] Valenta, R.[1] Vrtala, S.[1,2]
Affiliation	[1.]Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria; [2.]Christian Doppler Laboratory for the Development of Allergen Chips, Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria.
Presenter	Huang, H-J.

House dust mites are one of the most important allergen sources. Der p 1, Der p 2, Der p 5, Der p 7, Der p 21 and Der p 23 are the clinically most important house dust mite (HDM) allergens. The aim of this study was to define a mix of non-allergenic T cell epitope-containing peptides of these allergens for tolerance induction. According to the amino acid sequences of these allergens, we synthesized and purified 33 overlapping peptides covering the complete sequences of Der p 1, 2, 5, 7, 21 and 23. The peptides were tested for IgE, IgG reactivity with sera from HDM allergic patients in ELISA. PBMCs from 27 HDM allergic and 10 non-HDM allergic individuals were incubated with the synthetic peptides and T cell proliferation was measured using a CFSE dilution-based assay. The peptides could be purified in large amounts. They lacked secondary structure but most of them remained soluble in physiological buffers. ELISA assays indicated that most peptides from Derp1, 2, 5, 7, 21 and 23 lacked IgE reactivity and thus were non-allergenic. T cell proliferation assays identified 12 predominant epitopes in the Der p allergens. Our data indicates that a reasonable number of non-allergenic peptides including the sequences and thus T cell epitopes of the clinically most relevant house dust mite allergens can be defined for prevention of HDM allergy by tolerance induction.

Supported by projects F4602, F4605 of the Austrian Science Fund (FWF) and by a research grant from Biomay AG, Vienna, Austria.

PII_5

Abstract Title	Major allergens from fish and peanut interact with plasma membranes of intestinal and bronchial epithelial cells and induce differential gene expression of cytokines
Authors Family name, initials	Kalic, T.[1], Ellinger I. [1], Palladino, C. [1], Gepp, B. [1], Waltl E. [2], Niederberger, V. [2], Breiteneder, H. [1]
Affiliation	[1]Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria [2]Department of Otorhinolaryngology, Medical University of Vienna, Vienna, Austria
Presenter	Kalic, T.

Background: Fish and peanut allergies are among the most dangerous food allergies. Symptoms occur by allergen exposure via gastrointestinal or respiratory tracts. Food matrix components may contribute to the immune response. We explored interactions of intestinal and bronchial epithelial cells with the major Atlantic cod allergen Gad m 1, or a peanut allergen Ara h 1, with or without codfish-derived food matrix or peanut lipids.

Methods: Caco-2 and 16HBE14o- cells were used as *in vitro* models for human intestinal and bronchial epithelial cells, respectively. Confluent cells were treated with fluorescently labelled Gad m 1 with or without codfish matrix, or with Ara h 1 with or without peanut lipids. Labelled allergens were detected by confocal microscopy. Furthermore, mRNA levels of IL-6, IL-8 and TSLP were determined by qRT-PCR.

Results: In Caco-2 cells, both allergens bound to the apical plasma membrane. In 16HBE14o- cells, Gad m 1 localized to the lateral membrane domain (bellow ZO-1 level), while Ara h 1 interacted with the apical and lateral (above ZO-1 level) membrane domains. In both cell lines, treatments with the allergens and codfish matrix/peanut lipids induced differential gene expression of explored cytokines.
Conclusion: Fish and peanut allergens interact with plasma membranes of intestinal and bronchial epithelial cells, but are not internalized. This interaction induces signalling in the cells, which is further modulated by food matrix components and may contribute to allergic sensitization and reaction.

Supported by the Austrian Science Fund grants SFB 4608 and 4613 and the doctoral program W1248-B1.

PII_6

Abstract Title	Possible implications of soluble Fc-epsilon RI presence in different populations
Authors Family name, initials	Moñino Romero S.[1], Bannert C.[1,] Schmidthaler K.[1], Eiwegger T.[1], Dehlink E.[1], Fiocchi A.[2], Amoah A. S.[3], Yazdanbakhsh M.[3], Bohle B.[4], Fiebiger E.[5], Szépfalusi Z.[1]
Affiliation	[1]Department of Pediatrics and Adolescent Medicine, Medical University Vienna, Vienna, Austria [2]Hospital Bambino Gesù in Rome, Rome, Italy [3]Department of Parasitology, Leiden University Medical Center, Leiden, Netherlands [4]Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria [5]Division of Gastroenterology and Nutrition, Children's Hospital Boston, and the Department of Pediatrics, Harvard Medical School, Boston, MA
Presenter	Moñino Romero, S.

IgE-mediated allergies are potentially life threatening immunologic reactions towards otherwise harmless environmental antigens and serum IgE is the common marker for allergy diagnosis. However, allergen-specific IgE (sIgE) levels not always correlate with allergic reactions. The recently discovered soluble form of the high affinity receptor for IgE (sFcεRI) present in serum may interfere with IgE levels.

Serum sFcεRI levels of patients from different regions (Austria, Ghana and Italy) were measured by a newly developed ELISA model. Total and pre-complexed sFcεRI were compared between groups. Individuals were well defined and classified according to diagnostic tests as well as clinical symptoms in groups such as: healthy controls, non-sensitized and sensitized with or without clinical manifestations. The blocking capacity of sFcεRI was analysed by FAB-like (Facilitated Antigen Binding) tests using a MelJuSo cell line stably transfected with functional FcεRI. Change on FcεRI surface expression and bound-IgE were measured by flow cytometry.

We found high positive correlations (r^2 0.861-0.972) between total and complexed-sFcεRI among all groups irrespective of their disease phenotype. However, the Ghana group showed a lower positive correlation (r^2 0.661). Moreover, they also showed different expression pattern of the complex form between groups.

The presence of sFcεRI among different populations and its capacity of forming complexes with free IgE suggest a potential modulatory function in IgE-mediated responses. Furthermore, this potential inhibitory capacity was confirmed with MelJuSo cells. Thus sFcεRI could be an important player in the complex signalling pathway of the allergic response.

Supported by the Austrian Science Funds. Doctoral Program W 1248-B13.

PII_7

Abstract Title	Modulatory capacities of soluble Fc-epsilon RI in the IgE-mediated immune response
Authors Family name, initials	Moñino Romero S.[1], Bannert C.[1,] Schmidthaler K.[1], Eiwegger T.[1], Dehlink E.[1], Fiocchi A.[2], Amoah A. S.[3], Yazdanbakhsh M.[3], Bohle B.[4], Fiebiger E.[5], Szépfalusi Z.[1]
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Presenter	Moñino Romero, S.

IgE-mediated allergies are potentially life threatening immunologic reactions towards otherwise harmless environmental antigens and serum IgE is the common marker for allergy diagnosis. However, allergen-specific IgE levels not always correlate with allergic reactions. The recently discovered soluble form of the high affinity receptor for IgE (sFcεRI) present in serum may interfere with IgE levels.

The modulatory capacities of sFcεRI were analysed using a MelJuSo cell line stably transfected with functional FcεRI. Cells were stimulated with chimeric IgE (cIgE) plus its specific ovalbumin (NP-OVAL). To measure the blocking capacity, FAB-like (Facilitated Antigen Binding) tests were performed with sFcεRI collected from the supernatants. Change on FcεRI surface expression and bound-IgE were measured by flow cytometry. Serum sFcεRI levels from defined food allergic patients from different regions were measured by ELISA.

FcεRI surface expression and sFcεRI expression in the supernatant increased in a dose-dependent manner upon cIgE stimulation (5-30μg/mL) and cIgE together with NP-OVAL stimulation (1-100μg/mL) respectively. The blocking capacity of sFcεRI reached 57-63% inhibition in the cIgE-FcεRI binding. There is a high positive correlation between total and complexed-sFcεRI measured in serum, confirming that sFcεRI maintains its binding affinity.

MelJuSo cells are useful to characterise the modulatory capacity (surface expression, release, cleavage) of the sFcεRI. Furthermore, recent data suggest that sFcεRI present in the serum may have a blocking capacity on IgE binding to FcεRI. Thus sFcεRI could be an important player in the complex signalling pathway of the allergic response.

Supported by the Austrian Science Funds. Doctoral Program W 1248-B13.

PII_8

Abstract Title	The impact of peanut lipids on Ara h 1-induced immune responses in MoDCs
Authors Family name, initials	Palladino, C.[1],Gepp, B.[1], Sirvent, S.[2], Angelina, A.[2], Bublin, M.[1], Radauer, C.[1], Lengger, N.[1], Eiwegger, T.[3], Palomares, O.[2], and Breiteneder, H.[1]
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Presenter	Palladino, C.

It is still not clear whether Ara h 1, major peanut allergen, is able to sensitize by itself or whether there are other molecules involved. Evidence of the role of small molecules in the allergic sensitization, such as lipids, directly bound as ligands by the allergen or present in the allergen source, is emerging. Peanuts contain a significant amount of lipids. Therefore, we aimed to assess whether peanut lipids can bind to Ara h 1, and whether they can modulate the allergen-induced response in monocyte-derived dendritic cells (MoDCs). Lipid binding to Ara h 1 was evaluated by 1-anilinonaphthalene-8-sulfonic acid (ANS) displacement assay. ANS was used at 5 μM, and peanut lipids were added to Ara h 1 at 5, 50, and 100 μM. We observed that Ara h 1 incubated with peanut lipids at the highest concentration, showed a reduction of ANS fluorescence by 30% compared with Ara h 1 incubated with ANS alone. Furthermore, in MoDCs of non-allergic individuals Ara h 1 increased the TNF-α production and, interestingly, peanut lipids reduced TNF-α levels in response to Ara h 1 stimulation by 65%. These data indicate that peanut lipids do play a role in the modulation of the immune response to Ara h 1.

Supported by the Austrian Science Fund Doctoral Program MCCA W 1248-B13.

PII_9

Abstract Title	Neutrophils are potential APC in IgE- mediated Allergy
Authors Family name, initials	Dominika Polak, Birgit Nagl, Claudia Kitzmüller, Barbara Bohle
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Presenter	Dominika Polak

Background: Neutrophils are present in large numbers in allergic late-phase reactions. However, it is not yet clear whether they contribute to allergic inflammation. These professional phagocytes might present allergen to allergen-specific T cells since they express MHC class II molecules upon stimulation with certain cytokines, chemokines and bacterial factors, such as GM-CSF, TNF-α, IL-8, IFN-γ and LPS, respectively. In fact, murine neutrophils have been shown to process and present antigens to $CD4^+$ T-cells.

Aim: To assess whether human neutrophils act as antigen-presenting cells for allergen-specific T-cells.

Methods: Neutrophils isolated from the peripheral blood of allergic donors were cultured under different conditions and analyzed for the expression of MHC class II, CD40, CD80, and CD86, by flow cytometry. Surface binding, internalization and intracellular degradation of fluorescence-labelled Bet v 1 by neutrophils were compared with monocytes. Microsomal proteases were isolated from both cell types and incubated with Bet v 1. The resulting proteolytic fragments were sequenced using mass spectrometry. Finally, neutrophils and monocytes were cocultured with Bet v 1-specific T-cell cultures generated from birch-pollen allergic donors in the presence or absence of Bet v 1 and proliferative responses of T-cells were assessed.

Results: A cocktail of IL-3, GM-CSF and IFN-γ enhanced the expression of HLA class II and CD80 on neutrophils. Neutrophils effectively internalized Bet v 1 and their uptake and endolysosomal degradation of the allergen was faster than by monocytes. In addition, neutrophils processed longer peptides of Bet v 1 than monocytes. Neutrophils pulsed with Bet v 1 induced proliferation in Bet v 1- specific T- cells specific for different epitopes distributed over its entire amino acid sequence. However, monocytes were the more potent antigen-presenting cells.
Conclusions Our data provide evidence that neutrophils may serve as antigen-presenting cells for allergen specific T-cells and thereby, play a role in the late phase reaction of IgE-mediated allergy.

Supported by the Austrian Science Funds, project W1248 and SFB F4610.

PII_10

Abstract Title	Assessing basophil activation pathways via flow cytometry in the context of food allergy
Authors Family name, initials	Ponce, M.[1], Diesner, S.C.[1], Moñino-Romero, S.[1], Schmidthaler, K.[1], Szépfalusi, Z.[1], and Eiwegger, T.[1,2]
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Presenter	Ponce, M.

Introduction: Food allergy occurs at a prevalence of up to 5% in children within the first years of life. Some food allergies are associated with a high likelihood of tolerance development up to the age of five whereas others are not. The reasons are not fully understood. Currently, markers used to confirm food allergy such as specific IgE levels are not appropriate to monitor tolerance development or define sensitized non-allergic individuals. Basophil Activation Test using CD63 as a readout parameter has been described to be superior in assessing tolerance in peanut sensitized, tolerant individuals as compared to other tests.

Aim: To assess optimal kinetics of basophil activation pathways *in vitro* via flow cytometry in addition to CD63 measurement.

Methods: Children´s basophils were evaluated for CD63 and CD203c surface expression as well as for phosphorylation of ERK1/2 and p38 MAPK, ALK and PLCγ1 upon FcεRI crosslinking using flow cytometry.

Results: Kinetics of phosphorylation pathway demonstrate 1 minute (ERK1/2) and 3 minutes (p38) as optimal time points to measure IgE-related basophil activation.
Conclusions: To understand the effect of the phosphorylation of these key intracellular proteins that contribute to basophil activation and therefore elicit allergic symptoms is of great interest and may allow a precise delineation of events taking place during desensitization and tolerance development.

PII_11

Abstract Title	Phenotyping of allergen-reactive $CD8^+$ T cells in type I allergy
Authors Family name, initials	Nazanin Samadi[1], Claudia Kitzmüller[1], Rene Geyeregger[2], Barbara Bohle[1], Beatrice Jahn-Schmid[1]
Affiliation	[1]Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna [2]Department of Clinical Cell Biology and FACS Core Unite, CCRI, Vienna, Austria
Presenter	Samadi, N.

T cells play a main role in the induction and maintenance of IgE-mediated allergy. The function of CD4+ T cells in the pathophysiology of allergic disorders has been extensively investigated while the role of CD8+ T cells is still poorly understood and controversial. The aim of this project is to characterize allergen-specific CD8+ T cells in patients with different allergic manifestations (rhinoconjunctivitis, atopic dermatitis and atopic bronchial asthma). Different seasonal (birch pollen and grass pollen) and perennial allergens (cat dander and house dust mite) were included. PBMCs from allergic patients were stained with the proliferation dye efluor 670 and incubated with allergen. Proliferating CD3+CD8+ cells were then assessed for the expression of differentiation markers (CD27, CD28, CD45RO, CXCR3, CRTh2, PD-1), homing markers (CCR4, CD62L, CD29b), intracellular cytokines (IL-4, IL-5, IL-13, IL-17, IL-22 and IFN-γ, TNF-α), and cytotoxic proteins (granzyme B and perforin) by flow cytometry and compared to non-proliferating CD8+ cells. We found allergen-reactive CD8+ T cells in all allergic manifestations. Moreover, largest numbers were detected upon stimulation with house dust mite and grass pollen extracts. Proliferating cells contained higher numbers of cells producing IL-4, granzyme B and perforin than non-proliferating CD8+ T cells. In addition, a significantly higher expression of CD27, CD45RO, CD62L, and CD29b was detected in allergen-reactive CD8+ T cells indicating central memory T cells of mucosal origin. Thus, we could demonstrate allergen-reactive IL-4+ CD8+ T cells in different allergic manifestations which produce cytotoxic proteins. Their functional activity will be investigated in future experiments.

PII_12

Abstract Title	The role of PHL P 5 specific IgG antibodies for allergen presentation
Authors Family name, initials	Gabriela Sánchez Acosta[1], Sandra Faustmann[1], Margarete Foceke-Tejkl[1] and Barbara Bohle[1]
Affiliation	[1] 1Department of Pathophysiology and Allergy Research, Division of Immunopathology, Center of Pathophysiology, Infectiology and Immunology. Medical University of Vienna.
Presenter	Gabriela Sánchez Acosta[1]

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

Supported by Austrian Science Fund, projects SFB F4610 and W1212

PII_13

Abstract Title	Humanized mice as *in vivo* model for therapy of IgE-mediated allergy
Authors Family name, initials	Vizzardelli, C.[1], Nagl, B.[1], Neunkirchner, A.[2], Zimmann, F.[1], Kitzmüller, C.[1], Jahn-Schmid, B.[1], Bohle, B.[1]
Affiliation	[1]Department of Pathophysiology and Allergy Research and [2]Institute of Immunology, Medical University of Vienna, Vienna, Austria.
Presenter	Vizzardelli, C.

Allergen-specific immunotherapy (AIT) is the only treatment curing IgE-mediated allergy with long-term benefit. Unfortunately, AIT fails in a substantial number of treated patients. Therefore, one major focus of allergy research is the development of allergy vaccines with improved efficacy. Current mouse models are successful in a prophylactic but not in a therapeutic approach. To better imitate the therapy of humans in an *in vivo* model we humanized NOD-SCID IL-2Rγc$^{-/-}$ (NSG) mice with PBMC from allergic patients according to Martin *et al.* (JACI. 2012; 129:521). To this aim, PBMC from birch pollen-allergic donors and birch pollen extract (BPE) were concomitantly injected intraperitoneally (i.p.) in NSG-mice, followed by an i.p. boost with BPE after 7 days. NSG-mice received either PBS or BPE intranasally (i.n.) from day 20-22 and at day 24 the lung function was evaluated by invasive measurement of airway resistance performed on anesthetized, intubated mice by mechanical ventilation in response to increasing doses of methacholine. In parallel, the presence of human cells in lungs and spleens was analysed by flow cytometry. We could detect human CD3^{+}CD45^{+} T cells in lung, spleen and BAL fluid as well as a higher percentage of murine eosinophils in the lung of NSG-mice i.n. challenged with BPE. As a next step we will set-up a therapeutic approach to establish a suitable *in vivo* model for testing novel allergy vaccines developed in our laboratory.

Supported by Austrian Science Fund project SFBF4610.

PII_14

POSTER SESSION III_Treatments in Allergy

Abstract Title	Are birch pollen AIT induced blocking antibodies protective for cross-reactive allergens?
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Affiliation	[1]Department of Molecular Biology, University of Salzburg, Austria [2]Department of Dermatology, Paracelsus Private Medical University Salzburg, Austria
Presenter	Asam, C.

Pollen from Fagales trees, especially birch, is the main cause of early seasonal allergy in the temperate climate zone. Birch pollen allergic patients also frequently develop allergies towards various fruits, vegetables and nuts. Allergen immunotherapy (AIT) is currently the only strategy to effectively cure allergies. The success of the treatment is influenced by the development of specific blocking antibodies.

The aim of this study was to investigate whether blocking antibodies induced by successful birch pollen AIT using conventional allergen extracts would inhibit IgE binding to Bet v 1-related pollen and food allergens.

Therefore, a panel of eight Fagales pollen allergens from alder, birch, hornbeam, chestnut, hazelnut, beech, hop-hornbeam, oak as well as two related food allergens from apple and hazelnut were recombinantly produced in E. coli, purified to homogeneity and physico-chemically analyzed. Serum samples from 5 different birch pollen allergic patients were collected before, after reaching maintenance dose and one-year after AIT initiation with birch pollen extracts. Treatment efficacy was analyzed by nasal provocation tests. Facilitated antigen binding assays were performed to determine the blocking activity of AIT induced IgG.

All five patients included in the study showed an improvement of nasal provocation scores during AIT. Moreover, all patients developed Bet v 1 specific blocking antibodies. However, FAB assays revealed that not all donors developed blocking antibodies against the whole panel of Bet v 1 related allergens. Therefore, we believe that successful birch pollen AIT cannot always ameliorate allergic symptoms towards related allergens emphasizing the need for improved treatment strategies.

Supported by the University of Salzburg and the Austrian Science Funds projects P27589 and P26125

PIII_1

Abstract Title	Dissection of specific IgG responses with recombinant house dust mite allergens reveals that poor effect of specific immunotherapy is due to failure of blocking antibody induction towards certain allergens
Authors Family name, initials	Chen, KW[1], Zieglmayer, R.[2], Zieglmayer, P.[2], Lemell, P.[2], Horak, F.[2], Valenta, R.[1], Vrtala, S.[1]
Affiliation	[1]Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Austria [2]Vienna Challenge Chamber, Allergy Center Vienna West, Austria
Presenter	Chen, KW.

Introduction: Allergen-specific immunotherapy (SIT) is the only disease-modifying treatment for allergy but clinical efficacy is often low in the case of house dust mite (HDM) allergy.

We monitored allergen-specific antibody responses and clinical responses in HDM allergic patients undergoing SIT with a HDM extract-based vaccine using a panel of micro-arrayed HDM allergens and objective allergen provocation in the Vienna challenge chamber (VCC), respectively.

Material and Methods: Hundred HDM allergic patients were treated with the HDM extract-based vaccine or placebo for 47 weeks. Blood samples were taken before (week 0), during (week 23) and after SIT (week 47) and analyzed for allergen-specific IgE and IgG responses with micro-arrayed allergens. The total nasal symptom scores (TNSS) of the patients were determined at the same time points of SIT in the allergen provocation chamber.

Results: Allergen micro-array analysis showed that most of the patients exhibited IgE towards several other HDM allergens in addition to the Der p 1 and Der p 2 allergens but the vaccine induced only Der p 1 and Der p 2-specific IgG responses. Only those patients who were sensitized to Der p 1 and/or Der p 2 showed a reduction of TNSS in response to SIT.

Conclusions: Our study indicates that clinical failure of HDM SIT can be due to lack of induction of protective IgG antibodies towards certain important HDM allergens most likely due to poor immunogenicity and/or poor representation in the administered vaccine.

This study was supported by grants F1803, F1815, F4602, F4605 of the Austrian Science Fund and by the Christian Doppler Research Association

PIII_2

Abstract Title	Hepatitis B-specific immune responses in grass pollen allergic patients immunized with the preS-based grass pollen allergy vaccine BM32
Authors Family name, initials	Cornelius, C.[1], Schöneweis, K.[2], Weber, M.[1], Niespodziana, K.[1], Trauner, M.[3], Hofer, H.[3], Urban, S.[2], Valenta, R.[1]
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Presenter	Cornelius, C.

Introduction:
The grass pollen allergy vaccine BM32 contains four recombinant fusion proteins consisting of hypoallergenic peptides of the major timothy grass pollen allergens, fused to preS (preS1 + preS2), a domain of the Hepatitis B Virus (HBV) large surface protein, bearing the potential attachment site of HBV to hepatic cells. In the present study HBV-specific antibody and T cell responses were analyzed in grass pollen allergic patients who were vaccinated with BM32 and in patients suffering from HBV infection.

Materials and Methods:
Recombinant preS (Genotype A2) was expressed in *Escherichia coli* and purified by affinity chromatography. We assessed the cellular and humoral immune response towards preS and synthetic overlapping peptides, encompassing the entire preS-protein chain by analyzing serum and peripheral blood mononuclear cells of BM32-vaccinated individuals (n=30) and in patients with HBV infection (n=19). The HBV inhibitory capacity of BM32 was examined by an *in vitro* virus neutralization assay, using HepG2-hNTCP cells.

Results:
Epitope mapping experiments demonstrated that BM32 vaccinees but not infected patients developed strong serum IgG responses against motifs within the preS1 domain, containing the attachment sites of HBV to hepatocytes. Significant increases in preS-specific T cell proliferation over the period of immunization were observed. Furthermore, sera of BM32-vaccinated individuals, who have never received a conventional HB vaccination (n=7) showed inhibitory capacity regarding *in vitro* Hepatitis B infection by an average of 80%.

Conclusion:
This study demonstrated that the preS-based allergy vaccine BM32 may have the potential to protect from infection with HBV.

PIII_3

Abstract Title	Immunotherapy of allergic patients with the B cell epitope-based recombinant grass pollen allergy vaccine BM32 induces allergen-specific IgG antibodies which inhibit immediate allergic inflammation and allergen-specific T cell responses
Authors Family name, initials	Focke-Tejkl, M[1], Weber, M.[1], Zieglmayer, P.[2], Zieglmayer R.[2], Lemell, P. [2], Horak, F.[2], Neubauer, A.[3], Stolz, F.[3], Huber, H.[3] Henning, R.[3], Valenta, R.[1]
Affiliation	1 Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology Medical University of Vienna 2 Vienna Challenge Chamber 3 Biomay AG, Vienna
Presenter	Focke-Tejkl, M

Allergen-specific immunotherapy reduces immediate and late phase allergic inflammation with disease-modifying and long-lasting effects. BM32 is a novel grass pollen allergy vaccine based on four fusion proteins consisting of non-allergenic peptides from the IgE binding sites of the four major grass pollen allergens and hepatitis B-derived PreS as carrier. The immunological effects of immunotherapy of grass pollen allergic patients with BM32 were investigated.
Grass pollen allergic patients (n=70) received three subcutaneous injections of three doses of BM32/placebo in 4 week intervals. Before and after the treatment blood was taken and changes of allergen-specific antibody and T cell responses were measured using micro-arrayed allergens and T cell proliferation assays.

Vaccination led to a dose-dependent increase of allergen-specific IgG1>IgG4>IgG2 responses without IgE boosts. The measurement of allergen-specific IgE with the micro-arrayed allergens, an assay performed with low concentration of immobilized allergens, revealed a competition of IgG with IgE binding which was associated with reductions of allergen-specific skin inflammation and immediate nasal symptoms. Addition of post-treatment sera containing therapy-induced allergen-specific IgG inhibited IgE-facilitated allergen presentation to T cells and thus allergen-specific T cell responses in actively treated patients

The recombinant grass pollen vaccine BM32 induced allergen-specific IgG responses which inhibit IgE binding to the allergens and thus IgE-mediated immediate type allergic inflammation as well as T cell activation. The blocking activity of therapy-induced IgG on IgE binding to the allergens can be revealed with micro-arrayed allergens.

Supported by the Austrian Science Fund (FWF: F4605), by Biomay AG, Vienna, Austria and Thermofisher, Uppsala, Sweden.

PIII_4

Abstract Title	Oral tolerance induction to the major fish allergen parvalbumin in a mouse model of fish allergy
Authors Family name, initials	Freidl, R.[1], Baranyi, U.[2], Swoboda, I.[1], Focke-Tejkl, M.[1], Valenta, R.[1], Linhart, B.[1]
Affiliation	[1]Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria, [2]Department of Surgery, Medical University of Vienna, Vienna, Austria.
Presenter	Freidl, R.

IgE-mediated food allergy to fish is a severe, persistent hypersensitivity disease caused predominantly by one single major allergen, the fish parvalbumin. Oral tolerance induction to parvalbumin represents an attractive option as a prophylactic strategy. Thus BALB/c mice (n=5) received a single high dose of natural (n) Cyp c 1-enriched extract by intragastric feeding before subcutaneous sensitization to aluminium hydroxide-adsorbed n Cyp c 1. Control groups were either sensitized to n Cyp c 1 or received gavage only. Cyp c 1-specific antibody and cellular responses were studied by ELISA, rat basophil leukemia (RBL) assay and splenocyte proliferation assay. Mice receiving the tolerizing gavage showed strongly decreased Cyp c 1-specific IgG and IgE levels and reduced allergic mediator release in RBL assay. Intragastric feeding only did not induce any Cyp c 1-specific responses. Hence, prophylactic tolerance induction protected mice from the development of fish allergy. Further hypoallergenic derivatives of Cyp c 1 will be applied to induce oral tolerance in this mouse model of fish allergy.

PIII_5

Abstract Title	Novel drug design for birch pollen and associated food allergies
Authors Family name, initials	Hofer, H.[1], Asam, C.[1], Hauser, M.[1], Briza, P.[1], Himly, M.[1], Ebner, C.[2], Ferreira, F.[1], Wallner, M.[1]
Affiliation	[1] University of Salzburg, Department of Molecular Biology, Salzburg, Austria [2] Allergieambulatorium Reumannplatz, Vienna, Austria
Presenter	Hofer, H.

Introduction: Birch pollen allergy is the main cause of pollinosis in early spring time in the temperate climate zone of Europe. Patients are predominantly sensitized to the major birch pollen allergen Bet v 1. Many of them also suffer from adverse reactions after ingestion of various foods, primarily after consumption of apple and hazelnut. The respective food allergens structurally related to Bet v 1 are able to cross-link IgE originally produced against the birch pollen allergen. In this study we designed a low-IgE binding, immunogenic hybrid molecule for the combined treatment of birch pollen and associated food allergies towards apple and hazelnut.

Methods: Parental allergens Bet v 1 from birch, Cor a 1.04 from hazelnut, and Mal d 1 from apple, as well as the hybrid molecule MBC4 were purified and characterized physico-chemically. IgE-binding properties of MBC4 were determined in IgE ELISA and mediator release assays. Furthermore, its immunological behavior was monitored in a mouse immunization model.

Results: IgE data from patients' sera depict a significantly reduced IgE-binding capacity of MBC4 when compared to its parental allergens. Moreover, MBC4 was able to induce cross-reactive IgG antibodies in mice. In ELISpot assays, MBC4 was able to re-stimulate T cells from mice immunized with each parental allergen, which indicates a cross-reactive T cell response.

Conclusion: Due to reduced IgE binding but conserved immunogenicity, we conclude that MBC4 represents a suitable vaccine candidate for the parallel treatment of birch pollen and associated food allergies.

Supported by FWF L688 and ÖNB 12533 grants.

PIII_6

Abstract Title	A novel glutarimide derivative XC8 suppresses acute experimental allergic asthma in BALB/c mice
Authors Family name, initials	S. Kazemi[1], D. Reiner[1], R. Lee[1], G. Dekan[2], T.Kromova[3], A. Rydlovskaya[3], O. Proskurina[3], V. E. Nebolsin[3], M. M. Epstein[1]
Affiliation	
Presenter	Kazemi,S.

Introduction: Asthma is a chronic inflammatory disease characterized by lung inflammation, mucus hypersecretion and airway obstruction. Although corticosteroids are effective in most asthmatics, there are patients resistant and adverse effects may preclude long-term use. To test the efficacy of a novel biogenic compound, glutarimide histamine (XC8; 1-(2-(1H-imidazol-4-yl)ethyl)piperidine-2,6-dione) in acute onset ovalbumin (OVA) induced-allergic asthma in BALB/c mice.

Methods: We treated mice orally with titrated doses of XC8 and corticosteroid dexamethasone (Dex) daily for 10 consecutive days following the sensitization of mice with ovalbumin (OVA) and before and during aerosol challenge to the airways and measured lung inflammation, mucus secretion, OVA-specific serum antibodies, and methacholine-induced airway hyperresponsiveness (AHR).

Results: When we treated mice with XC8 orally with 0.3 mg/kg daily for 10 consecutive days, eosinophilic lung inflammation was inhibited at disease onset compared to diluent-treated controls. XC8 also suppressed mucus hypersecretion, sera antigen-specific IgE or IgG1 titres, and AHR. Remarkably, XC8 was significantly more effective than a clinically relevant dose of Dex for all disease parameters.

Conclusion: Our results demonstrate that XC8 efficiently suppresses experimental allergic asthma at low doses. These data highlight properties of XC8, which may have potential for clinical testing in this indication.

This research was supported by HSCD GmbH, Tulln, Austria.

PIII_7

Abstract Title	Fusion proteins of the TLR5 ligand flagellin and the major birch pollen allergen Bet v 1 show intrinsic adjuvanticity
Authors Family name, initials	Kitzmüller C., Kalser J., Mutschlechner S., and Bohle B.
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Presenter	Kitzmüller C.

Toll-like receptor (TLR) ligands are considered ideal candidates to enhance the effectiveness of allergen immunotherapy. The TLR5 ligand flagellin has already been shown to be a potent adjuvant in a number of vaccines. It is efficient at picomolar concentrations and of low toxicity. Antigens can be fused directly to its sequence. To reduce the size of the protein, we developed a truncated version of flagellin. We substituted the hypervariable region, which is not necessary for immunostimulation, with a short turn motif. The remaining N- and C-terminal conserved regions form a domain essential for TLR5 activation. The truncated protein, termed NtCFlg, activated TLR5 even better than full length flagellin. NtCFlg was fused to the major birch pollen-allergen, Bet v 1, either at its N- or C-terminus. The resulting fusion proteins, Bet v 1-NtCFlg and NtCFlg-Bet v 1 activated hTLR5 and induced maturation in human dendritic cells. The constructs were hypoallergenic in comparison to Bet v 1, but activated Bet v 1-specific T cells to a higher degree than Bet v 1. Importantly, the fusion constructs induced antibodies in mice in the absence of adjuvant. Comparing the N- and C-terminally fused constructs, we found that Bet v 1-NtCFlg showed a much better reactivity in all the assays used. Collectively, our data demonstrate that a fusion construct of an allergen and a TLR ligand can have immunogenicity and intrinsic adjuvanticity, however, usefulness as a therapeutic can be different between constructs consisting of the same components arranged in different orders.

Supported by Biomay AG, the Christian Doppler Research Association, Austria and the Austrian Science Fund, project SFB F4610

PIII_8

Abstract Title	Rhinovirus infections rather than allergen exposure trigger wheezing attacks in preschool children
Authors Family name, initials	Niespodziana, K.[1], Stenberg-Hammar, K.[2,3], Cabauatan, C. R.[1], Lupinek, C.[1], Melén, E.[4,5], Söderhäll, C.[6], van Hage, M.[7], Hedlin, G.[2,3], and Valenta, R.[1]
Affiliation	[1] Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria [2] Astrid Lindgren Children´s Hospital, Karolinska University Hospital, Stockholm, Sweden [3] Department of Women´s and Children´s Health, Karolinska Institutet, Stockholm, Sweden [4] Institute of Environmental Medicine, Karolinska Institutet, and [5] Sachs´Children´s Hospital, Södersjukhuset, Stockholm, Sweden [6] Department of Biosciences and Nutrition, and Center for Innovative Medicine (CIMED), Karolinska Institutet, Stockholm, Sweden [7] Clinical Immunology and Allergy Unit, Department of Medicine, Solna, Karolinska Institutet and University Hospital, Stockholm, Sweden
Presenter	Niespodziana, K.

Allergic sensitization and rhinovirus (RV) infections are the most common triggers of acute wheezing/asthma exacerbations during early childhood. However, it is often unclear whether these exacerbations had been caused by a rhinovirus infection and/or by allergen exposure. We have previously found that rhinovirus infections induce increases of IgG and IgA responses against an N-terminal portion of the rhinovirus VP1 protein and that respiratory allergen exposure induces increases in allergen-specific IgE levels. These increases of specific antibody levels can be detected several weeks after infection and allergen exposure, respectively. In this study we analyzed IgE responses to multiple allergen components determined by the MeDALL-allergen chip and IgG antibody responses to recombinant RV-derived proteins measured by ELISA in sera from 120 preschool children obtained during an acute episode of wheeze and at follow-up several weeks after. We found that the majority of children mounted increases of IgG responses towards the rhinovirus proteins whereas in none of the children with an IgE-sensitization, increases of IgE responses to inhalant allergens were detected. Our findings thus indicate that rhinovirus infections but not respiratory allergen exposure were responsible for the elicitation of wheezing attacks among the preschool children investigated.

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PIII_9

Abstract Title	Characterization of NET responses to adjuvants used in allergy vaccines
Authors Family name, initials	Manuel Reithofer[1], Dominika Polak[1], Claudia Kitzmüller[1], Barbara Bohle[1], Beatrice Jahn-Schmid[1]
Affiliation	[1] Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria
Presenter	Manuel Reithofer

Most subcutaneous allergy vaccines in Europe contain alum as adjuvant, and a few monophosphoryl lipid A (MPL), a TLR-4 agonist. In mice, the adjuvant activity of alum seems to be mediated by neutrophil-derived DNA. Human neutrophils are the most abundant leukocyte population and are an important part of innate immunity. Their repertoire includes the ability to trap, phagocytose and kill pathogens extracellularly by releasing DNA and granular material sticking to this DNA, so-called neutrophil extracellular traps (NETs). We intend to characterize the NET response of human neutrophils to alum and MPL and their possible role in the immune response induced by allergen-specific immunotherapy.

Freshly isolated human neutrophils are stimulated with known NET-inducing factors (PMA or LPS), alum or MPL. The formation of NETs is evaluated by staining of DNA and granular proteins and fluorescence microscopy. In supernatants the amount of extracellular DNA and elastase activity are determined in time course experiments.

The response to MPL showed expected similarity to LPS-triggered NET-formation with DNA-fibers, granular myeloperoxidase, elastase or LL-37 sticking to them. In contrast, alum induced cloud-like NETs associated with granular proteins. None of the adjuvants caused general cell death after 3 hours, as observed after PMA stimulation. Alum induced increased amounts of extracellular DNA, and increased extracellular elastase activity was observed with both adjuvants.

Alum and MPL induce the release of DNA and associated granular proteins by neutrophils typical for NETS. We will further investigate the stimulatory capacities of these NETs, in co-cultivation experiments with different APCs.

Supported by FWF W1248 MCCA PhD Program

PIII_10

POSTER SESSION IV_Immune Cells

Abstract Title	Molecular Analysis of MAZR Function in $CD4^+$ T cells
Authors Family name, initials	Andersen L., Gülich A., Schebesta A., Sakaguchi S. and Ellmeier W.
Affiliation	Division of Immunobiology, Institute of Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria
Presenter	Andersen L.

Transcriptional and epigenetic mechanisms play a key role in the regulation of T cell development and function. We identified the BTB zinc finger protein MAZR (also known as Patz1) as an important regulator of Cd8 gene expression in DN thymocytes and revealed its essential role in CD4/CD8 lineage choice of DP thymocytes. However, the role of MAZR in peripheral T cell development has not been elucidated so far. To comprehensively analyze the in vivo and in vitro role of MAZR in CD4+ T cells, we are employing conditional gene targeting approaches (using the Cd4-Cre deleter strain) as well as gain-of-function studies using retroviral-mediated overexpression strategies. Preliminary results suggest a role for MAZR in modulating regulatory T cell development and differentiation. Moreover, we show that MAZR inhibits the suppressive capacity of Tregs in an in vitro system. Data from our ongoing experiments will be presented.

This work is supported by the Austrian Science Fund (FWF P23641FW)

PIV_1

Abstract Title	Abatacept (CTLA-4Ig) treatment reduces T cell apoptosis and regulatory T cell suppression in patients with rheumatoid arthritis (RA).
Authors Family name, initials	Bonelli M[1], Göschl L[1], Blüml S[1], Karonitsch T[1], Steiner G[1], Smolen JS[1] and Scheinecker C[1].
Affiliation	[1]Division of Rheumatology, Internal Medicine III, Medical University of Vienna, Vienna, Austria.
Presenter	Scheinecker C.

OBJECTIVE:
Abatacept (CTLA-4Ig) blocks CD28-mediated T cells activation by binding to the costimulatory B7 ligands CD80/CD86 on antigen presenting cells (APC). Costimulatory molecules, however, can also be expressed on T cells upon activation. Therefore the aim of our study was to investigate direct effects of CTLA-4Ig on distinct T cell subsets in RA patients.

METHODS:
Phenotypic and functional analyses of $CD4^+$ T cells, including $CD4^+FoxP3^+CD25^+$ regulatory T cells (Treg), from RA patients were performed before and during CTLA-4Ig therapy. In addition T cells from HC were analysed upon in vitro culture with CTLA-4Ig or anti-CD80 and anti-CD86 antibodies. Apoptotic DNA fragmentation in $CD4^+$ and $CD4^+FoxP3^+$ T cells was measured by TUNEL staining.

RESULTS:
We observed an increase in T cells, including Treg cells, after initiation of CTLA-4Ig therapy, which was linked to a downregulation of activation associated marker molecules and CD95 on $CD4^+$ T cells and Treg cells. CTLA-4Ig decreased CD95-mediated cell death in vitro in a dose dependent manner. Functional analysis of isolated Treg cells from RA patients further revealed a diminished suppression of responder T cell proliferation. This was found to be due to CTLA-4Ig mediated blocking of CD80 and CD86 on responder T cells that led to a diminished susceptibility for Treg cell suppression.

CONCLUSION:
CTLA-4Ig therapy in RA patients exerts effects beyond the suppression of T cell activation, which has to be taken into account as an additional mechanism of CTLA-4Ig treatment.

PIV_2

Abstract Title	Modulation of Th17 responses by the protein tyrosinae kinase TEC
Authors Family name, initials	Boucheron, N.[1], Sharif, O.[2], Van Greuningen, L.[3], Knapp, S.[2] and Ellmeier, W.[1]
Affiliation	[1]Division of Immunobiology, Institute of Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, 1090 Vienna, Austria. [2]CeMM, Center for Molecular Medicine of the Austrian Academy of Sciences & Dept. of Medicine 1, Div. of Infectious Diseases & Tropical Medicine, Medical University of Vienna, 1090 Vienna, Austria. [3]University of Applied Sciences, Leiden, Netherlands.
Presenter	Boucheron, N.

Members of the Tec kinase family play an important role during lymphocyte development and activation and mutations in Tec family kinases are linked with immunodeficiencies in humans and mice. Three members of the Tec kinase family are expressed in T cells: Tec, Itk and Rlk. Itk–/– mice have defects in T cell development, affecting in particular conventional T cells. Moreover, TCR-mediated activation and proliferation of naïve T cells as well as their polarization into Th2 effector T cells is impaired in the absence of Itk. Several studies implied a role for Rlk in Th1 cell polarization. The analysis of Itk–/–Rlk–/– T cells revealed that Rlk can compensate for loss of Itk in TCR-mediated signaling events. Taken together, these studies showed that Itk and Rlk play an important role in the development and function of T cells. With respect to Tec, studies using a murine T cell hybridoma line and antisense oligonucleotide treatment in primary murine splenocytes implicated Tec in TCR/CD28 mediated pathways and in the activation of the Il2 and Il4 promoters. Recently, it was reported that Tec expression is up-regulated in effector T cells, in particular Th2 cells. Taken together, the function of Tec in primary T lymphocytes is poorly understood. In this study we performed a comprehensive analysis of the helper T cell lineages in Tec–/– mice.

Supported by FWF grant APP24265FW

PIV_3

Abstract Title	STAT1-S727 - the license to kill
Authors Family name, initials	Putz, E.M., Didara Z., Gotthardt D., Sexl V.
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Presenter	Didara, Z.

The JAK-STAT signaling cascade constitutes a paradigmatic pathway transporting extracellular signals from the cell membrane to the nucleus. Interferons (IFNs) bind to the interferon receptor and induce the phosphorylation of the transcription factor STAT1 on tyrosine 701. Subsequently, activated STAT1 proteins translocate to the nucleus, where they are subjected to an additional phosphorylation on serine 727 (S727). STAT1-S727 phosphorylation was proven indispensable for the ultimate response to type II IFNs and the establishment of cytotoxic T lymphocyte effector functions in vivo. Thus, STAT1-S727 is generally accepted as an activating phosphorylation site.
Natural killer (NK) cells are large granular lymphocytes and important members of the innate immune system. They play a pivotal role in the eradication of virally infected and tumor cells. IFN-induced STAT1 signaling provides essential signals for the development of fully functional NK effector cells. Thus, NK cells derived from Interferon-α/β receptor 1 (Ifnar1)- or Stat1-deficient mice display severe defects in final maturation and cytotoxicity. However, mutating the STAT1-S727 phosphorylation site (Stat1-S727A) unexpectedly enhances NK cell cytotoxicity towards various tumor cell lines. Accordingly, Stat1-S727A mice are highly resistant to NK cell-surveilled tumors. Our experiments indicate a novel inhibitory role of STAT1-S727 phosphorylation. Kinase inhibitors and knockdown experiments identified the cyclin-dependent kinase (CDK)8 as relevant kinase phosphorylating STAT1-S727 and restraining NK cell cytotoxicity. Thus, inhibiting CDK8-mediated STAT1-S727 phosphorylation may represent a novel therapeutic strategy to stimulate NK cell-mediated tumor surveillance.

PIV_4

Abstract Title	The role of STAT5A and STAT5B in natural killer cells
Authors Family name, initials	Edlinger, L.[1], Putz, E.M.[2], Prchal-Murphy, M.[1], Gotthardt, D.[1], Tigan, A.S.[1], Hölbl-Kovacic, A.[1], and Sexl, V.[1]
Affiliation	[1] Institute of Pharmacology and Toxicology, University of Veterinary Medicine, Vienna, Austria [2] Queensland Institute of Medical Research, Brisbane, Australia
Presenter	Edlinger, L.

Natural killer (NK) cells are part of the first line defense against cancer, and their development and function depends on the Janus kinase / signal transducer and activator of transcription (JAK-STAT) signaling pathway. We could show that NK cell-specific deletion of *Stat5* in mice led to abrogated NK cell maturation at the NK cell precursor stage in the bone marrow and a dramatic reduction of mature NK cells in peripheral lymphoid organs. There are two known isoforms of STAT5, designated STAT5A and STAT5B, with approximately 90% sequence homology. We hypothesize that STAT5A and STAT5B have non-redundant functions in NK cells. By using mice, in which *Stat5a* or *Stat5b* is specifically deleted, we want to investigate the role of either protein in NK cell development, maturation, and functionality.

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PIV_5

Abstract Title	MAZR and Runx factors synergistically repress ThPOK during CD8+ T cell lineage development
Authors Family name, initials	Sakaguchi, S.[1], Hainberger, D.[1], Tizian, C.[1], Tanaka, H.[2], Okuda, T.[3], Taniuchi, I.[2], Ellmeier, W.[1];
Affiliation	[1]Division of Immunobiology, Institute of Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria [2]Laboratory for Transcriptional Regulation, RIKEN Center for Integrative Medical Sciences (IMS-RCAI) Yokohama, Kanagawa, Japan [3]Department of Biochemistry and Molecular Biology, Kyoto Prefectural University of Medicine Kyoto, Kyoto, Japan
Presenter	Hainberger, D.

ThPOK is a key commitment factor for CD4 lineage development and the maintenance of CD4+ T cell lineage integrity, while ThPOK expression has to be repressed during CD8 lineage development to allow the generation of the cytotoxic branch of T cell-mediated adaptive immunity. We previously showed that the transcription factor MAZR is part of the transcriptional network that represses ThPOK during CD8+ T cell lineage differentiation.

Here we investigated how ThPOK repression is regulated by MAZR during CD8+ T cell lineage development. Conditional gene targeting approaches revealed that MAZR and Runx1 together repressed ThPOK in pre-selection DP thymocytes, while MAZR acted in synergy with Runx3 in the repression of ThPOK in CD8+ T cells. Moreover, MAZR-Runx1 as well as MAZR-Runx3 double-mutant mice showed enhanced derepression of Cd4 in DN thymocytes and in CD8+ T cells in comparison to Runx1 or Runx3 single-deficient mice, respectively, indicating that MAZR modulates Cd4 silencing. Thus, our study shows developmental stage-specific synergistic activities between MAZR and Runx/CBFβ complexes. Finally, retroviral Cre-mediated conditional deletion of MAZR in peripheral CD8+ T cells led to the derepression of ThPOK, indicating that MAZR is also part of the molecular machinery that maintains a repressed state of ThPOK in CD8+ T cells.

The work in the laboratory of W.Ellmeier was supported by the Austrian Science Fund FWF (P19930, P23641, I00698), S.Sakaguchi was supported by the FWF (P23669). The work in the laboratory of I.Taniuchi was supported by the RCAI International Collaboration Award Program and JPSP Joint Research Projects.

PIV_6

Abstract Title	Molecular imaging of the antigen recognition dynamics in $CD8^+$ ctotoxic T-cells
Authors Family name, initials	Markus Kraller, René Platzer, Paul Spechtl, Johannes Huppa and Hannes Stockinger
Affiliation	Molecular Immunology Unit, Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna
Presenter	Kraller Markus

Cytotoxic T-cells (CTLs) are of paramount importance in immune defense against tumors and viruses. They are exquisitely sensitive towards antigen as they can detect with their T-cell antigen receptors (TCRs) the presence of even a single antigenic peptide-loaded MHC molecule I (pMHCI) among thousands of structurally related yet non-stimulatory pMHCs (Marco Purbhoo, 2004). Antigen recognition takes place within the special constraints of the immunological synapse between a T-cell and an antigen presenting cell. Here receptors and their ligands are pre-oriented, possibly clustered in specific membrane domains and subjected to cellular forces. Since biochemical experimentation invariably requires the destruction of at least one of the synaptic membranes it does not account for the specific microenvironment in which T-cell antigen recognition occurs. We are therefore employing a molecular imaging approach in which we confront cytotoxic TCR transgenic T-cells with a glass-supported lipid bilayer (SLB) functionalized with accessory molecules (adhesion and/or co-regulatory) and a wild-type (WT) pMHCI or CD8 deficient pMHCI. In this defined system we can set the concentration and composition of the molecules in order to determine the influence of CD8 and accessory molecules on antigen-recognition in the case of high and low -, agonistic and antagonistic antigen and correlate TCR-pMHCI engagement with downstream signaling events.

PIV_7

Abstract Title	The role of AMP-activated protein kinase in T helper cell differentiation
Authors Family name, initials	Gualdoni, G.A.[1], Mayer, K.A.[1], Göschl, L.[2], Gerner, M.[3], Schmetterer, K.G.[3], Zlabinger, G.J.[1]
Affiliation	[1]Institute of Immunology, Medical University of Vienna, Austria [2]Department of Rheumatology, Internal Medicine 3, Medical University of Vienna, Austria [3]Department of Laboratory Medicine, Medical University of Vienna, Austria
Presenter	Mayer, K.A.

Recent research has established a delicate interplay between cellular energy metabolism and immune cell function. The key sensing molecule of cellular energy supply, AMP-activated protein kinase (AMPK), is activated in conditions of low ATP-levels and inhibits anabolic processes, amongst other by inhibiting the mammalian target of rapamycin (mTOR). As such, the kinase has been proposed to play a role in T helper cell function and differentiation. Here, we have investigated the role of this molecule in murine CD4+ T helper cell polarization and analyzed the underlying metabolic alterations. We found that the AMP-analogue AICAR, but not other AMPK-activators such as 2-deoxyglucose, A-769662 or Metformin, enhanced the differentiation of Foxp3+CD25+GITR+ regulatory T cells (Treg). Since AICAR and rapamycin exhibited additive effects, a role of AMPK in T cell polarization independent of mTOR is conceivable. AICAR specifically induced expansion of Treg while dampening the proliferation of Foxp3- cells. Strikingly, AICAR treated Foxp3+ Treg enhanced their fatty acid uptake and interference with fatty acid oxidation inhibited AICAR-induced Treg polarization, indicating a role of altered energy consumption patterns in AICAR's effects. With this work, we further elucidate the modulating effect of metabolic pathways on T-cell differentiation.

PIV_8

Abstract Title	Nuclear receptor corepressor 1 (NCoR1) in T cell development and homeostasis
Authors Family name, initials	Müller, L.[1], Hassan, H.[1], Hainberger, D.[1], Villunger, A.[2], Auwerx, J.[3], Ellmeier, W.[1]
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Presenter	Müller, L.

NCoR1 (nuclear receptor corepressor 1) has been identified as a regulator of nuclear receptor mediated gene repression. Interestingly, studies with NCoR1 knockout mice revealed important functions for NCoR1 during stages of early embryonic development, such as neural cell differentiation, progression of erythrocytes and fetal thymocyte development. NCoR1 facilitates transcriptional repression through the interaction with chromatin modifying enzymes and is recruited to target gene loci via binding to transcription factors. Among them, a set of BTB zinc finger (BTB-ZF) transcription factors (e.g. PLZF, BCL6 and MAZR), which are key regulators of T cell development and function, are in a complex with NCoR1. Together, this implies important roles for NCoR1 in T cells.

To study the role of NCoR1 in T cells, we have crossed *Ncor1*$^{f/f}$ mice with T cell-specific cre deleter transgenic mouse lines to determine the function of NCoR1 during thymocyte development and in peripheral T cells. Preliminary results indicate an essential role for NCoR1 in maintaining a proper T cell lineage developmental program and in the regulation of T cell homeostasis.

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PIV_9

Abstract Title	Characterization of B-cell subsets in follicular structures: from classical germinal centers to ectopic follicles at tumor site
Authors Family name, initials	Mungenast, F.[1], Meshcheryakova, A.[1], Oswald, A.[1], Bajna, E.[1], Tamandl, D.[2], Bergmann, M.[3], Koperek, O.[4], Birner, P.[4], Mechtcheriakova, D.[1]
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Presenter	Mungenast, F.

B cells are known to possess multifaceted biological functions; still there is a need for detailed characterization of individual B-cell subsets attributed to development and function of follicular structures in secondary lymphoid organs as well as at ectopic sites within diseased tissues. Recently, our group demonstrated that $CD20^+$ B cells organized into follicular structures at the metastatic site of patients with colorectal cancer (CRC) are strongly associated with better prognosis suggesting their anti-tumoral role. Yet, the biological mechanisms for development and maintenance of germinal center (GC)-like ectopic structures within malignant tissue are not well defined; limited knowledge is available on the magnitudes of post-germinal memory and/or plasma cell subsets. We developed a computerized microscopy-based algorithm allowing quantitative assessment of memory and plasma B cells across large-scale tissue specimens. We used CD20, AID, IgM, CD27, CD73, and CD138 as B-cell subset markers. Given the broader expression pattern of CD27 and CD73, we discriminated the $IgM^+/CD27^+$, $IgM^+/CD73^+$ or $CD20^+/CD27^+$ memory cells and $CD138^+/CD27^{high}$ plasma cells. We first assessed the distribution and quantities of B-cell subsets in different pre-defined compartments of classical follicles (germinal center, mantle zone, surrounding 100 μm rim) within the tonsil tissues. Furthermore, we applied the established strategy to characterize B-cell aggregates and ectopic follicles formed within primary CRC tissue and the metastatic site in the liver (matched n=23). The results indicate the presence of various B-cell memory subsets both at primary and metastatic CRC which in addition show the tumor anatomy-attributed distribution and organization patterns.

Supported by FWF P22441-B13/P23228-B19

PIV_10

Abstract Title	The role of HDAC2 in T cells
Authors Family name, initials	Preglej, T.[1], Göschl, L.[1,2], Andersen, L.[1], Mathias, P.[3], Seiser, C.[4], Ellmeier, W.[1]
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Presenter	Preglej, T.,

The interplay of histone acetylation and deacetylation serves as a key regulatory mechanism in T cell development and function by modulating cellular gene expression. The dynamic changes in the acetylation of core histones are mediated through the activity of two large families of antagonistic proteins, namely histone acetyltransferases (HATs) and histone deacetylases (HDACs), which modify chromatin structure through transfer of acetyl-groups to and from lysine residues of histones, respectively. Moreover, HATs and HDACs also act on non-histone targets regulating protein activity, stability, localization and protein-protein interaction. The application of HDAC inhibitors (HDACi) revealed important immunological processes and T cell functions that are dependent on the activity of HDACs. To date, 18 individual HDACs have been identified that act in numerous cellular pathways, frequently through their repressive influence on gene transcription. However, the specific roles of individual HDAC family members in T cells are still subject of ongoing research.

Our group previously demonstrated that HDAC1 controls the magnitude of a Th2-type inflammatory response by modulating cytokine expression in effector T cells. During the last years we showed that conditional deletion of HDAC1 in T cells leads to enhanced airway inflammation and increased Th2 cytokine production and that HDAC1 controls antiviral immune responses. However, the role of the highly HDAC1-related HDAC2 protein in T cells is only poorly understood. Here we aim to elucidate the role of HDAC2 in T cells. Results of our ongoing studies will be presented.

Supported by FWF, grant P26193.

PIV_11

Abstract Title	Rapid multiplex analysis of lipid raft components with single cell resolution
Authors Family name, initials	Schatzlmaier, P.[1], Huppa, J.B., Stockinger, H.[1]
Affiliation	[1] Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Austria
Presenter	Schatzlmaier, P.

Lipid rafts, a distinct class of highly dynamic cell membrane microdomains, are integral to cell homeostasis, differentiation and signalling. Biochemical analysis of lipid raft components usually involves cell lysate fractionation via sucrose density gradient ultracentrifugation, demonstrating raft-association of important lymphocyte receptor signalling molecules as well as stimulation-induced alterations in raft composition. Current protocols are laborious, time-consuming and need a large number of cells, thus complicating the examination of rare cells, developmentally heterogeneous cell populations or weakly raft-associated factors. Here we present a fast and reliable method, which is based on the passage of cells through a detergent gradient at low g centrifugation, requiring little starting material and effort. Our protocol enables multidimensional and sensitive flow cytometric quantitation of raft-resident proteins with single cell resolution, e.g. prior and post stimulation of the T-cell receptor. Thus it is possible to precisely assess membrane components from a few cells in complex isolates as well as their dynamics due to cell signalling, differentiation, and mutation.

Supported by the Cell Communication in Health and Disease (CCHD) PhD Program.

PIV_12

Abstract Title	The novel mTOR inhibitor AZD8055 shuts-off T helper 1 and 2 but not interleukin-2 secretion early upon activation of allergen-specific T cells
Authors Family name, initials	Tauber, P.[1], Candia, M. R.[1], Trapin, D.[1], Neunkirchner, A.[1] and Pickl, W. F.[1]
Affiliation	[1]Institute of Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria
Presenter	Tauber, P.

AZD8055 is a newly established adenosine triphosphate-competitive inhibitor of mTOR complexes 1 and 2. Here we examined AZD8055 for its effects on viability, allergen-specific activation, growth and factor production of major mugwort (*Artemisia vulgaris*) pollen allergen-specific T lymphocytes derived from double transgenic allergy mice. Proliferation and T cellular activation were determined by measuring ^{3}H-thymidine uptake and evaluation of CD69, CD25, CD154 and CD49e expression on $CD3^{+}CD4^{+}$ T cells. Factor production was determined by multiplexing of supernatants after T cell activation with titrated amounts of allergen. Using the IC_{50} dose for proliferation inhibition, viability of AZD8055 treated splenocytes was not affected. Furthermore, AZD8055 treatment did not cause negative effects on expression of activation markers studied. After 24 hours, AZD8055 strongly inhibited IFN-γ (73.9±11.4%, $p<0.05$) secretion, while moderately decreasing IL-13 (49.1.±17.4%, $p<0.01$), TNF-α (50.7±19.4%, $p<0.001$) and GM-CSF (60.1±16.6%, $p<0.01$) secretion and weakly suppressing IL-5 (25.5±42.9%, $p<0.05$). Furthermore, from 48 hours onwards it almost completely inhibited IFN-γ (85.1±4.7%, $p<0.05$), TNF-α (74.4±13.1%, $p<0.01$), IL-4 (86.9±5.1%, $p<0.05$), IL-5 (70.9±13.0%, $p<0.001$), IL-13 (86.0±2.9%, $p<0.01$), IL-17 (72.6±13.2%, $p<0.05$) and GM-CSF (84.9±6.1%, $p<0.01$). IL-10 secretion was moderately inhibited after 48 hours peaking at 72 hours (90.5±5.8%, $p<0.05$). Noteworthy, IL-2 levels where only weakly and non-significantly (ns) affected 20.5±10.5% (ns) after 72 hours. Other substances tested with comparable inhibition of proliferation drastically suppressed IL-2. In summary, AZD8055 shows potentially interesting features since it inhibits Th1 and Th2 cytokines while keeping IL-2 secretion intact.

Supported by the Austrian Science Fund SFB-F4609-B19, DK W1248-B13 & Christian Doppler-Research Association and Biomay AG

PIV_13

Abstract Title	The 3-phosphoinositide-dependent kinase-1 targeting drug BX795 promotes interleukin-2 but shuts off T helper 1 and 2 cytokine secretion upon activation of allergen-specific T cells
Authors Family name, initials	Tauber, P.[1], Candia, M. R.[1], Trapin, D.[1], Neunkirchner, A.[1] and Pickl, W. F.[1]
Affiliation	[1]Institute of Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria
Presenter	Tauber, P.

BX795, an inhibitor of 3-phosphoinositide-dependent kinase-1 (PDK-1), was investigated for its effects on viability, allergen-specific activation, growth and factor production of major mugwort (*Artemisia vulgaris*) pollen allergen-specific T lymphocytes derived from double transgenic allergy mice. We assessed proliferation and T cellular activation by measuring ^{3}H-thymidine uptake and evaluation of CD69, CD25, CD154 and CD49e expression on $CD3^{+}CD4^{+}$ T cells. Factor production was determined by multiplexing of supernatants after T cell activation with titrated amounts of allergen. Using the IC_{50} dose for proliferation inhibition, no alteration in viability of BX795-treated splenocytes was observed. Furthermore, BX795 treatment did not negatively affect activation marker expression studied. Noteworthy, BX795 almost completely inhibited IFN-γ secretion (72.9±10.0%, $p<0.01$) early on (24 hours) and inhibited IL-4 (88.3±6.5%, $p<0.05$), IL-13 (75.8±8.7%, $p<0.001$) and IL-10 (80.8±13.1%, $p<0.001$) secretion at 48 hours. Moreover, at 48 hours BX795 partially inhibited IL-5 (35.8±23.2%, $p<0.05$) and TNF-α (45.2±24.8%, $p<0.05$) secretion with maximal inhibition seen after 72 hours (70.6±15.5% $p<0.001$ and, 68.0±17.8% $p<0.05$, respectively). BX795 did not influence IL-17 and GM-CSF levels, however, it significantly stimulated IL-2 secretion at 48 and 72 hours (2.3±0.6-fold and 4.7±1.4-fold, $p<0.01$ and $p<0.001$, respectively). Other substances tested, displaying an equal level of proliferation inhibition, such as the ERK2 inhibitor Vx-11e drastically inhibited IL-2 secretion. In summary, BX795 specifically shuts-off Th1 and Th2 cytokines while it strongly stimulates interleukin-2 secretion. A potential clinical application is currently being evaluated in *in vivo* experiments using double transgenic allergy mice.

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PIV_14

Abstract Title	The role of B-cells and humoral immunity in the lung
Authors Family name, initials	M. Watzenboeck[1,2], S. Saluzzo[1,2], K. Lakovits[1,2], A. Hladik[1,2] and S. Knapp[1,2]
Affiliation	[1.]CeMM, Research Center for Molecular Medicine of the Austrian Academy of Sciences, Austria [2.]Department of Medicine I/Research Laboratory of Infection Biology, Medical University of Vienna, Austria
Presenter	M. Watzenboeck[1]

B-cells contribute substantially to the total pool of immune cells in healthy lungs. Yet, compared to other cell types, relatively little is known about the characteristics and functions of pulmonary B-cells. The aim of this project to understand to which degree B-cells contribute to lung homeostasis and to host defense against acute bacterial infections.

Using flow cytometry, we found that the B-cell population accounted for about 20% of total viable immune cells within the lungs of healthy adult C57/BL6 mice. The B-cell population appears shortly after birth and expands within the first 2 weeks of life. In adult mice, around 60% of these cells exhibit an IgDhigh/IgMlow/ to intermed surface marker phenotype, while surface markers compatible with B1 cells were expressed on 0.5-1% of total lung B-cells

Our goal is to better understand the functions of these B-cells within the lung, and to evaluate antibody-mediated effects in lung tissue To this end, we discovered that B-cell deficient mice showed increased CFU counts in lung tissues as early as 6h after induction of pneumococcal infection, suggesting a contribution of B-cells to the innate immune defense against this pathogens. We are currently addressing the potential contribution of natural antibodies and innate B-cells to this phenotype. Our future aims are to elucidate the functional characteristics of pulmonary B-cell population and their contribution to lung homeostasis using various approaches, including cell-depletion and adoptive transfer strategies.

PIV_15

POSTER SESSION V_Inflammation and Clinical Immunology

Abstract Title	Diminished CD19-expression on newly produced $IgD^{+}/CD27^{-}$ B cells after B cell depletion is associated with clinical response in rheumatoid arthritis and scleroderma
Authors Family name, initials	Brezinschek, H.P. [1], Fürst-Moazedi, F. [1], Kielhauser, S. [1], Stradner, M. [1], Graninger, W.B. [1]
Affiliation	[1] Division of Rheumatology and Immunology, Department of Internal Medicine, Medical University Graz, Austria.
Presenter	Brezinschek, H.P.

CD19 is a membrane glycoprotein of the immunoglobulin superfamily and part of the hetero-oligomeric complex comprising the complement receptor type 2, which positively regulates BCR activation. In this study we analyzed the effect of B cell depletion by CD20 antibodies in rheumatoid arthritis (RA) and systemic sclerosis (SSc) patients on the CD19-expression on peripheral blood B cells. The MFI±SEM on B cells of RA and SSC patients before RTX was 40,890 ± 2,240 and 32,129 ± 6,718, respectively. After RTX the MFI of CD19 was significantly reduced on B cells with the greatest reduction in SSC B cells (9,016 ± 845 and 3,804 ± 281, $p \leq 0.0001$), followed by B cells from RTX-responders (9,156 ± 719 and 5,975 ± 585, $p \leq 0.008$) and non-responders (9,492 ± 603 and 6,712 ± 818, $p \leq 0.031$). Interestingly, analyzing CD19 expression on the B cell subsets demonstrated that naïve B cells in RTX-non responders had no significant reduction in MFI (9,596 ± 677 and 6,890 ± 1,302, $p=0.1663$) compared to RTX responders in RA (9,214 ± 745 and 6,466 ± 635, $p \leq 0.018$) or SSc (8,672 ± 977 and 3,673 ± 607, $p \leq 0.016$). These data suggest, that RTX affects disease activity not only by eliminating B cells but also by reducing the expression of the co-stimulatory surface molecule CD19. Interestingly, in RTX-non responders newly produced naïve B cells do not significantly down regulate CD19. Thus, they might still be able to activate T cells or produce pro inflammatory cytokines.

PV_1

Abstract Title	Testing biocompatibility of thermo-sensitive elastin-like recombinamer (ELR) biogels for bone repair and regeneration *in vivo* in BALB/c mice
Authors Family name, initials	Changi, K.[1]*, Bosnjak, B.[1], Ibáñez, A.[2], Gonzalez-Obeso C.[2], Rodríguez-Cabello, J.[2], Epstein, M. M.[1]
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Presenter	Changi, K.

Introduction: The use of novel biomaterials for tissue repair and regeneration is a burgeoning field. However, the potential to induce adverse inflammatory and allergic responses limit their use. A novel thermo-sensitive elastin-like recombinamer (ELR) biogel was developed for use in large non-union bone lesions. We established an *in vivo* biocompatibility evaluation platform to test these biogels.

Materials and methods: ELRs were tested using short-term intraperitoneal (i.p.) and long-term subcutaneous (s.c.) mouse models. Female BALB/c mice were implanted with ELRs or a currently available biomaterial for bone repair: Vitoss Foam (OVF, Orthovita, USA). We evaluated biocompatibility by 1) enumerating inflammatory cells and measuring cytokine levels in peritoneal lavage fluid 7 days after i.p. implantation and 2) by H&E and Masson's trichrome blue-stained tissue sections and qPCR of the implant site at 3 and 8 weeks after s.c. implantation. Serum antigen-specific antibody titres were measured.

Results: Following i.p. implantation, OVF induced inflammation and high levels of IL-1β, IL-2 and IL-4 compared with ELR-implanted and sham control mice. Similarly, s.c. OVF, induced chronic inflammation with giant cells and collagen deposition compared with mild inflammation and few collagen fibers induced by ELRs. qPCR assays revealed high upregulation of inflammation-, fibrosis- and wound healing-related genes following OVF compared to ELRs. OVF and ELRs generated antigen-specific IgG1 and IgE titres.

Conclusion: Our data demonstrate that ELRs induce minimal immune responses compared with OVF and suggest that the ELRs may be a useful for tissue repair.

This research was supported by the EC-FP7-InnovaBone project, grant no. 263363.

PV_2

Abstract Title	IgE Autoreactivity in Bullous Pemphigoid
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Presenter	Freire, P.

Bullous pemphigoid (BP) is an auto-immune disease typically associated with old age. It is characterized by bullae at the dermal-epidermal junction (DEJ) that are thought to be induced by the binding of auto-antibodies. These antibodies can recruit inflammatory cells through complement activation, culminating in the proteolytic destruction of cell adhesion structures. While IgG has been the class consistently associated with the disease, more recent studies point to a potential involvement of IgE. Consistent with previous literature, we have detected IgE in the perilesional skin of 22 out of 33 (67%) BP patients. This IgE was not found at the DEJ, but instead on the surface of mast cells and eosinophils, most likely bound as an immune complex. We have evidence that the high-affinity receptor for IgE is the primary molecule involved in this interaction and that eosinophils are expressing FcεRI in BP patients. Furthermore, using whole skin lysates for immunoblotting, we have demonstrated peripheral BP IgE reactivity against antigens with approximately 60, 120, 180 and 230 kD. These likely represent intra- and extra-cellular domains of BP180 and the full-length BP180 and BP230 proteins, respectively. Given that the clinical picture of BP consists of erythema and bullae, appearing alone or concomitantly, an association between self-reactive IgE and urticarial-like lesions is therefore plausible and suggests an alternative pathway of disease pathogenesis. Uncovering the dominant epitopes for both IgG and IgE in different presentations of the disease could further clarify this question and additionally argue for the development of new IgE-based therapeutic approaches.

PV_3

Abstract Title	Molecular insights into chimeric antigen receptors (CARs) targeted against leukemia's: A major breakthrough in adoptive immunotherapy
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Affiliation	[1] Medical University of Vienna, [2] Universitätsklinikum Würzburg
Presenter	Gudipati.V

Chimeric antigen receptors (CAR) are recombinant receptors designed to target tumor cells expressing a specific antigen. A typical CAR consists of an extracellular single chain variable fragment (scFv) fused to a transmembrane domain followed by intracellular activation and co-stimulatory domain. Despite the success of CAR therapy, it is not well understood how the combination of domains influences the signaling, cytotoxicity and proliferation of these cells. Additionally, despite being capable of binding to the antigen with higher affinities than T cell receptor, CARs require a higher threshold of antigens (10^3-10^4 vs. 1-10 for TCR) to initiate signaling and exhibit cytotoxicity. In order to understand the functioning of CAR-T cells, we aim to study synapse formation, internalization, signaling, dimerization and nano-scale organization by means of TIRF and super-resolution microscopy. Along these lines, we have established a lipid bilayer system on to which target antigens (CD19 & Ror1) and co-stimulatory molecules (ICAM1 & B7-1) can be immobilized to investigate CAR-T cells *in situ*. Our experiments have indicated that a CAR-T cell synapse is distinct from a typical T cell synapse. We have also observed that CARs are internalized after activation. In the near future we will visualize CAR synapse in high resolution. We expect to extend the above mentioned results to different versions of CD19 and Ror1 CARs to understand how the differences in their design affect the function.

PV_4

Abstract Title	Autoimmune encephalomyelitis in humans: what can we learn about B-cells in multiple sclerosis?
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Presenter	Höftberger, R.

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system. Immunological studies suggest that it is a T-cell mediated autoimmune disease, although an MS-specific target antigen for autoimmunity has so far not been identified. Models of experimental autoimmune encephalomyelitis in part reproduce features of MS, but none of the models so far covers the entire spectrum of pathology and immunology. Autoimmune disease of the nervous system has occasionally been observed in humans after active sensitization with brain tissue or brain cells, giving rise to acute demyelinating polyradiculoneuritis, acute disseminated encephalomyelitis and in rare cases inflammatory demyelinating conditions similar to acute MS. In this study we analyzed in detail the immunopathology in archival autopsy tissue of a patient who died with an MS like disease after repeated exposure to subcutaneous injections of lyophilized brain cells. The pathology of this patient fulfilled all pathological diagnostic criteria of MS. Demyelination and tissue injury was associated with antibody (IgM) deposition at active lesion sites and complement activation. Major differences to classical EAE models were seen in the composition of inflammatory infiltrates, being dominated by B-cells, infiltration of IgM positive plasma cells, profound infiltration of tissue by $CD8^+$ T-lymphocytes and nearly complete absence of $CD4^+$ T-cells. Our study shows that auto-sensitization of humans with brain tissue can induce a disease, which closely reflects the pathology of MS, but that the mechanisms leading to demyelination and tissue injury differ from those, generally implicated in the pathophysiology of MS through studies in EAE.

PV_5

Abstract Title	Investigating the Role of EGFR in myeloid cells in inflammatory diseases
Authors Family name, initials	Holcmann, M. Sohn S. Martins R. Goecen B.V. Rakob L. Komposch K. Sibilia M.
Affiliation	Institute for Cancer Research, Dept. of internal Medicine I, Medical University of Vienna
Presenter	Holcmann M.

We have recently discovered a novel role for Epidermal Growth Factor Receptor (EGFR) in macrophages. In a mouse model of hepatocellular carcinoma the production of tumor promoting Interleukin (IL)-6 by Kupffer cells in response to IL-1b was dependent on autocrine EGFR signaling. We were therefore interested in investigating a potential role of EGFR in regulating macrophage function in other inflammatory diseases. Mice conditionally lacking Egfr in myeloid cells were challenged in different disease models. Lack of EGFR had no impact on Imiquimod induced skin inflammation in conditional Egfr(fl) x LysCre mice and did not rescue the skin phenotype induced by lack of EGFR in the skin. Conversely, EGFR deficient mice infected with E. coli showed a higher bacterial load in peritoneal lavage and liver. Along that line we observed inhibition of phagocytosis in RAW 264.7 cells treated with the EGFR inhibitor Cetuximab. We are therefore currently analyzing phagocytosis of bone marrow derived macrophages, peritoneal macrophages and Kupffer cells of mice lacking EGFR in macrophages. To get further insight into potential mechanisms underlying the observed in vivo phenotypes the effect of EGFR on macrophage polarization is investigated in vitro. This study may contribute to a better understanding of the role of EGFR on immune cells and reveal potential new mechanisms underlying the (side)effects of EGFR inhibitors.

PV_6

Abstract Title	Role of the AP-1 protein c-Jun in Imiquimod mediated tumor clearance.
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Presenter	Novoszel P.

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

PV_7

Abstract Title	The Role of Plasmacytoid Dendritic Cells in Imiquimod Mediated Skin Inflammation and Melanoma Clearance in Mice
Authors Family name, initials	Stulnig, G.[1], Novoszel, P.[1],Holcmann M.[1],Sibilia M.[1]
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Presenter	Stulnig, G.

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

PV_8

Abstract Title	Examining virus-recognizing receptors in Langerhans cellsfollowing human skin barrier disruption and stimulation with synthetic RNA
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Presenter	Tajpara P.

Classic epitheliotropic viruses are able to infect both Langerhans cells (LCs) and keratinocytes (KCs). However, the expression and function of virus-sensing receptors in LCs is still not fully understood. Poly(I:C) is a synthetic analogue of viral double-stranded RNA, which occurs as an important metabolite during viral infection. It isinternalized into cells through endocytosis and activatesthe endosomal TLR3 (Toll-like receptor 3)as well asthecytoplasmic receptors MDA5 (melanoma differentiation-associated gene5)and PKR (protein kinase R).We found that rhodamine-labeled poly(I:C) was rapidly taken up by freshly isolated, FACS-sorted human LCs and KCs and induced the production of the proinflammatory cytokine IL-6 in KCs with the same potency as unlabeled poly(I:C). To test whether poly(I:C)is able to induce LC maturation in situ, we applied it topically onto barrier-disruptedfull-thickness human skin explants in vitro. Twenty four hours after poly(I:C) treatment CD83 and CD86 expression was significantly induced on LCs. Analysis of PRRs recognizing double-stranded RNA in untreated and poly(I:C) treated skin explants, revealed a highbaseline expression ofTLR3andPKR in KCs and a weak MDA5 expression exclusively in LCs. In addition, all three receptors were further upregulated by poly(I:C) treatment in the respective cell types.Our data suggest that MDA5 but not TLR3 and PKR may play a key role in the innate immune response of LCs to viral infections.

PV_9

Abstract Title	The effect of anti-CD40L mAb induction therapy on graft survival in a CTLA4Ig based murine heart transplantation model
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Presenter	Unger, L.

Introduction: Costimulation blockade using CTLA4Ig has been proven as an effective therapy in kidney transplantation but a higher incidence of acute rejection episodes has been noticed in clinical studies. We therefore developed a murine model mimicking the human setting and investigated the effects of interference with an additional costimulation pathway, namely CD40/CD40L.

Materials and Methods: Balb/c donor hearts were transplanted heterotopically into 10-12 week old C57Bl6 mice. 0.25mg CTLA4Ig was administered intraperitoneally on days 0, 4, 14, 28, 56, 84. Where indicated, mice received 0.2mg or 0.5mg anti-CD40L mAb (MR1) intraperitoneally on days 0, 4 as induction therapy. Grafts were harvested on day 100 or at the time of rejection. Serum samples for assessment of donor specific antibodies were collected 3-4 weeks after transplantation and at the time of rejection. All experiments were performed under specific pathogen free (SPF) conditions.

Results: CTLA4Ig treated mice rejected their grafts at early and late time points with a median survival time (MST) of 22 days. Additionally administered induction therapy using 0.2mg MR1 (MST 53 days) and 0.5mg MR1 (MST 64 days) seemed to prolong graft survival, but did not lead to permanent graft acceptance. Interestingly, donor-specific antibodies were detected in all groups, although not in all mice that rejected their grafts.

Conclusion: Short-term induction therapy using anti-CD40L mAb is not sufficiently potent to achieve permanent allograft survival in CTLA4Ig-treated heart graft recipients.

PV_10

Abstract Title	Comparison of five different damaging models of the respiratory epithelium
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Presenter	Waltl, E.E.

(Abstract also handed in for ECI congress 2015)

The respiratory epithelium with its tight junctions is an important barrier against inhaled exogenous factors. Damaged epithelium can be penetrated by allergens and pollutants more easily, thus facilitating allergic reactions and inflammation. Here we aimed to establish different models for damage in order to enable us to investigate protective factors for the epithelium.

Three different cell culture models were investigated. A bronchial epithelial cell line (16HBE14o-) and primary human nasal epithelial cells were cultured in monolayers cultivated in six well plates and analysed by microscopy. Second, a transwell system was used to investigate permeability through the monolayer, using transepithelial resistance as an endpoint. Third, the xCELLigence DP system was employed for continuous real-time monitoring of impedance-based cell responses.

The cellular response to the following damaging conditions was analysed: i) Physical damage by scratching the cell layer, ii) infection with human rhinovirus (RV), iii) incubation with standardised cigarette smoke extract and iv) exposure to the TH1 cytokine interferon-γ (IFN-γ) and v) exposure to house dust mite (HDM) extract.
The ability of cells to recover after physical damage by scratching within 24 (16HBE14o-) or 72 (primary cells) hours was shown both in cell culture and the xCELLigence DP system. Barrier function decreased in a time- and dose-dependent manner after infection of cells with RV, exposure to cigarette smoke extract, to IFN-γ and to HDM extract.

We established and compared various models for damage of respiratory epithelial cells using three different cell culturing systems and five different damaging conditions.

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PV_11

Abstract Title	Discovery of mimotopes for pathogenic antiaquaporin 4 autoantibodies in neuromyelitis optica via phage display
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Presenter	Zeka B.

Neuromyelitis optica (NMO) is a severe autoimmune astrocytopathy of the central nervous system (CNS) driven by the presence of autoantibodies (NMO IgG) that target astrocytic water channel aquaporin 4 (AQP4). The conformational epitope of NMO IgG remains to be elucidated. This study addresses the identification of epitope mimics (so-called mimotopes) for samples of NMO IgG from 6 NMO patients. For this purpose, we used a random phagedisplayed 12-mer peptide library for biopanning with purified polyclonal immunoglobulin G (IgG) of an NMO-IgG-seropositive patient as target. After increasing target specificity within three rounds of panning, single phage clones were picked and their reactivity with NMO-IgG was evaluated via ELISA. For further characterization, mimotope-based prediction softwares were employed to map the peptides obtained by DNA sequencing of the single phage clones on human AQP4 in silico. Promising candidates were selected and tested for their potential to block the binding of NMO-IgG to its target, AQP4, in a FACS-based analysis using AQP4-transfected HEK293A cells. The FACS measurements revealed peptides to be capable of reducing the binding of NMO-IgG to the extracellular loops of AQP4, indicating that these peptides successfully mimic epitopes of the autoantibody.

The identification of these mimotopes provides a solid basis from which the development of several applications is conceivable in the long term. Mimotopes could enable the characterization and classification of patients' antibody pools according to their reactivity with different mimotopes or could be used for active immunization strategies.

PV_12

Abstract Title	AQP4-specific T cells and NMO IgG orchestrate NMO-like lesions in Lewis rat
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Presenter	Zeka B.

Neuromyelitis optica (NMO) is an inflammatory, astrocytopathic disease of the central nervous system in which pathogenic aquaporin 4 (AQP4)-specific antibodies gain access to the CNS. These antibodies belong to a T cell-dependent subgroup of immunoglobulins, and since NMO lesions contain activated CD4+ T cells, it is tempting to speculate whether AQP4-specific T cells might not only provide T cell help for antibody production, but also play a role in the induction of NMO lesions. We show here that highly pathogenic, AQP4-peptide-specific T cells exist in Lewis rats, which recognize AQP4268–285 as their specific antigen and cause severe panencephalitis. These T cells are re-activated behind the blood–brain barrier and deeply infiltrate the CNS parenchyma of the optic nerves, the brain, and the spinal cord, while T cells with other AQP4-peptide specificities are essentially confined to the meninges. AQP4268–285-specific T cells have NMO-typical “hotspots” for infiltration, i.e. periventricular and periaqueductal regions, hypothalamus, medulla, the dorsal horns of spinal cord, and the optic nerves.

Remarkably, together with NMO-IgG, they initiate large astrocyte-destructive lesions which are located predominantly in spinal cord gray matter.
We conclude that T cells specific for AQP4268–285 are part of the immune repertoire of normal Lewis rats and can be readily expanded in culture, and that AQP4268–285-specific T cells produce NMO-like lesions in the presence of NMO-IgG

PV_13

AUTHORS INDEX