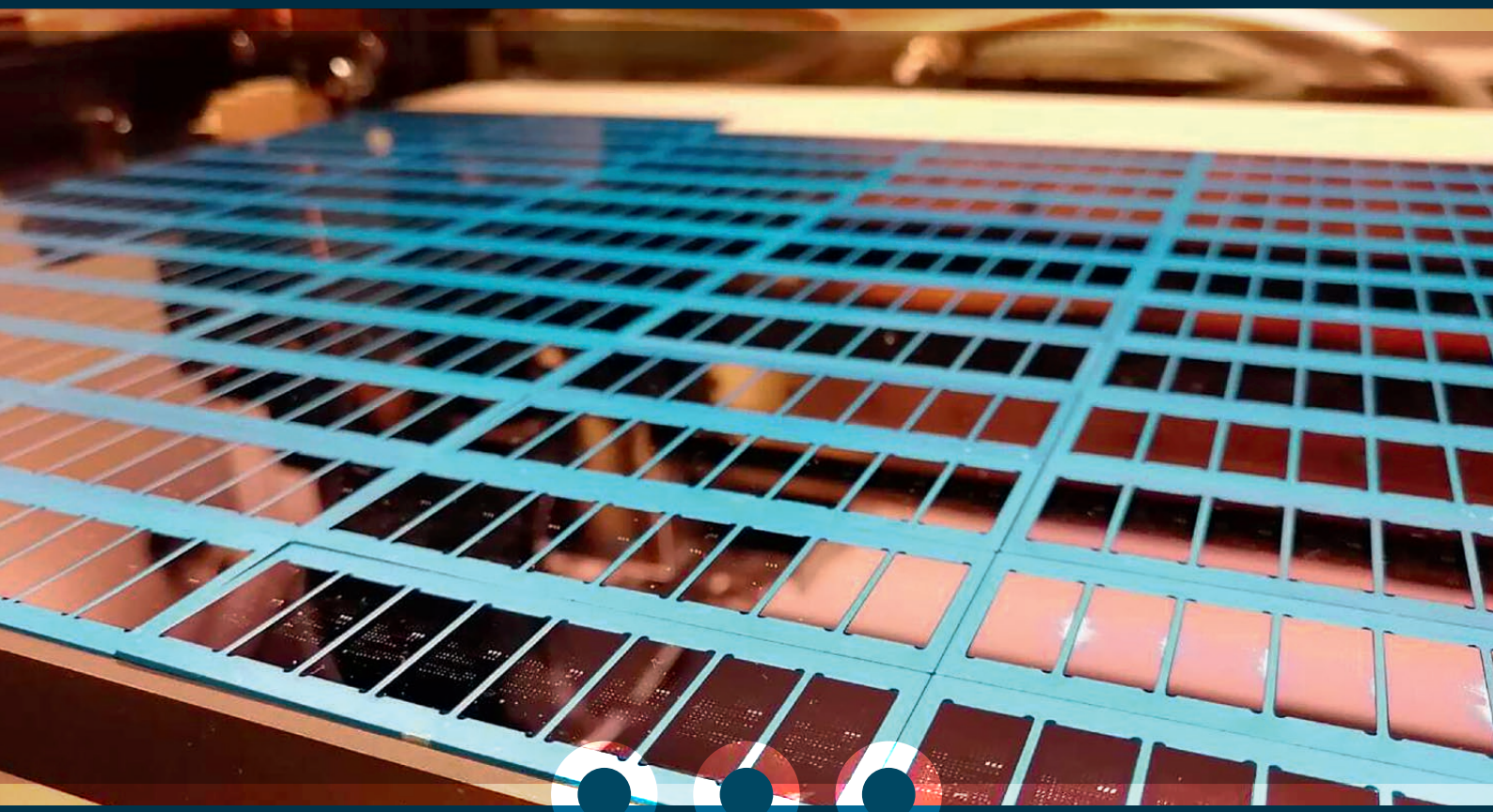
Karl Landsteiner Symposium
From B cells to antibodies
Danube Allergy Research Cluster (Danube ARC)

July 10 - 11, 2023

&

Retreat of the Immunology Research Cluster (IRC)
and the
Research Platform Transplantation (RPT)
of the Medical University of Vienna

July 12, 2023



Venue:
Luberegg Castle
Luberegg 18
3644 Emmersdorf an der Donau, Lower Austria
Seminar Hall Kaiser Saal
www.pichler-wachau.com/luberegg/schloss-uebersicht-wachau.html

TABLE OF CONTENTS

ORGANIZING TEAM

Aleksandra Podzhilkova
MCCA Student Speaker

Anna Guentcheva
Administrative Assistant
to Prof. Valenta

Maja Bajic
MCCA Deputy Student Speaker

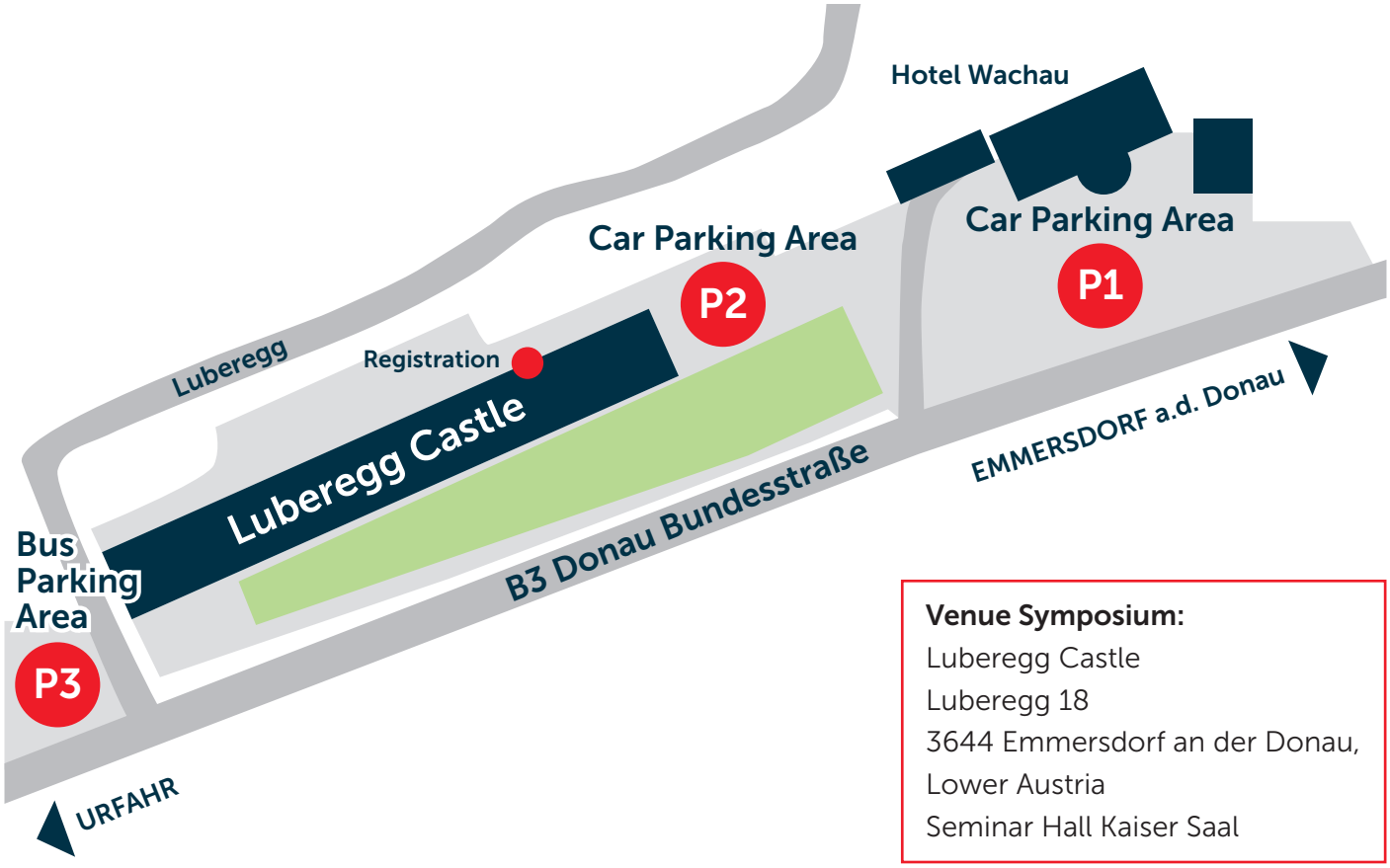
Claudia Oppitz
Administrative Assistant
Danube ARC Prof. Valenta

Metka Luckmann-Wagner
Administrative Assistant
to IRC

Doris Werjant-Locmele
Administrative Assistant
to Prof. Valenta

Kerstin Pöschl
Administrative Assistant
to RPT

TRAVEL ARRANGEMENTS AND VENUE



Venue Symposium:
Luberegg Castle
Luberegg 18
3644 Emmersdorf an der Donau,
Lower Austria
Seminar Hall Kaiser Saal

Departure from Vienna to Luberegg (bus transfer):
Meet in front of Hotel Regina
1090 Vienna, Rooseveltplatz 15

- *Monday, July 10, 2023; Departure 7:15 am (2 Busses)*
- *Tuesday, July 11, 2023; Departure 7:15 am*
- *Wednesday, July 12, 2023; Departure 7:15 am*

Departure from Luberegg to Vienna (bus transfer):
Meet at Bus Parking Area P3

- *Monday, July 10, 2023; Departure 7:30 pm (Bus #1), will stop on its way to Vienna at Hotels Donauhof and Pritz.*
- *Monday, July 10, 2023; Departure approximately 10:45 pm (Bus #2), will stop on its way to Vienna at Hotels Donauhof and Pritz.*
- *Tuesday, July 11, 2023; Departure 5:30 pm, will stop on its way to Vienna at Hotels Donauhof and Pritz.*
- *Wednesday, July 12, 2023; Departure 5:00 pm (Bus #1), will stop on its way to Vienna at Hotels Donauhof and Pritz.*
- *Wednesday, July 12, 2023; Departure 5:00 pm (Bus #2), will bring guests to Farewell at wine tavern Flößerei, Rossatz, before departing from there to Vienna at about 9.30 pm (arrival planned for 10:45 pm)*

WELCOME ON BEHALF OF THE DANUBE ARC STUDENTS

On behalf of the organizing committee, it is our great pleasure to warmly welcome you to the Danube ARC Symposium. This symposium represents a collective effort to explore, learn, and collaborate in the fascinating field of immunology.

Today, we are fortunate to be surrounded by an exceptional lineup of speakers, whose expertise and groundbreaking research have paved the way for advancements in allergy and immunology. As students, we are honored to have the opportunity to learn from our unique guests, who are the leaders in the field. Your expertise, guidance, and mentorship are invaluable to us as we navigate our own scientific journeys.

This symposium is a testament to the collaborative spirit that underpins scientific progress. It is a space where great researchers and students from different backgrounds can come together, exchange ideas, and foster meaningful connections. We have the unique opportunity to learn from one another, challenge experience, and fuel our collective passion for advancing allergy and immunology.

We would like to express our deepest appreciation to the doctoral program speaker (DK-MCCA), Univ. Prof. Winfried Pickl, as well as the speaker of the Danube Allergy Research Program (Danube ARC), Univ. Prof. Rudolf Valenta, for their support, guidance and tireless efforts for organizing the respective research programs and also this top-notch symposium. Their dedication and meticulous planning have created an environment where students can actively participate in shaping the future of scientific research. Further, we would like to acknowledge the support of the funding organizations, all our sponsors and all colleagues involved in organizing this event.

Finally, we wish you an enjoyable symposium with stimulating talks, fascinating insights into state-of-the-art allergy and immunology research and interesting discussions. Thank you all for your participation, and we look forward to memorable experience together.

For the organizing committee

Aleksandra Podzhilkova

Maja Bajic

WELCOME ON BEHALF OF THE DANUBE ALLERGY RESEARCH CLUSTER (DANUBE-ARC) „TOWARDS CURE OF ALLERGY" AND THE IMMUNOLOGY RESEARCH CLUSTER (IRC) OF THE MEDICAL UNIVERSITY OF VIENNA

Everything started with the idea to invite some of the most iconic speakers in the field of Immunology, with special emphasis on B cells, antibodies and plasma cells to the scientific retreat of the Danube Allergy Research Cluster (Danube-ARC).

Danube-ARC (https://www.kl.ac.at/en/university/scientific-organisational-units/danube-arc-danube-allergy-research-cluster) is a research program combining allergy research performed by research groups in the Country of Lower Austria and Vienna along the river Danube. The goal of Danube-ARC is to perform basic research in the field of allergy and to translate the scientific results into better diagnosis and care for the good of allergic patients. Danube-ARC consists of research groups with complementary expertise, an international PhD program "Molecular, Cellular and Clinical Allergology" MCCA and an international post-graduate program which aims to bring modern Molecular Allergology into clinical practice.

One cannot imagine how excited I was when almost all of the invited international speakers agreed to come to this retreat and to exchange their knowledge with the researchers and students in the Danube-ARC.

Accordingly, the idea grew further to invite also the members of the Immunology Research Cluster (IRC) of the Medical University of Vienna and those of the Research Platform Transplantation (RPT) to join and come to Luberegg Castle in the beautiful region of the Wachau in the Country of Lower Austria.

In fact, the country of Lower Austria is not only one of the nine states of Austria. It may be also considered as a cradle of Austrian history in many aspects and recognizes science, art and culture as important areas. It is the hope of this joint meeting to continue uniting research in the field of Allergy and Immunology in the Danube region by bringing scientists from this area together. Importantly, we wish to learn from our international guests, who are some of the best in the world how to further grow the area of allergy and immunology research for the well-being of people. Furthermore, it is hoped that this joint meeting will not only reinforce already existing collaborations but establish new interactions at the local and international level. The beautiful and remote location may facilitate this process and should guarantee that everybody will feel well and be ready for joining forces for improving the lives of people who suffer from immunologically-mediated diseases such as allergy and to develop innovative immunological strategies for diagnosis, treatment and prevention of diseases.

I am most grateful to everybody who will attend and wish to everybody a rewarding stay in terms of scientific and personal interactions as well as good memories.

Univ. Prof. Dr. Rudolf Valenta, MD
(Speaker of the Danube Allergy Research Cluster & Speaker of the Immunology Research Cluster of the Medical University of Vienna)

WELCOME ON BEHALF OF THE DOCTORAL PROGRAM MOLECULAR, CELLULAR AND CLINICAL ALLERGOLOGY, MCCA

The Doctoral Program (DK) **Molecular, Cellular and Clinical Allergology, MCCA**, (www.phd-mcca.at) was established by a consortium of 16 Faculty Members in 2013, with the mission to integrate molecular, cellular and clinical aspects of allergic diseases and to offer an excellent, state-of-the-art research setting along with a structured teaching environment. We aimed to promote highly talented, internationally selected PhD Students, thereby fostering interdisciplinarity and translational research in molecular, cellular and clinical allergology. The program contributes to the development of innovative strategies for diagnosis, therapy and prevention of allergic diseases. The faculty members mentoring the individual PhD-projects have been carefully selected and embedded into the program to cover the entire field of allergology starting from the disease-causing allergen molecules, the allergen-specific immune responses *in vitro* and *in vivo*, to the clinical application and thereby guarantee an educational program spanning the entire field of allergology. The PhD program MCCA is therefore unique in its conception and structure and addresses a central aspect of health care, as almost 50% of the population is sensitized to at least one allergen and at least 30% suffer from a clinically manifest allergy. Due to the active research activities, among others in the DK-MCCA, the Center for World University Rankings (CWUR) has ranked the Medical University of Vienna in its latest subject ranking in the field of allergology among the top 10 universities worldwide, in fact on the outstanding 7th place! Furthermore, the Medical University of Vienna was selected by the Excellence Selection Committee of the World Allergy Organization (WAO) as one of the 30 WAO Centers of Excellence, this distinction was renewed again only recently.

In its first period from 2013-2020 the DK-MCCA has been generously supported by the Austrian Science Fund (FWF), the Medical University of Vienna and the Veterinary University of Vienna. In 2020, the DK-MCCA became part of a larger research network, the Danube Allergy Research Cluster (Danube ARC), which is generously supported by the Federal Government of Lower Austria. Within this framework, the DK-MCCA teams up with research institutions in the larger "Danube Region" in Lower Austria, i.e., AIT: Austrian Institute of Technology, Tulln; Department of Agrobiotechnology, IFA-Tulln; Karl Landsteiner University of Health Sciences, Krems, St. Pölten, Tulln). Along those lines, the DK-MCCA and its members contribute to sustainable excellence in allergy research at the Medical University of Vienna and its partner institutions in Vienna and Lower Austria, where allergic diseases have been a central field of research.

Since 2013, the DK-MCCA enrolled and educated 72 PhD Students (53 female, 19 male) from 26 different countries from all over the world (Austria: 24; EU: 17; non-EU: 31), 26 directly financed by FWF, 19 paid by the generous support of the Medical University of Vienna, 10 financed by peer-reviewed third party funds and, recently, 17 funded by the Danube ARC program. Seven of the 72 PhD Students held an MD degree when entering the DK-MCCA. The international recruitment in the form of concerted international calls was only possible due to the generous funding provided and has now become a best practice tool at the Medical University of Vienna. Until today, 35 students have already finished their PhD, while 32 are still working on their thesis and only 5 students changed their career path. Of the PhD Students who finished, the vast majority continued their scientific career (mostly in academia but also in industry) while a few followed a management career in the pharmaceutical industry.

Since August 2020, our students within the Danube Allergy Research Cluster (Danube ARC) published 97 peer reviewed research papers, with a considerable number of them in the highly-prestigious journals of the discipline amounting to an overall impact factor of 904.9 (average IF 9.3 per publication), with 32% of publications having an impact factor >13.

Besides the ambitious and intensive training program, the PhD-program MCCA offers a personalized mentoring-environment for the individual students, which relies on intra- and extramural scientists and members of the international scientific advisory board (ISAB) providing support, advice as well as further career planning. Along those lines, 30+ PhD-Students already have made 'stays-abroad' and were committed by the MCCA program to top-notch international research laboratories to perform complementary experiments within the framework of their research projects.

The Karl Landsteiner Symposium, under the auspices of the Danube Allergy Research Cluster (Danube ARC), provides an excellent opportunity for our PhD Students to meet and interact with top-notch scientists who have contributed significantly to our better understanding of the biology of B cells, their beneficial and pathological potential, and the ways to modulate and manipulate their function for the well-being of patients suffering from B-cell-driven diseases.

My deep gratitude goes to all PhD Students, Faculty Members, Guest Speakers, companies, institutions and funding bodies, who made the Karl Landsteiner Symposium on *'From B cells to antibodies'* a reality. I personally wish all participants a most memorable scientific meeting, lots of new ideas and insights for your ongoing and future research as well as best personal experiences.

Univ. Prof. Dr. Winfried F. PICKL, MD
(DK MCCA-Speaker)

WELCOME ON BEHALF OF THE RESEARCH PLATFORM TRANSPLANTATION (RPT)

We warmly welcome you to the first joint meeting of the Research Platform Transplantation and the Immunology Research Cluster of the MedUni Vienna.

While transplantation research spans a wide range of fields and topics, immunology has always taken a central position. Immunological topics not only dominate basic and translational research efforts in transplantation, but also play a crucial part in clinical transplantation medicine. Thus, it is only natural that the two fields come together in a joint meeting.

We hope you enjoy the presentations and discussions and wish you a fruitful meeting!

Yours sincerely,

Univ. Prof. Dr. Rainer Oberbauer, MD, PhD and Univ. Prof. Dr. Thomas Wekerle, MD
(Coordinators, Research Platform Transplantation)

Karl Landsteiner Symposium: From B cells to antibodies

PROGRAM

Monday: July 10, 2023

Venue of the Symposium: Luberegg Castle, Luberegg 18,
3644 Emmersdorf on the Danube, Lower Austria; Seminar Hall Kaiser Saal
(www.pichler-wachau.com/luberegg/schloss-uebersicht-wachau.html)

	Bus Transfer from Hotel Regina, 1090 Vienna to Luberegg Castle (Symposium Venue)
07:15	Departure: Hotel Regina, Rooseveltplatz 15, 1090 Vienna
08:45 – 09:00	Arrival at Symposium Venue Luberegg Castle Luberegg 18, 3644 Emmersdorf an der Donau, Lower Austria, Austria *(early check-in Landhotel Wachau & Luberegg Castle; Luggage for hotel guests at Hotel Donauhof & Pritz can be taken to the respective hotels after disembarking at Luberegg Castle)*
09:30 – 10:00	Registration and Poster Mounting
10:00 – 10:30	Opening of the event: **Rudolf Valenta and Winfried Pickl,** Danube Allergy Research Cluster and Medical University of Vienna **Alexander Auer, Martina Höllbacher,** Department for Science and Research, Federal Government of Lower Austria **Rudolf Mallinger, Sabine Siegl,** Karl Landsteiner University, Krems, Lower Austria **Wolfram Hoetzenecker,** Austrian Society for Allergology and Immunology, University of Linz, Upper Austria
Session 1 Chairs: Aleksandra Podzhilkova and Maja Bajic	
10:30 – 11:15	**Keynote Lecture 1:** **Max D. Cooper, MD,** Georgia Research Alliance, Professor of Pathology and Laboratory Medicine, Member of the Vaccine Center at the Emory University School of Medicine, Atlanta, GA, USA *Evolution of adaptive immunity in vertebrates.*
11:15 – 11:30	**Short Presentation 1: Katarzyna Niespodziana.** *Antibodies specific for the membrane-proximal site of ICAM-1 inhibit rhinovirus infection.*
11:30 – 11:45	**Short Presentation 2: Tanja Schwickert.** *The essential role of Ikaros and Aiolos in plasma cell differentiation and homeostasis.*
11:45 – 12:00	**Short Presentation 3: Inna Tulaeva.** *Vaccination with recombinant grass pollen allergy vaccine BM325 is safe and induces protective Phl p 5-specific immune response in non-allergic subjects.*
12:00 – 13:30	Lunch Break: 'Students meet Professors'
13:30 – 15:00	Guided Poster Sessions *(for poster chair details please refer to pages 31ff)*
Session 2 Chairs: Zicheng Liu and Daria Trifonova	
15:00 – 16:00	**Keynote Lecture 2:** **Jacques Banchereau, PhD,** Professor of Immunological Sciences, Chief Scientific Officer at Immunai, New York, NY, USA *Four decades of wandering with her Highness: The B cell and her courtesans: T cells, DCs, macrophages and cytokines.*
16:00 – 16:45	**Keynote Lecture 3:** **Virginia Pascual, MD,** Ronay Menschel Professor of Pediatrics, Weill Cornell Medical College, Director, Drukier Institute for Children's Health Weill Cornell Medical College, New York, NY, USA *Follicular and extra-follicular B cells in childhood-onset autoimmunity.*
16:45 – 17:00	**Short Presentation 4: Nishelle Dsouza.** *Recombinant Blomia tropicalis allergens allow differential diagnosis of genuine IgE sensitization to Blomia tropicalis in allergic patients.*
17:00 – 17:30	Coffee break

Karl Landsteiner Symposium: From B cells to antibodies

PROGRAM

Monday: July 10, 2023

	Session 3 Chairs: Tianchi Jiang and Hilal Demir
17:30 – 18:15	**Keynote Lecture 4:** **Rita Carsetti, MD,** Professor of General Pathology and Laboratory Methods, Ospedale Pediatrico Bambino Gesù, Division of Immunology, Rome, Italy *Human B Cell memory in health and disease.*
18:15 – 18:30	**Short Presentation 5: Armin Kraus.** *Investigation of mechanisms for binding, uptake and processing of virus-like nanoparticles (VNP) by antigen presenting cells (APC).*
18:30 – 18:45	**Short Presentation 6: Lisa Prickler.** *Transfer of allergen-expressing immune cells for tolerance induction in grass pollen allergy.*
18:45 – 19:00	**Short Presentation 7: Lena D. Roßbacher.** *Differences in cross-reactive IgG responses after flavivirus infections.*
	Bus Transfer from Luberegg Castle to Vienna
19:30	Departure: Luberegg Castle (event location: Bus Parking Area P3; *will stop on its way to Vienna at Hotels Donauhof and Pritz*)
19:30	**Gala Dinner in Restaurant Luberegg Castle**
	Bus Transfer from Luberegg Castle to Vienna
approximately 22:45	Departure: Luberegg Castle (event location: Bus Parking Area P3; *will stop on its way to Vienna at Hotels Donauhof and Pritz*)

KARL LANDSTEINER UNIVERSITY OF HEALTH SCIENCES

WISSENSCHAFT · FORSCHUNG NIEDERÖSTERREICH

MEDICAL UNIVERSITY OF VIENNA

DANUBE ALLERGY RESEARCH CLUSTER

cellular molecular MCCA clinical allergology

Karl Landsteiner Symposium: From B cells to antibodies

PROGRAM

Tuesday: July 11, 2023

07:15	**Bus Transfer from Hotel Regina, 1090 Vienna to Luberegg Castle** (Symposium Venue)

Session 4 Chairs: Mohammed Zghaebi and Lisa Prickler	
09:00 – 09:45	**Keynote Lecture 5:** **Meinrad Busslinger, PhD,** Professor of Molecular Genetics, Deputy Director Science, Research Institute of Molecular Pathology (IMP), Campus-Vienna-Biocenter 1, Vienna, Austria *Generation of a broad antibody repertoire by cohesin-mediated control of V (D) J recombination.*
09:45 – 10:30	**Keynote Lecture 6:** **Facundo Batista, PhD,** Phillip T. and Susan M. Ragon Professor of Microbiology and Immunology, Harvard Medical School, Associate Director and Scientific Director Ragon Institute of Mass General, MIT and Harvard, Cambridge, MA, USA *Informing vaccine design by defining the rules of antibody responses.*
10:30 – 11:15	**Keynote Lecture 7:** **Andreas Radbruch, PhD,** Professor of Experimental Rheumatology, Scientific Coordinator Deutsches Rheuma-Forschungszentrum (DRFZ), A Leibniz Institute, Berlin, Germany *Memory B and plasma cells of the bone marrow.*
11:15 – 12:00	**Keynote Lecture 8:** **Marta Rizzi, MD, PhD,** Professor of Clinical and Experimental Immunology, Institute of Immunology, Medical University of Vienna, Vienna, Austria *B cell fate decisions in Autoimmune Lymphoproliferative Syndrome (ALPS): a FAScinating story.*
12:00 – 12:15	**Short Presentation 8: Joshua Tobias.** *From antibodies to the clinical development of several B-cell peptide-based vaccine candidates.*
12:15 – 12:30	**Short Presentation 9: Srinidhi Sudharson.** *Transcriptome analysis reveals the nasal provocation–associated cellular responses in birch pollen allergic and non-allergic subjects.*
12:30 – 12:45	**Short Presentation 10: Franziska Schmidt.** *Dysregulation of the B cell compartment and low BAFF-R in AAV patients.*
12:45 – 14:30	Lunch Break

Session 5 Chairs: Varsha Dwivedi and Eszter Sarzsinszky	
14:30– 14:45	**Short Presentation 11: Mohammed Zghaebi.** *Controlled nasal allergen exposure in allergic patients expands IgE+ plasmablasts expressing pre-existing IgE VH regions.*
14:45 – 15:00	**Short Presentation 12: Angelika Wagner.** *Two-year follow-up of immune responses to primary and booster vaccination with COVID-19 mRNA vaccines in immunocompromised individuals.*
15:00 – 15:15	**Short Presentation 13: Daria Trifonova.** *Novel recombinant hypoallergenic cat allergy vaccine for immunotherapy.*
15:15 – 16:00	**Keynote Lecture 9:** **Winfried F. Pickl, MD,** Professor of Translational Immunology, Institute of Immunology, Medical University of Vienna, Vienna, Austria *Karl Landsteiner (1868-1943) and his major contributions to Immunology and Immunohematology.*
16:00 – 16:30	**Closing ceremony with awards to for best poster and oral presentations as well as appreciation awards for invited speakers**
16:30 – 17:00	Farewell

	Bus Transfer from Luberegg Castle to Vienna
17:30	Departure: Luberegg Castle (event location: Bus Parking Area P3; *will stop on its way to Vienna at Hotels Donauhof and Pritz*)

Retreat of the Immunology Research Cluster and the Research Platform Transplantation of the Medical University of Vienna

PROGRAM

Wednesday: July 12, 2023

Venue: Luberegg Castle, Seminar Hall Kaiser Saal

07:15	**Bus Transfer from Hotel Regina, 1090 Vienna to Luberegg Castle** (Symposium Venue)
08:30 – 08:55	Registration and Poster Mounting
08:55 – 09:00	Introduction of Program and Guests: **Rudolf Valenta (IRC) and Thomas Wekerle (RPT)**
Session 1 Chairs: Ursula Wiedermann and Gregor Bond	
09:00 – 09:20	**Keynote Lecture 1:** **Thomas Wekerle, MD,** Professor of Transplantation Immunology, Department of General Surgery, Medical University of Vienna, Vienna, Austria *Transplantation tolerance through combined cell therapy.*
09:20 – 09:40	**Keynote Lecture 2:** **Wilfried Ellmeier, PhD,** Professor of Immunobiology, Institute of Immunology, Medical University of Vienna, Vienna, Austria *Histone deacetylases and the control of CD4+ T cell-mediated immunity.*
09:40 – 10:00	**Keynote Lecture 3:** **Gregor Bond, MD, PhD,** Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria *Torque Teno virus - on the quest for an immunometer.*
10:00 – 10:10	**Short Presentation 1: Paul Ettel.** *MacDeath: A cell death screen identifies novel macrophage-depleting agents for the therapy of macrophage activation syndrome and cancer.*
10:10 – 10:20	**Short Presentation 2: Tobias Frey.** *The adaptor protein TRAT1 modulates Th17 and Treg effector functions.*
10:20 – 10:30	**Short Presentation 3: Moritz Muckenhuber.** *Bone marrow resident long-lived plasma cells as source of chronic DAS.*
10:30 – 10:40	**Short Presentation 4: Andreas Heinzel.** *Changes of the TCRβ repertoire after CMV infection in CMV naive kidney transplant recipients.*
10:40 – 10:50	**Short Presentation 5: Małgorzata S. Małys.** *Small extracellular vesicles (sEV) are released ex vivo from residual leukocytes and platelets remaining in plasma.*
10:50 – 11:00	**Short Presentation 6: Vanessa Mühlgrabner.** *How differential TCR-engagement triggers differential T-cell activation – a molecular imaging approach.*
11:00 – 13:00	Guided poster session and lunch break
Session 2 Chairs: Sylvia Knapp and Hannes Vietzen	
13:00 – 13:10	**Short Presentation 7: Michael Bergmann.** *FasL- induced cell death and inflammation contribute to disease severity and lethality in a new mouse-adapted SARS-CoV-2 model.*
13:10 – 13:20	**Short Presentation 8: Alina Fokina.** *The impact of maternal SARS-CoV-2 infection on offspring immunity.*
13:20 – 13:30	**Short Presentation 9: Laura Gebetsberger.** *The role of host cellular proteins in SARS-CoV-2 innate immune evasion.*
13:30 – 13:40	**Short Presentation 10: Pia Gattinger.** *Better induction of broadly neutralizing antibodies with recombinant fusion protein combining HBV PreS with wildtype and omicron-derived RBD than with strain-specific fusion proteins.*

Retreat of the Immunology Research Cluster and the Research Platform Transplantation of the Medical University of Vienna

PROGRAM

Wednesday: July 12, 2023

13:40 – 13:50	**Short Presentation 11: Thomas Vogl.** *Deciphering human antibody repertoires against the microbiome in health and disease.*
13:50 – 14:00	**Short Presentation 12: Michael Thaler.** *The role of Schistosoma mansoni cysteine proteases in the modulation of the immune system.*
14:00 – 14:20	**Keynote Lecture 4:** **Sylvia Knapp, MD, PhD,** Professor of Infection Biology, Department of Internal Medicine I, Medical University of Vienna, Vienna, Austria *Determinants of pulmonary immune homeostasis.*
14:20 – 14:40	**Keynote Lecture 5:** **Andreas Heinzel, MSc,** Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria *Non-HLA immunity.*
14:40 – 15:00	**Keynote Lecture 6:** **Hannes Vietzen, PhD,** Center for Virology, Medical University of Vienna, Vienna, Austria *(HCMV-specific) Natural Killer Cells: Friend or Foe in Lung Transplant Recipients?*
15:00 – 15:30	Coffee break
Session 3 Chairs: Sylvia Knapp and Hannes Vietzen	
15:30 – 15:50	**Keynote Lecture 7:** **Talin Barisani-Asenbauer, MD,** Professor of Tropical Medicine, Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Vienna, Austria *New insights into immunological-based approaches for ocular surface diseases.*
15:50 – 16:10	**Keynote Lecture 8:** **Ursula Wiedermann, MD, PhD,** Professor of Vaccinology, Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Vienna, Austria *Primary and booster vaccine responses in immunocompromised patients.*
	Bus Transfer from Luberegg Castle to Vienna
17:00	Departure: Luberegg Castle Bus #1 (event location: Bus Parking Area P3; *will stop on its way to Vienna at Hotels Donauhof and Pritz*)
	Farewell (at own expense): Bus Transfer from Luberegg to Vine Tavern Flößerei, Rossatz 186, 3602 Rossatz
17:00	Departure: Luberegg Castle Bus #2 (event location: Bus Parking Area P3; *will stop on its way to Flößerei at Hotels Donauhof and Pritz);* *Return from Rossatz for Vienna, Hotel Regina is planned for 21:30 (arrival planned for 22:45).*

RESEARCH PLATFORM TRANSPLANTATION MEDICAL UNIVERSITY OF VIENNA

IMMUNOLOGY RESEARCH CLUSTER MEDICAL UNIVERSITY OF VIENNA

KEYNOTE SPEAKERS DANUBE ARC

Jacques Banchereau, PhD

Jacques Banchereau is a human immunologist with more than four decades of experience in discovery and translational science in both the pharmaceutical industry and academia.

After graduating as a pharmacist from the University of Angers, France, Jacques transferred to the University of Paris, earning certification in pharmacology and immunology, as well as clinical biochemistry, microbiology, and parasitology, before completing a Ph.D. in biochemistry at the same institution. Jacques then joined the Schering Plough's Laboratory for Immunology Research near Lyon, France and went on to become its Director for 12 years, a career that expanded the understanding of the biology of cytokines and explored their therapeutic potential. His research group studied the biology of recently identified human cytokines and was the first to discover how to grow dendritic cells (DCs), and one of the first to develop human monoclonal antibodies using novel *in vitro* strategies to grow human B cells.

Jacques next became the Founding Director of the Baylor Institute for Immunology Research located in Dallas, TX and held the W.W. Carruth Chair for Transplantation Immunology Research. While at Baylor, he was appointed director of the first INSERM unit in the United States, the French National Institute for Health and Medical Research.

Jacques moved on to serve as Senior Vice President, Head of Inflammation & Virology and Chief Science Officer of the Roche Nutley site, where he oversaw research and early development up to late-stage development in the fields of inflammation and virology, tackling autoimmune diseases, chronic pulmonary disease, as well as infectious diseases including hepatitis B and C, and influenza.

Jacques subsequently served as Deputy Director of the Jackson Laboratory of Genomic Medicine (JAX-GM) and as a Professor and Director of Immunological Sciences at The Jackson Laboratory. There, he developed studies to monitor the immune system throughout the life span, from infants to elderly individuals, in the steady state and upon perturbations with vaccines or infections.

Currently, as Chief Scientific Officer of a biotech company, Immunai, Jacques guides its team of experts in genomics, bioinformatics, immunology, and software engineering, who use single-cell genomics and machine learning approaches to discover and develop novel therapeutics that could reprogram the immune system.

Jacques has made significant contributions to our understanding of human immunology through the identification and characterization of novel interleukins (GM-CSF, IL-4, IL-5, IL-10, IL-13 and IL-17), studies on B and T lymphocytes, and in particular DCs, the main initiators and regulators of immune responses. He also developed the use of DC-based immunotherapy in patients suffering from cancer as well as HIV infection. Jacques also dedicated much effort to the understanding of autoimmune diseases, in close collaboration with Dr. Pascual, with a focus on lupus, where they found the critical role of Type I Interferon, which is now an FDA-approved target. Another important contribution of Jacques', together with Dr. Pascual, was the discovery of the pathophysiological role of IL-1 in the Systemic Onset Juvenile Arthritis, leading to a therapy that controlled the disease in three quarters of the patients. Jacques has been very active in the field of infectious diseases including influenza, RSV, HIV and SARS-CoV-2 infections and response to vaccines.

Over his career, Jacques has mentored over 70 students and trainees, many of them being eminent immunologists in their own right today. He is the inventor or co-inventor of over 40 patents, has authored over 400 peer-reviewed scientific articles, and more than 250 book chapters and reviews. Jacques' work has been cited more than 135,000 times, giving him an h-index of 166. He has been an invited speaker at more than 300 international meetings and conferences. He has held a Board position in several biotechnology companies and academic institutions.

During his academic career, Jacques was awarded numerous grants from the NIH (National Institutes of Health) and private foundations, and in 2001 he was awarded a contract with DARPA (Defense Advanced Research Projects Agency). In 2009, Jacques was honored with the Dana Foundation Award for Human Immunology Research (now the AAI-Steinman Award) from the American Association of Immunologists for significant and sustained achievement in immunology pertinent to human disease, pathogenesis, prevention, and therapy.

Facundo D. Batista, PhD

Facundo D. Batista earned his PhD in Immunology in 1995 from the International School of Advanced Studies in Trieste, Italy, after completing his undergraduate studies at the University of Buenos Aires, Argentina. From 1996–2001 he was an European Molecular Biology Organization (EMBO) Postdoctoral Fellow at the MRC Laboratory of Molecular Biology of Cambridge University. He first became a Group Leader at the London Research Institute, Cancer Research UK (now a part of the Francis Crick Institute) in 2002 and was tenured in 2006; during his time at the LRI, he was also a Professor at Imperial College, London. In 2016, he joined The Ragon Institute of Mass General, MIT, and Harvard as its first Associate and Scientific Director, and joined Harvard Medical School as the Philip T. and Susan M. Ragon Professor of Microbiology and Immunology.

His scientific career has focused on unraveling the molecular and cellular events underlying B cell activation, fate determination and antibody production in response to immune challenges. Over the course of more than 25 years and more than 100 research articles, his lab has made major contributions to the field of B cell biology: (i) establishing the identity of the B cell synapse and a mechanism for antigen extraction; (ii) identifying key features of B cell receptor (BCR) and co- receptor organization; (iii) determining dynamics and signaling mechanisms that tune BCR function; and (iv) elucidating the central principles that drive *in vivo* B cell immune responses. Recently, he has developed a new approach to mouse model generation that now forms the basis of preclinical vaccine research for diseases including malaria, HIV, and influenza.

Key Service

Prof. Batista is the co-lead PI of the Scripps Consortium for HIV/AIDS Vaccine Development (CHAVD) Scientific Research Support Unit. He served on the editorial board of *Science* for seven years and has participated in numerous other editorial boards; he is currently Chief Editor for *The EMBO Journal*. He has chaired or organized a number of major conferences; most recently, he organized the 2023 Keystone Symposium, "B Cell Biology in the Context of Infectious Diseases, Autoimmunity and B Cell Cancers / HIV Vaccines, Immunoprophylaxis and Drugs", and chaired the 2022 Immunochemistry & Immunobiology Gordon Research Conference.

Major Awards and Honors

Facundo D. Batista is an elected member or fellow of the American Academy of Microbiology, the Academia de Ciencias de América Latina (ACAL), the UK Academy of Medical Science, and the European Molecular Biology Organization (EMBO). He is a recipient of the Royal Society Wolfson Research Merit and EMBO Young Investigator Awards.

Webpage: https://ragoninstitute.org/batista/

Full List of Publications: https://www.ncbi.nlm.nih.gov/pubmed/?term=facundo+d+batista

Meinrad Busslinger, PhD

Meinrad Busslinger studied biochemistry at the ETH Zürich and obtained a doctoral degree in molecular biology from the University of Zürich. Following postdoctoral studies at the MRC Institute Mill Hill, London, he became a group leader at the University of Zürich. In 1987, he followed Max Birnstiel as a Senior Group Leader to the newly founded IMP in Vienna. Busslinger is Director of Academic Affairs at the IMP, and since 2013 also Scientific Deputy Director of the institute. He is a Professor at the University of Vienna as well as a member of the Austrian Academy of Sciences and the European Molecular Biology Organization. He has published over 190 papers in peer-reviewed journals, served on different scientific advisory boards as well as on editorial boards of several scientific journals.

Scientific achievements

Meinrad Busslinger is known for his work in the field of molecular immunology with a strong focus on the transcriptional control of B lymphopoiesis. He discovered and characterized the transcription factor Pax5. Gene inactivation experiments in the mouse demonstrated that Pax5 controls all aspects of B cell immunity, as it is essential for B-lineage commitment as well as the generation of all mature B cell types. Notably, Pax5 is also required for V_H-DJ_H recombination by controlling chromatin loop extrusion across the 2.8-Mb long *Igh* locus in pro-B cells. It does so by downregulating the expression of the cohesin-release factor Wapl, which in turn leads to extended loop extrusion across the entire *Igh* locus. As a consequence, all VH genes can participate in V_H-DJ_H recombination, thus leading to a broad antibody repertoire able to fight all possible pathogens. Notably, the Busslinger group identified Pax5 as a tumor suppressor in the mouse and deciphered its role in B-cell acute lymphoblastic leukemia (B-ALL) by generating and characterizing mouse models for *PAX5* translocations occurring in human B-ALL. More recently, the Busslinger group, together with collaborators at the Erasmus Medical Center (Rotterdam), could demonstrate that mutations in *PAX5* are causally involved in the generation of autism spectrum disorder in humans.

By using conditional mutagenesis in the mouse, the Busslinger group identified the molecular functions of other transcription factors in regulating important aspects of B cell development, such as Ebf1 and E2A in the control of early and late B cell development, Bhlhe41 in the regulation of the innate B-1 cell lineage, Blimp1 in plasma cell differentiation, and Ikaros in the control of pre-BCR signaling in early B cells and in the suppression of autoimmunity in mature B cells. In summary, Meinrad Busslinger made major contributions to our current understanding of the role of Pax5 in B cell commitment, immunity and cancer and the function of other key regulators of B lymphopoiesis and autoimmunity and also discovered how V(D)J recombination is controlled by cohesin-mediated spatial regulation of the *Igh* locus.

Awards

Busslinger received two ERC Advanced Grants and was awarded the Wittgenstein prize of the Austrian Government in 2001, the Virchow Medal by the University of Würzburg in 2010 and the Prize of the City of Vienna for Natural Sciences in 2020 and is an honorary member of the Swiss Society for Allergology and Immunology since 2015.

Rita Carsetti, MD

Rita Carsetti studied Medicine at the University La Sapienza in Rome (Italy) and then was trained in Obstetrics and Gynecology. During the studies and more clearly when she was a post-Doctoral fellow in the United States, at the M.D. Anderson Hospital in Houston (Texas), she realized that science was her real interest. For this reason, she came back to Europe for a second post-Doc at the Max-Plank Institute for Immunobiology in Freiburg (Germany), in the department of Georges Koehler, who had received the Nobel price for the discovery of monoclonal antibodies. She spent 13 years in Germany working on the basic mechanisms of molecular immunology. After the post-Doc, she first became responsible for the Cytofluorimetry Facility at the MPI and then group leader. At the end of the year 2000, she went back to Rome, to the Bambino Gesù Children Hospital. With a strong background in basic immunology of murine B cells, she moved to a more patient-orientated research approach. Thanks to the close contact to clinicians and her role as head of the Diagnostic Immunology Unit, Rita Carsetti investigated how human B cells change with age, immunodeficiency, infection, transplantation, and vaccination.

The COVID-19 pandemic and the global vaccination campaign gave her the possibility to learn more about the development, function, and persistence of human memory B cells after vaccination in healthy subjects and patients with primary or secondary immunodeficiencies. She also demonstrated a novel mechanism by which breastfeeding actively instructs and prepare neonatal immunity.

Throughout her career, Rita Carsetti has made a number of important discoveries regarding the development of human and murine B cell populations and the effect changes in such populations have on infection and immunodeficiency in humans. Her recent work on SARS-CoV-2 and RNA vaccination has been vital in demonstrating how human memory B cells aid immune protection and how mucosal immunity is induced in neonates born to SARS-CoV-2 infected mothers. Altogether, Rita Carsetti's work is extremely relevant to both basic and clinical immunologists.

Other Scientific Activities

2012-date	Member SIICA
2021-date	SIICA Steering Committee
2016-date	Member ISCCA and ESCCA
2014-date	Advisory Board Centre International de Recherche en Infectiologie, Lyon, France
2012-date	Edit. Board, Frontiers in Mucosal Immunology
2015-date	Edit. Board Frontiers in Pediatrics
2020-date	Edit. Board Immunotherapy Advances
2021-date	Edit. Board Frontiers in Immunology
2022-date	Edit.Board Frontiers in Medicine
2019-date	Vice Chair Vaccine Committee IUIS
2019-date	Chair Pub Committee IUIS
2017-date	Founder European B cell network ECB net
2021-date	Chair of the Committee for Gender quality SIICA
2022-date	Delegate of SIICA to the EFIS Diversity Task Force
2022-date	Member of Austrian Society of Vaccinology

Max D. Cooper, MD

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 antibody class switching by 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.

Key Memberships/Offices in National and International Societies

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, National Academy of Medicine, American Academy of Arts and Sciences, a foreign member of the French Academy of Sciences and the Royal Society of London.

Professional Awards and Honors

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), Robert Koch Prize (2010), AAI Excellence in Mentoring Award (2012), Japan Prize (2018), Albert Lasker Basic Medical Research Award (2019), and National Academy of Inventors Fellow (2021).

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/max.cooper.1/bibliography/40464297/public/?sort=date&direction=descending

Maria Virginia Pascual, MD

I am a pediatric rheumatologist with long standing experience in clinical, basic and translational research related to inflammatory and autoimmune diseases. My laboratory is focused on understanding the pathogenesis, finding biomarkers to guide therapeutic interventions and identifying therapeutic targets for diseases such as Systemic Lupus Erythematosus (SLE), Juvenile Dermatomyositis (JDM), and various forms of Arthritis that appear in childhood. I am also interested in elucidating immune responses to a broad variety of infections and vaccinations, especially in infants and children. I am currently the Program Director of an NIAID-funded Autoimmunity Center of Excellence and a NIAMS-funded Center for Lupus Research. Pioneering studies from my laboratory identified the role of dendritic cells and Interferon in SLE, and of cytokines such as IL-1 in Systemic-onset Juvenile Arthritis, which have led to successful therapeutic interventions in these diseases. We also developed a personalized approach to identifying molecular drivers of disease activity in pediatric SLE patients. This approach permitted us to stratify patients into major molecular subgroups towards improved design of SLE clinical trials. My team also identified endogenous (mitochondrial) nucleic acids extruded by neutrophils or retained by red blood cells as potent activators of the immune system in SLE. Finally, we have reported the expansion of an unconventional $CD4^+$ T helper cell population in the blood and kidney of SLE patients that lacks follicular markers and helps B cells through a cytokine program distinct from those of follicular helper (Tfh) and peripheral helper (Tph) cells.

Complete List of Published Work in My Bibliography:
https://pubmed.ncbi.nlm.nih.gov/?term=Maria%20Pascual&sort=date&ac=yes

Winfried F. Pickl, MD

Graduated from the Medical University of Vienna as M.D., he is board certified immunologist, professor for translational immunology at the Medical University of Vienna and guest professor at the Karl Landsteiner University, Krems, head of the Division of Cellular Immunology and Immunohematology and the Immunodiagnostic Laboratory at the Institute of Immunology, Medical University of Vienna, Austria. Dr. Pickl received postdoctoral training in immunology (Walter Knapp, Vienna) and molecular biology (Brian Seed, Boston, MA). He has critically contributed to the molecular and functional characterization of T cell activation antigens and monocyte-derived dendritic cells. More recently he has centered his scientific interests towards the better definition of the immunological synapse. Hence, he and his group have created novel forms of antigen-presenting platforms based on virus-like nanoparticles (immunosomes, VNP) and novel, human-relevant model systems for allergy research based on humanized double transgenic mice (human TCR and HLA). Dr. Pickl is now seeking to develop novel forms of allergy and SARS-CoV-2 treatment. Winfried Pickls work is highly cited (h-index: 52, citations: 8.811 according to Scopus, 21/05/2023), he has published more than 190 original scientific publications, reviews and book chapters.

Key Memberships/Offices in National and International Societies

Winfried Pickl serves as member of the Clinical Immunology and the Education Committee of the Austrian Society for Allergology and Immunology, ÖGAI (www.oegai.org/oegai), is Steering Member of the Vaccine Committee (VAC) of the International Union of Immunological Societies, IUIS (www.iuisonline.org), and treasurer (term 2015-2024) of the European Federation of Immunological Societies, EFIS (www.efis.org). Moreover, Dr. Pickl was president of the Austrian Society for Allergology and Immunology (ÖGAI, term 2014-2016; www.oegai.org) and Congress President of the 4th European Congress of Immunology Vienna (www.eci-vienna2015.org).

Most Important Scientific/Scholarly Research Achievements

Since 2013, Winfried Pickl is speaker of the PhD-program *Molecular, Cellular and Clinical Allergology, MCCA* (www.phd-mcca.at), which is currently funded by the State of Lower Austria (www.kl.ac.at/en/university/scientific-organisational-units/danube-arc-danube-allergy-research-cluster).

Professional Awards and Honors

Karl Landsteiner Award of the Austrian Society for Allergology and Immunology 2007.

Links:

www.phd-mcca.at

https://immunologie.meduniwien.ac.at

www.kl.ac.at/en/university/scientific-organisational-units/danube-arc-danube-allergy-research-cluster

https://pubmed.ncbi.nlm.nih.gov/?term=pickl+w&sort=date

www.efis.org

www.scopus.com/results/authorNamesList.uri?st1=Pickl&st2=&origin=searchauthorlookup

Andreas Radbruch, PhD

A biologist by education, Andreas Radbruch has focussed his scientific curiosity on the immune system, and in particular immunological memory, and the way it provides immunity and generates immunopathology.

Andreas Radbruch obtained his PhD at the Genetics Institute of the Cologne University, Germany, with Klaus Rajewsky in 1980. He later became Associate Professor there and was a visiting scientist with Max Cooper and John Kearney at the University of Alabama, Birmingham. In 1996, he became Director of the German Rheumatism Research Center in Berlin, now a Leibniz Institute, and in 1998, Professor of Rheumatology at the Charité Medical Center and Humboldt University of Berlin.

Andreas Radbruch has developed a line of research aiming at a cellular and molecular understanding of immune reactions and immunological memory. His research approach is based on the analysis of individual cells, developing and using cutting-edge technologies for cytometry and cell sorting. His lab developed the MACS technology, cytokine cytometry, the cytometric secretion assay, magnetofluorescent liposomes and other tools to analyze fate decisions and imprinting of lymphocytes. He initially focused on the transcriptional regulation of antibody class switch recombination, the shaping of antibody specificity by somatic mutation in B lymphocytes, and the epigenetic imprinting of cytokine gene expression in T lymphocytes. The discovery of long-lived (memory) plasma cells in 1997 initiated a line of research that so far has generated a new understanding of immunological memory, as maintained by functionally imprinted memory plasma cells, and memory B and T lymphocytes, which, as this group has shown, individually reside, rest and survive in niches provided by mesenchymal stromal cells, mostly in the bone marrow, but also in other tissues. Of clinical relevance, memory plasma cells secreting pathogenic antibodies have been recognized as being refractory to conventional therapies and a critical and so far unmet therapeutic target in antibody-mediated diseases. The same is true for memory T lymphocytes driving chronic inflammation, for which the group has identified molecular adaptations and novel therapeutic targets, like the transcription factor Twist1, which dampens pathogenicity and promotes persistence of Th lymphocytes in chronic inflammation.

Andreas Radbruch has received various scientific awards and honors, the Carol Nachman Prize for Rheumatology (2011), an Advanced Grant of the European Research Council (ERC, 2011), and the Avery Landsteiner Award (2014). He is a member of the Berlin Brandenburg Academy of Sciences, the European Molecular Biology Organization (EMBO) and the Leopoldina, the German National Academy of Sciences. He has been president of the International Society for the Advancement of Cytometry (ISAC; 2014-2016), of the German Societies for Immunology (2009-2010) and for Rheumatology (2007-2008), and of the European Federation of Immunological Societies (EFIS) (2019 -2021).

Marta Rizzi, MD, PhD

After studying Medicine at the University of Genoa/Italy, Marta Rizzi completed her PhD in Clinical and Experimental Immunology in Genoa/Italy and San Diego/California in 2004. In 2007, she completed her residency training in Allergology and Clinical Immunology. Since 2006, she has been conducting research at the University Medical Center Freiburg - initially as a postdoctoral fellow, and later as head of a research group since 2014. In 2014, she was awarded the prestigious Margarete von Wrangell fellowship.

Since 2022 she is Professor of Clinical and Experimental Immunology at the Medical University of Vienna.

She has been awarded the prestigious Heisenberg Professorship of the German Research foundation (DFG) and since 2023 she is also Professor for Experimental and Translational Rheumatology (Heisenberg) at the Faculty of Medicine of the University of Freiburg.

Marta Rizzi research focus is human B-lymphocytes development, maturation and activation in physiological and pathological conditions. She has developed unique expertise in *in vitro* modelling of early and late human B cell development, that she uses: to uncover mechanisms of disease in monogenic defects leading to autoimmunity; to study the B cell function in more complex rheumatological diseases; to assess the specific impact on human B lymphocytes of novel targeted therapies.

Marta Rizzi is elected member of the DFG Study group 204 'Microbiology, Virology and Immunology', she is member of the organizing faculty of the European B cell network (EBCnet) school; of the Executive Board Member of Hans Hench Stiftung for rheumatology research; of the selection committee for clinician scientist programs (IMMEDIATE) and of the medical scientist program (Hans A. Krebs) from the Medical Faculty of the University of Freiburg, of the steering committee of the SFB1160 IMPATH and of the SFB/TRR 353 Apoptosis. Between 2014-2023 she has been speaker of the B-cell study group of the German Society of Immunology (DGfI); from 2010-2014 sha has been executive Board Member of European Society of Immunodeficiency (ESID) and Chair of ESID Junior working party.

Talin Barisani, MD

Talin Barisani-Asenbauer has studied Medicine at the University of Vienna. She started her career at the Department of Ophthalmology, University of Vienna, as scientific coworker in 1987; where she thereafter also completed her residency in ophthalmology. In 1997 she became the director of the Uveitis unit and the working group "ocular immunology & infectiology". In 2001 she was awarded the habilitation degree by the University of Vienna, the topic of her habilitation thesis being `Molecular methods to identify microbes in ocular tissues´ and became Fellow of the European Board of Ophthalmology.

In 2009 she succeeded to obtain a Laura Bassi Centre of Expertise grant, enabling her to establish the working group OCUVAC at the Institute of Specific Prophylaxis & Tropical Medicine, Center for Pathophysiology, Infectiology and Immunology. OCUVAC aimed at achieving a multidisciplinary understanding of neglected and orphan ocular infections and inflammations underpinning the more translational research in the center. International cooperations are key to progress in the management of orphan diseases, Talin Barisani-Asenbauer joined several global networks to tackle the puzzle of ocular infections as tuberculosis and herpetic infections. These global efforts resulted in several medical guidelines.

In November 2022 she was appointed as professor in the field of immunology. Within the framework of this professorship it will be a priority to extend the global networks and improve the diagnostics of ocular diseases.

Talin Barisani-Asenbauer occupied several chairwoman positions; she became chairwoman of the Educational Committee of the IOIS in 2011 and in the same year of the Educational Board of the European Board of Ophthalmology. 2010-2019 she acted as Vice-Chairwoman of the Commission for International Ophthalmology of the Austrian Ophthalmology Society (ÖOG) and from 2009 to 2011 and from 2017-2021 Chairwoman of the ÖOG Commission for Ocular Inflammation, infection and allergy. In 2009 she was President of the Viennese Ophthalmological Society and from 2007 to 2011 she was General-Secretary of the International Ocular Inflammation Society-IOIS. Since 2017 she occupies the treasury of the IOIS being responsible for the budget of 4 international ocular inflammation conferences. She represented Austria from 2008 -2016 at the European Board of Ophthalmology (EBO) and at the Union Europeenne des Medecins Specialistes (UEMS).

Her research interests are: translational ophthalmology, rare and neglected ocular diseases, ocular immunology, inflammation & infection, cross-border health care, drug delivery to ocular tissues and ocular vaccine development.

She is member of the Academia-Net, Association for Research in Vision and Ophthalmology (ARVO), American Academy of Ophthalmology (AAO), International Ocular Inflammation Society (IOIS), Austrian Ophthalmology Society (ÖOG) and the Royal Society of Medicine.

Gregor Bond, MD, PhD

Gregor studied medicine at the Medical University Vienna and Louisiana State University. He subsequently served as a post-doctoral fellow at the MRC Centre for Transplantation in London and pursued further studies in clinical study design at the London School of Hygiene and Tropical Medicine. Currently, he holds the position of Associate Professor of Transplant Nephrology and serves as the head of the Doctoral Program for Organ Failure, Replacement, and Transplantation at the Medical University Vienna. Gregor also works as a consultant in Nephrology and Intensive Care Medicine at the Vienna General Hospital.

In addition to his academic and clinical roles, Gregor is an active member of various professional organizations. He is a member of the Council of Medicine and Science for EUROTRANSPLANT, as well as a member of the Infectious Disease Task Force of the European Society of Transplantation.

Gregor's primary scientific focus lies in the personalization of immunosuppressive drugs and the optimization of drug adherence after kidney transplantation. Currently, he is leading a European Union-funded randomized controlled interventional trial that aims to evaluate the potential benefits of using the TT virus to guide immunosuppression. The TT virus, a non-pathogenic and widespread virus, has been found to correlate with the level of immunosuppression in the host.

Outside of his professional endeavours, Gregor enjoys participating in outdoor sports and embarks on mushroom-hunting adventures in the woods with his son.

Wilfried Ellmeier, PhD

Wilfried Ellmeier is Professor of Immunobiology and Head of the Institute of Immunology at the Medical University of Vienna, Austria. He studied biochemistry at the University of Vienna, carried out his doctoral thesis at the Research Institute of Molecular Pathology (IMP Vienna) and performed postdoctoral studies at the Skirball Institute (NYU Medical Center) in New York. His research interests focus on epigenetic and transcriptional control mechanisms that regulate the development and function of T cells. He is also coordinating the Special Research Program (SFB F70) "Histone deacetylases as regulators of T cell-mediated immunity in health and disease", which is a multi-group research network funded by the Austrian Science Fund. He received several prizes including the START program award for highly-qualified young scientists from the Austrian Science Fund and the Novartis award for biology. Wilfried Ellmeier is a corresponding member of the Austrian Academy of Sciences and a board member of the Austrian Science Fund. He is president-elect (2023-2024) of the Austrian Society of Allergology and Immunology (ÖGAI). He also has a strong interest in science policy and served as the President (2020-2021) of the BioMed Alliance in Europe.

Institute: immunologie.meduniwien.ac.at/

Lab homepage: www.meduniwien.ac.at/immunobiology

SFB: www.meduniwien.ac.at/HIT/

Andreas Heinzel, MSc

Andreas Heinzel graduated with a MSc in Bioinformatics from the University of Applied Sciences Upper Austria. Already during his studies, he joined emergentec biodevelopment GmbH in Vienna where he was introduced to the field of immunoinformatics and conceptualized and implemented computational vaccinology workflows. From there Andreas naturally transitioned to the field of biomarker research and pursued the quest for predictive and prognostic biomarkers for diabetic kidney disease in the EU FP7 project SysKid. In 2017, he joined the research group of Rainer Oberbauer at the Medical University of Vienna where he works on prognostic biomarkers for diabetic kidney disease in the IMI2 project BEAt-DKD. His work in the research group of Rainer Oberbauer allowed Andreas to expand into the field of transplant immunology in which both his interests for risk factors for disease and adaptive immunity combine. Andreas current focus lies on non-HLA incompatibility between kidney transplant donors and recipients and its association with long term graft survival.

Selected publications

Heinzel A, Schrezenmeier E, Regele F, et al. Three-Month Follow-Up of Heterologous vs. Homologous Third SARS-CoV-2 Vaccination in Kidney Transplant Recipients: Secondary Analysis of a Randomized Controlled Trial. Front Med (Lausanne). 2022 Jul 22;9:936126.

Reindl-Schwaighofer R, **Heinzel A**, et al. Comparison of SARS-CoV-2 Antibody Response 4 Weeks After Homologous vs Heterologous Third Vaccine Dose in Kidney Transplant Recipients: A Randomized Clinical Trial. JAMA Intern Med. 2022 Feb 1;182(2):165-171.

Reindl-Schwaighofer R, **Heinzel A,** Kainz A, et al. Contribution of non-HLA incompatibility between donor and recipient to kidney allograft survival: genome-wide analysis in a prospective cohort. Lancet. 2019 Mar 2;393(10174):910-917

Heinzel A, Kammer M, Mayer G, et al. Validation of Plasma Biomarker Candidates for the Prediction of eGFR Decline in Patients With Type 2 Diabetes. Diabetes Care. 2018 Sep;41(9):1947-1954.

Söllner J, **Heinzel A**, Summer G, et al. Concept and application of a computational vaccinology workflow. Immunome Res. 2010 Nov 3;6 Suppl 2(Suppl 2):S7.

Sylvia Knapp, MD, PhD

Sylvia Knapp, MD, PhD, is Professor of Infection Biology at the Medical University of Vienna. Sylvia studied Medicine in Vienna and Berlin, is a board-certified internist and obtained her PhD at the University of Amsterdam. In 2006, she joined CeMM as a Principal Investigator and until recently, she continued her clinical duties while also running her own lab. Sylvia's research focuses on the innate immune response to infections in general, focusing specifically on the comprehensive repertoire of macrophage functions in health, development and disease.

Sylvia is highly committed to bridging academic medicine and basic science. She is elected full member of the Austrian Academy of Sciences and member of the Academia.Net circle of excellent female scientists. In 2018, Sylvia was appointed to the University Board of the Medical University of Graz and elected vice president of the Ludwig Boltzmann Society. Since 2022, Sylvia is the founding director of the inter-university Ignaz Semmelweis Institute for research in Infectious Diseases.

Hannes Vietzen, PhD

Hannes Vietzen (DOB: 15th January 1991) studied *Biological Sciences* as well as *Molecular Microbiology, Microbial Ecology and Immunobiology* at the University of Constance (Germany) and the University of Vienna. He then completed his Ph.D. in the Ph.D. Program for *Organ Failure-, Replacement and Transplantation (POET)* in 2020 at the Medical University of Vienna and is since then a Postdoc in the research group of Prof. Elisabeth Puchhammer-Stöckl at the Center for Virology, Medical University of Vienna.

Hannes Vietzen has been working in the field of transplant virology, host-virus interactions, and herpesvirus-specific immune responses. He characterized SARS-CoV-2, human Cytomegalovirus (HCMV), and Epstein-Barr Virus (EBV)-specific T cells, NK cells and antibodies in immunocompetent individuals as well as immunosuppressed transplant recipients to identify protective virus-specific immune responses. He is the first author of 14 publications with an average impact factor of 11.4 (May 2023). Hannes Vietzen presented his work in eleven talks and four posters. He received four awarded travel grants. He is a reviewer for numerous journals, *e.g. Cell Reports Medicine* as well as national and international funding organization *e.g.* the *L'Agence nationale de la recherché*.

For his work, he received the Ph.D. Award 2020 (*Dissertationspreis, Vereines zur Förderung von Wissenschaft und Forschung in den neuen Wiener Universitätskliniken am Allgemeinen Krankenhaus der Stadt Wien*), the Clinical & Experimental Austrotransplant Awards 2022 (Österreichischen Gesellschaft für Transplantation, Transfusion und Genetik, *Austrotransplant*) and the Dora Brücke-Teleky Award for exceptional publications authored by Postdocs (Vienna Alumni Club and the Society of Physicians in Vienna) in 2022.

Hannes Vietzen received two Start-up Grants from the HHV-6 Foundation and the Research Platform Transplantation of the Medical University of Vienna. He is a member of the German Society for Virology (GfV), European Society of Clinical Virology (ESCV), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), German Society for Immunology (DGfI), Austrian Association of Molecular Life Sciences and Biotechnology (ÖGMBT) and the Austrian Society for Transplantation, Transfusion and Genetics (Austrotransplant).

Thomas Wekerle, MD

Professor of Transplantation Immunology,
Dept. of General Surgery, Medical University of Vienna, Austria.

Education:

M.D. Degree: University of Vienna
Post-doctoral training: Harvard Medical School/Massachusetts General Hospital and Inst. of Pathophysiology/Medical University of Vienna

Research Interests:

- Induction of tolerance in transplantation and allergy
- Costimulation blockade
- Cell therapy for immunomodulation in kidney transplantation (NCT03867617)

Biography:

Dr. Thomas Wekerle is currently Professor of Transplantation Immunology at the Medical University of Vienna. After graduating from medical school, he trained both in Vienna and Boston (Harvard Medical School, laboratory of Dr. Megan Sykes) and was certified as clinical immunologist in 2006. Since 2000 he is leading a research group at the Department of General Surgery at the Medical University of Vienna which has a long-standing interest in investigating ways to induce immunological tolerance in organ transplantation and in IgE-mediated allergy. The goal of his research is to avoid the problems and risks associated with lifelong treatment with immunosuppressive drugs in the setting of organ transplantation and to permanently tolerize the immune system towards selected allergens in the setting of allergy. In both settings cell therapy-based approaches are being investigated as promising strategies. Thomas Wekerle is Principal Investigator of the WWTF Life Science Project "Combination cell therapy for immunomodulation in kidney transplantation" and Coordinator of the *Research Platform Transplantation* together with Rainer Oberbauer at the Medical University of Vienna. Thomas Wekerle has been awarded several national and international awards, among them the Young Investigator Award of the American Society of Transplant Surgeons and the American Society of Transplantation. He has published more than 140 scientific articles.

Ursula Wiedermann, MD, PhD

Ursula Wiedermann, MD, MSc, PhD is Professor for Vaccinology, Medical Specialist for Immunology and Specific Prophylaxis and Tropical Medicine at the Medical University of Vienna, Austria.

She is among other things the Head of Centre for Pathophysiology, Infectiology and Immunology, Head of Institute of Specific Prophylaxis and Tropical Medicine and Head of Outpatient Clinic for Vaccination, Travel- and Tropical Medicine, Member of the Supreme Board of Health of the Austrian Ministry of Health, Member of the National Immunization Committee (NITAG) of the Austrian Ministry of Health, Chair of the Vaccine Committee of the International Union of Immunological Societies (IUIS), President of the Austrian Society of Vaccination, permanent guest of STIKO (permanent Vaccination Committee) at the Robert Koch Institute in Germany, Member of the working group Travel Medicine/STIKO.

Her main areas of research are development of new vaccines and vaccination strategies against infectious diseases, allergies and cancer, immunological characterization of vaccine responsiveness and vaccination failures, preclinical and clinical testing (Phase I – IV) of new vaccine candidates and adjuvant systems, host-pathogens interaction and microbiota-host interaction.

Danube ARC Poster Sessions

Poster Session 1 (Posters 1 - 9)

Chairs: Prof. Cooper and Prof. Bohle

Transcriptomic analysis of B cell subsets in common variable immunodeficiency

Shirin Pour Akaber[1], A. Tilevik[2], S. Barresi[3], D. Alex[2], A. Lundqvist[4], J. Karlsson[5], V. Friman[5], O. Grimsholm[1]

[1]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Pathophysiology and Allergy Research, Vienna, Austria

[2]University of Skövde, School of Bioscience, Skövde, Sweden

[3]Bambino Gesu Children Hospital (IRCCS), Genetics and Rare Diseases Research Division, Rome, Italy

[4]Södra Älvsborg Hospital, Borås, Sweden

[5]University of Gothenburg, Sahlgrenska Academy, Institute of Biomedicine, Department of Infectious Diseases, Gothenburg, Sweden

Background: Common variable immunodeficiency (CVID) is the most common symptomatic immunodeficiency and it is characterized by low levels of IgG, IgA and sometimes IgM. The clinical picture is heterogeneous and all patients respond poorly to vaccination and suffer from recurrent infections, but some patients also develop more severe inflammatory and autoimmune manifestations. Here, we investigated the transcriptomic landscape of ex vivo and in vitro activated naïve and CD27^{bright} memory B cells (MBCs) as well as cytokine analysis of the supernatants from in vitro cultures.

Methods: RNA sequencing data from ex vivo and in vitro activated naïve and CD27^{bright} MBCs were analysed for differentially expressed genes (DEGs) as well as biological pathways (using GO and KEGG tools). Changes in cytokine levels in culture supernatants of naïve and MBCs were analyzed by 27-plex cytokine kit from Biorad.

Results: We observed that there are already in ex vivo naïve B cells major differences between healthy controls and CVID patients in their transcriptomic profile. Similarly, we found that also in CD27^{bright} MBCs there were thousands of DEGs between the two groups. Next, we compared the naïve B cells from ex vivo and in vitro activation and we observed that more than 6000 DEGs in healthy controls and 5000 DEGs in CVID patients. With the DEGs we performed both GO and KEGG pathway analysis and found major differences between the two groups in e.g. the B cell receptor signaling pathway, mismatch repair machinery and histone modifications. In the cytokine analysis, we found that proinflammatory cytokines such as IL-8 and IL-1beta are increased in culture supernatants of naïve B cells from CVID patients.

Conclusion: We conclude that naïve B cells cannot be properly activated in CVID patients and that their cytokine profile is skewed towards a proinflammatory profile upon T-cell dependent activation.

Funding: This work was supported by the European Union's Horizon 2020 research and innovation program under Marie Sk1odowska-Curie grant agreement 754412 (to O.G.) and the Austrian Science Fund (FWF) grant no. P32953.

Novel airborne antigens from common outdoor basidiomycetes

Joan Bacqué[1], C. Pogner[1], I. Swoboda[2], S. Pfeiffer[2], C. Amsüss[3], V. Unterwurzacher[1], Z. Wessely[1], A. Sessitsch[1], M. Gorfer[1]

[1]Austrian Institute of Technology, Bioresources, Tulln, Austria

[2]University of Applied Sciences, FH Campus Wien, Vienna, Austria

[3]BOKU University of Natural Resources and Life Sciences, Vienna, Austria

Background: A considerable proportion of the population is sensitized against allergens from airborne fungi like *Alternaria* and *Aspergillus*. Many fungal allergens, mostly from ascomycetes, are well characterized. Basidiomycetes are, however, often prevalent in outdoor air but only few allergens from this phylum are described. Based on community data from air samples, common basidiomycetes were selected for characterization of their major allergens.

Methods: Airborne fungal community data from locations in Vienna and Lower Austria were analysed by a high-throughput sequencing approach. Extracts from common basidiomycetes available through the AIT strain collection were tested for major allergens by immunoblotting with patients' plasmas. IgE-reactive bands were sequenced and the corresponding cDNAs were then expressed in *E. coli* for further characterisation.

Results: Airborne fungal communities are highly diverse and differ with location, season and place (indoor vs. outdoor). Especially outdoor communities are often dominated by basidiomycetes and some types are associated with birch pollen aerosols. The omnipresent wood-rot fungi *Trametes versicolor* and *Irpex lacteus* harbour strongly IgE-reactive allergens that were identified as enolase, transaldolase, GAPDH and NDPK. Initial screens with a small set of patients' plasmas indicate that IgE against allergens of wood-rot basidiomycetes are commonly found in patients with known fungal allergies. Association of certain fungal spores with birch pollen suggest a co-occurrence of plant and fungal allergens.

Conclusion: Small-sized spores from basidiomycetes are widespread in outdoor air and have the potential to induce allergic reactions in sensitized patients.

Funding: This study received funding from Lower Austria through the Danube Allergy Research Cluster - DARC P11 of the Karl-Landsteiner University, Krems, Austria

Recombinant VP1 capsid protein target of Human Boca 1 (HBoV1)-specific antibodies

Maja Bajic[1,2], T. Schlederer[2], K. Borochova[2], R.Kiss[1], R.Valenta[1,2,3,4], P. Errhalt[5], K. Niespodziana[2]

[1]Karl Landsteiner University for Health Sciences, Krems, Austria

[2]Medical University of Vienna, Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectology and Immunology, Vienna, Austria

[3]Sechenov First Moscow State Medical University, Laboratory of Immunopathology, Department of Clinical Immunology and Allergy, Moscow, Russia

[4]National Research Center, Institute of Immunology Federal Medico-Biological Agency of Russia, Moscow, Russia [5]Karl Landsteiner University of Health Sciences, Department of Pneumology, University Clinics Krems, Krems, Austria

Background: Besides rhinovirus (RV) and respiratory syncytial virus (RSV), Human Bocavirus 1 (HBoV1) has been suggested as important virus, which may trigger acute exacerbations of chronic respiratory diseases, such as asthma and chronic obstructive pulmonary disease (COPD). However, there is currently no serological test available which could allow detecting antibody responses against different HBoV1 antigens and the diagnosis of HBoV1 infections is mainly performed by the direct detection of the virus by PCR-based methods. However, there are no serological tests which confirm by measurements of HBoV1-specific antibodies that infection has occurred.

Materials and Methods: The HBoV1 capsid proteins VP1 and VP2 were expressed in *Escherichia coli* as C-terminally hexahistidine-tagged recombinant proteins and purified by Nickel-affinity chromatography. The purity and identity of targeted proteins were analyzed by SDS-PAGE and by Western-blotting using a monoclonal anti-His-tag antibody, respectively. Synthetic peptides of approximately 30 amino acids length spanning the complete proteins were produced by solid phase synthesis, purified by HPLC and characterized by mass spectrometry. Enzyme-linked immunosorbent assay (ELISA) as well as microarray technology were used to study the occurrence of VP1- and VP2- as well as specific antibodies in serum samples from adult individuals.

Results: Recombinant proteins were expressed and purified as soluble proteins. VP1- and VP2-specific IgG responses were found in almost all tested serum samples, VP1-specific IgG antibody levels were higher than those measured specific for VP2. Furthermore, compared to rhinovirus (RV)-derived VP1 and respiratory syncytial virus (RSV)-derived G protein, the major targets of RV and RSV-specific humoral immunity. VP1-specific IgG responses were significantly lower. IgG reactivity to certain VP1-derived peptides were also detected but it was lower than IgG reactivity to complete HBoV1-derived VP1.

Conclusion: Recombinant HBoV1-derived VP1 can be used to investigate if HBoV1 infections induce increases of HBoV1-specific antibody responses and if such increases of HBoV1-specific antibodies may be related with exacerbations of chronic respiratory diseases.

Funding: „Danube-ARC" research program of the country of Lower Austria grant no. 330950005.

Unraveling temporal immune changes in atopic dermatitis through a mouse model

Parvaneh Balsini[1], S. Kazemi[1], D. Symmank[1], F. Assen[1], S. Tikoo[1], R. Jain[1], M. Buchberger[1], M. M. Epstein[1], K. Pfisterer[1], W. Weninger[1]

[1]*Medical University of Vienna, Department of Dermatology, Vienna, Austria*

Background: Atopic dermatitis (AD) is a chronic inflammatory skin disease that affects individuals of all ages, characterized by skin inflammation, epidermal hyperplasia, and immune cell infiltration. While the causes of AD are not completely understood, genetic, environmental, and immunological factors may play a role.

Methods: This study aims to investigate immediate and delayed immune responses in a mouse model of allergen penetration via dinitrofluorobenzene (DNFB) sensitization. Current knowledge suggests that AD patients have an increased amount of CD4 T cells and impaired cytotoxic T-cell function. Previous research also indicates that Th2 cells contribute to the acute phase of AD, while Th1 cells are involved in the chronic phase. In this study, we sought to explore the phenotype of T cells and T cell subsets that contribute to tissue inflammation in our mouse model using T cell activation markers (CD44 and CD62L), type 1 and type 2 cytokine markers (IFN-γ, IL-4, and IL-13), flow cytometry, and confocal microscopy.

Results: Our preliminary experiments revealed an increase in CD44 expression and a decrease in CD62L expression in the spleen and lymph nodes, along with higher levels of IFN-γ in the spleen, lymph nodes, and ear of mice. Conversely, we detected increased levels of IL-13 in the spleen and lymph nodes but lower IL-13 levels in the ear.

Conclusion: Our data provide initial valuable information regarding the expression levels of key molecules and cytokines in different tissues of mice, shedding light on the potential involvement of immune cell subsets in the development and progression of AD. These findings set the stage for further experiments that can help elucidate the underlying mechanisms and temporal regulation of AD pathogenesis.

Funding: The project was supported by the Country of Lower Austria's funded Danube Allergy research cluster (Danube ARC)

Bet v 1-specific nanobody trimers – a new tool for allergy treatment?

Clarissa Bauernfeind[1], I. Zettl[1], T. Ivanova[2], O. Goryainova[2], A. Drescher[3], M. Focke-Tejkl[1], J. Eckl-Dorna[4], S. Tillib[2], S. Flicker[1]

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

[2]*Russian Academy of Sciences, Institute of Gene Biology, Moscow, Russia*

[3]*Cytiva Europe GmbH, Freiburg, Germany*

[4]*Medical University of Vienna, Department of Otorhinolaryngology, Vienna, Austria*

Background: Around 20% of the European population suffers from allergic symptoms elicited by Bet v 1, the major birch pollen allergen, and cross-reactive allergens from related trees and food. Hence, new treatment strategies were elaborated demonstrating that blocking Bet v 1 with monoclonal IgG antibodies is effective in preventing IgE-mediated events. A recent study provided evidence that Bet v 1-specific nanobodies, reduce patients´ IgE binding to Bet v 1.

In order to increase the potential of nanobodies to outcompete IgE binding to Bet v 1 and related allergens, we developed Bet v 1-specific nanobody trimers and evaluated their capacity to suppress IgE binding and allergen-induced degranulation of effector cells.

Methods: Nanobody trimers were engineered by adding isoleucine zippers, to enable trimeric formation. Trimers were analyzed for successful post-translational trimerization, their cross-reactivity and affinity to Bet v 1 and Bet v 1-homologs (SEC, ELISA, SPR). Their efficacy to (cross)-inhibit patients' IgE binding and basophil degranulation was investigated (ELISA, RBL assay).

Results: Nanobody monomers and trimers bound to Bet v 1, Aln g 1 (alder), Cor a 1 (hazel), Car b 1 (hornbeam), Fag s 1 (European beech), and Dau c 1 (carrot), whereas only trimers recognized Mal d 1 (apple), Cor a 1.04 (hazelnut), Pru p 1 (peach), Pru du 1 (almond), Ara h 8 (peanut), Gly m 4 (soybean), and Api g 1 (celery). Furthermore, trimers exhibited significant reduction of IgE binding to all tested allergens and are potent competitors for allergen-induced mediator release. Trimers displayed slow dissociation rates with a remarkable complex stability with applied allergens.

Conclusion: We generated nanobody trimers that bind Bet v 1 and its cross-reactive allergens with high affinity. Our results revealed a superior inhibition of the IgE-allergen interaction of nanobody trimers compared to monomers and a strong prevention of IgE-mediated basophil activation induced by Bet v 1 and relatives.

Funding: Supported by Austrian Science Fund (FWF) grant I3946-B33 and the Russian Foundation for Basic Research (RFBR) grant 18-515-14003.

Natural IgE and IgG antibodies of allergic and non-allergic individuals recognize different epitopes of a major birch pollen allergen Bet v 1

Georgii Brazhnikov[1,2], E. Smolnikov[3,4], A. Litovkina[3,4], A. Shatilov[3], A. Poroshina[3], Y. Zhernov[5], M. Focke-Tejkl[1,6], M. Weber[1], O. Akinfenwa[1], T. Jiang[1], O. Elisyutina[3,4], S. Andreev[3], I. Shilovskiy[3], N. Shershakova[3], V. Smirnov[3], E. Fedenko[3], T. Lepeshkova[7], E. Beltyukov[8], V. Naumova[8], M. Kundi[9], M. Khaitov[3,4,10], U. Wiedermann[2], R. Valenta[1,3,6,11], R. Campana[1]

[1]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Immunology, Infectiology and Immunology, Division of Immunopathology, Vienna, Austria.

[2]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology Institute for Specific Prophylaxis, Vienna, Austria.

[3]National Research Center Institute of Immunology Federal Medical-Biological Agency of Russia, Moscow, Russian Federation.

[4]Peoples' Friendship University of Russia (RUDN University), Department of Immunology, Institute of Medicine, Moscow, Russian Federation.

[5]I.M. Sechenov First Moscow State Medical University (Sechenov University), F. Erismann Institute of Public Health, Moscow, Russian Federation.

[6]Karl Landsteiner University of Health Sciences, Krems, Austria.

[7]Ural State Medical University, Department of Polyclinic Pediatrics, Yekaterinburg, Russian Federation.

[8]Ural State Medical University, Department of Faculty Therapy, Endocrinology, Allergology and Immunology, Yekaterinburg, Russian Federation.

[9]Medical University of Vienna, Institute for Hygiene and Applied Immunology, Center for Public Health, Vienna, Austria.

[10]Pirogov Russian National Research Medical University, Moscow, Russian Federation.

[11]Sechenov First Moscow State Medical University (Sechenov University), Laboratory of Immunopathology, Department of Clinical Immunology and Allergology, Moscow, Russian Federation.

Background: The epitopes recognized by IgE antibodies from birch pollen allergic patients have been studied but the epitopes recognized by IgG antibodies from allergic and non-allergic subjects have not been characterized so far. The aim of the study was to compare IgE and IgG recognition of conformational and linear epitopes of Bet v 1 by subjects with and without birch pollen allergy.

Methods: Three groups of subjects were studied: Birch pollen allergic patients (n=66), allergic patients without birch pollen allergy (n=30) and non-allergic subjects (n=38) and tested for IgE, IgG, IgG_1 and IgG_4 reactivity by ELISA using folded recombinant Bet v 1, two unfolded Bet v 1 fragments and six linear peptides spanning Bet v 1 sequence. The effect of IgG antibodies from non-allergic subjects on IgE binding of allergic patients to Bet v 1 and Bet v 1-induced basophil activation was studied by ELISA and basophil activation, respectively.

Results: IgE antibodies from birch pollen allergic patients reacted only with folded Bet v 1 but not unfolded Bet v 1 fragments or linear Bet v 1 peptides. In contrast, IgG antibodies from all individuals reacted with folded Bet v 1 and with unfolded Bet v 1 fragments and peptides. Natural occurring Bet v 1-specific IgG antibodies from allergic and non-allergic subjects inhibited IgE binding of allergic patients to Bet v 1 and Bet v 1-induced basophil activation to a varying extent and in certain patients enhanced basophil activation.

Conclusion: IgE and IgG antibodies from birch pollen allergic patients and non-allergic subjects recognize different epitopes of Bet v 1 and therefore IgE and IgG-producing cells have different clonal origins.

Funding: This work was supported by the Austrian Science Fund (FWF) project P34472-B and by the Danube Allergy Research Cluster Program of the Country of Lower Austria.

A CRISPR/Cas9 *in vivo* screen for new regulators of plasma cells

Lesly Calderon[1], M. Schäfer[1], M. Jaritz[1], Q. Sun[1], R. Rauschmeier[1], T. Schwickert[1], M. Fellner[1], J. Zuber[1], M. Busslinger[1]

[1]Vienna Biocenter, Research Institute of Molecular Pathology, Vienna, Austria

Background: Plasma cells are generated once B lymphocytes encounter their cognate antigen in secondary lymphoid organs. While a very rare population of cells, they secrete huge amounts of antigen-specific antibodies and are essential for the humoral immunity against pathogens. To identify novel genes and pathways regulating the development, function, and survival of plasma cells within the microenvironment of secondary lymphoid organs, we have established an *in vivo* model system for pooled sgRNA CRISPR/Cas9 screenings.

Methods: The *in vivo* model system requires the *in vitro* transduction of Cas9-exppressing naïve B cells with lentiviral (LV) particles carrying gene-specific sgRNAs, the subsequent transfer of transduced naïve B cells to recipient mice, the immunization of these mice to induce a T-dependent or a T-independent immune response and, finally, the evaluation of sgRNAs abundance on the generated plasma cells.

Results: For the first screenings, 380 genes highly expressed in plasma cells were selected after comparing RNA-seq gene expression data of B cells with, those of plasma cells. As a result of these screenings, several tens potential positive and potential negative regulators were found. Many of these genes have not been previously described as regulators of B cell activation or plasma cell differentiation. The most interesting hits are currently being validated. They include genes involved in cell adhesion, enzymes, transporters, zinc finger proteins, receptor ligands or signaling molecules.

Conclusions: Our studies might uncover novel molecular mechanisms regulating antibody-mediated immune responses and plasma cells. Moreover, they might enlighten the rational design of vaccines and could potentially be beneficial for the treatment of antibody-mediated autoimmune diseases or plasma cell malignancies.

Nasal IL-13 production identifies patients with late phase allergic responses

Nicholas James Campion[1], S. Villazala-Merino[1], R.S. Thwaites[2], V. Stanek[1], H. Killick Fd[3], E. Pertsinidou[4], M. Zghaebi[1], J. Toth[1], R. Fröschl[5], T. Perkmann[5], K. Gangl[1], S. Schneider[1], R. Ristl[6], I.C. Scott[3], E.S. Cohen[7], M. Molin[4], M. Focke-Tejkl[8], G. Regelsberger[9], T.T. Hansel[2], R. Valenta[8,10], J. Eckl-Dorna[1] and V. Niederberger-Leppin[1]

[1]Medical University of Vienna, Department of Otorhinolaryngology, Vienna, Austria

[2]National Heart and Lung Institute, Imperial College of London, London, United Kingdom

[3]AstraZeneca, Translational Science and Experimental Medicine, Research and Early Development, Respiratory & Immunology, BioPharmaceuticals R&D, Cambridge, United Kingdom

[4]Thermo Fisher Scientific, Research and Development, Uppsala, Sweden

[5]Medical University of Vienna, Department of Laboratory Medicine, Vienna, Austria

[6]Medical University of Vienna, Center for Medical Statistics, Informatics and Intelligent Systems, Vienna, Austria

[7]AstraZeneca, Bioscience Asthma, Research and Early Development, Respiratory & Immunology, BioPharmaceuticals R&D, Cambridge, United Kingdom

[8]Medical University of Vienna, Divison of Immunopathology, Department of Pathophysiology and Allergy Research, Vienna, Austria

[9]Medical University of Vienna, Division of Neuropathology and Neurochemistry, Department of Neurology, Vienna, Austria

[10]Karl Landsteiner University of Health Sciences, Krems, Austria

Background: There is limited knowledge on how local cytokine secretion patterns after nasal allergen challenge correlate with clinical symptoms especially with regards to the "late allergic response" (LAR) which occurs in approximately 40-50% of allergic patients.

Objective: In this study we aimed to characterise the immunological and clinical nasal responses to birch pollen allergen challenge with a special focus on the LAR.

Methods: In this randomised double-blinded placebo-control trial, birch pollen allergic participants were challenged with pollen extract (n=20) or placebo (n=10) on three consecutive days. On days one and three nasal secretions were collected at selected time points over a 24h time course for the measurement of 33 inflammatory mediators. Clinical responses were determined through subjective symptom scores and objective nasal airflow measurements.

Results: Provoked participants had significantly greater clinical responses and showed significant increases in tryptase and sST2 within minutes compared to placebo. Eight out of 20 provoked participants displayed high IL-13 levels 2-8 hours after allergen provocation. This group also showed significant changes in clinical parameters, with a secondary drop in nasal airflow measured by peak nasal inspiratory flow and increased symptoms of nasal obstruction which significantly differed from IL-13 non responders at 6 hours.

Conclusion: IL-13 response status correlates with cytokine and clinical responses in the late phase after allergen provocation.

Funding: Supported by grants DK W 1248-B30 and SFB F4613 from the Austrian Science Fund (FWF) and by the DANUBE ARC program of the County of Lower Austria.

Molecular mechanisms driving Non-specific Lipid Transfer Protein (nsLTP) transport across the epithelial barrier

Nicola Cavallari[1], C. Nagl[1], A. Johnson[2], S. Geiselhart[1], K. Hoffmann-Sommergruber[1]

[1]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Department of Pathophysiology and Allergy Research, Vienna, Austria.

[2]Medical University of Vienna, Center for Anatomy and Cell Biology, Vienna, Austria.

Background: nsLTPs are a ubiquitous (pan-)allergen family described in all land plants and recently also in Algae. Sensitization to nsLTPs shows relevant geographical distribution mainly localized in but not restricted to the Mediterranean area. At the structural level, the protein presents an inner hydrophobic pocket hosting a lipid ligand, both playing a prominent role in allergic sensitization. While food allergic reactions are primarily located at the intestinal level, allergen absorption may start few minutes after ingestion in the oral mucosa. However, most of the molecular mechanisms driving nsLTP transport across the epithelium are still unknown. The present work aims to investigate allergen-epithelial cell interactions using three highly representative nsLTPs (the prototypic Pru p 3 from peach, the related Mal d 3 from apple and the phylogenetically distant but largely cross-reacting Cor a 8 from hazelnut) as models in cell endocytosis and signaling.

Methods: Fluorescent nsLTP transport and localization in epithelial cells was evaluated by trans-well plate system and confocal microscopy respectively. Co-immunoprecipitation of plasma membrane protein bound Cor a 8 was achieved with a polyclonal anti-Cor a 8 antibody immobilized on Protein A-agarose matrix.

Results: All nsLTPs were able to cross the intestinal epithelial cell monolayer *in vitro*, without affecting the integrity of the tight junctions, supporting an intra-cellular pathway hypothesis. Moreover, the observed fast saturating transport kinetics suggested the presence of a receptor mediated cellular uptake mechanism.

Accordingly, fluorescently labeled nsLTPs co-localized with early endosomes in cell lines of intestinal enterocytes and oral keratinocytes, only minutes after addition to the cell media. Remarkably, inhibition of both, Clathrin- and Caveolin-mediated endocytosis (two prominent although independent internalization pathways) largely reduced nsLTP intracellular localization.

Hence, a refined protocol for the isolation of plasma membrane proteins was developed to finally identify the elusive cell receptors triggering nsLTP internalization in epithelial cells.

Conclusion: Our mechanistic approach will define the molecular interactions between nsLTPs and epithelial cells and provide both a "road map" and the tools for further investigations on other allergens and cell lines.

Funding: This work was supported by the Danube Allergy Research Cluster P07.

Poster Session 2 (Posters 10 - 18)

Chairs: Prof. Banchereau and Prof. Wiedermann

The cross-blocking activity of immunotherapy-induced antibodies accords with allergen homology

Hilal Demir[1], M.R. Strobl[1], C. Radauer[1], S. Scheurer[2], T. Kinaciyan[3], B. Bohle[1]

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

[2]*Paul-Ehrlich-Institute, Molecular Allergology, Langen, Germany*

[3]*Medical University of Vienna, Department of Dermatology, Vienna, Austria*

Background: We recently reported that sublingual immunotherapy (SLIT) with recombinant (r) Bet v 1 (rBet v 1-SLIT) or its homolog in apple, Mal d 1 (rMal d 1-SLIT), resulted in differing types of IgE-blocking antibodies (Abs). rBet v 1-SLIT-induced Abs blocked IgE-reactivity to Bet v 1 but not to Mal d 1 whereas rMal d 1-SLIT-induced Abs blocked IgE-reactivity to Mal d 1 but not Bet v 1. Here, we employed Cor a 1 from hazelnut (highly homologous to Bet v 1), Pru av 1 from cherry (highly homologous to Mal d 1), and Dau c 1 from carrot to assess the cross-reactivity, cross-blocking capacity and avidity of SLIT-induced Abs.

Methods: Allergen-specific IgE, IgG1, and IgG4 levels were determined by ELISA in pre- and post-SLIT samples of 20 rMal d 1-SLIT and 17 rBet v 1-SLIT-treated individuals, respectively. To assess IgE-blocking, allergens were incubated with pre- and post-SLIT samples from 7 individuals of each group prior to their use in basophil activation tests. Avidity was compared by challenging the binding of SLIT-induced Abs to plate-bound allergens with acidic buffers. Potentially shared epitopes of SLIT-induced Abs on the different allergens were assessed by competition ELISA. Shared surface areas of the allergens were identified by using an in-house designed script based on structural alignments.

Results: rBet v 1-SLIT significantly enhanced IgG1 and IgG4 responses to all studied food allergens except Dau c 1. rMal d 1-SLIT significantly increased IgG1 levels specific for Mal d 1, Cor a 1, and Pru av 1, and IgG4 levels specific for Mal d 1 and Pru av 1. Post-rBet v 1-SLIT sera displayed higher blocking activity and avidity for Bet v 1 whereas post-rMal d 1-SLIT sera showed higher blocking activity and avidity for Mal d 1 and Pru av 1. Competition ELISA suggested that rMal d 1-SLIT-induced IgG1 Abs share more epitopes with Pru av 1 than those following rBet v 1-SLIT. Accordingly, highest surface identities were found between Pru av 1 and Mal d 1.

Conclusion: Our results with Bet v 1-related allergens indicate that IgE-cross-blocking depends on the homology of allergens. These findings are relevant to understand better why therapy with Bet v 1 has limited effects on associated food allergies.

Funding: This work was supported by the Danube Allergy Research Cluster project no. FA648A1903.

Recombinant *Blomia tropicalis* allergens allow differential diagnosis of genuine IgE sensitization to *Blomia tropicalis* in allergic patients

Nishelle Dsouza[1], M. Weber[1], E. Sarzsinszky[1], G. Pauli[2], M. Curin[1], M. Schaar[1], V. Garib[1], M. Focke-Tejkl[1], M. Tulaev[1], T. Schlederer[1], R. Valenta[1,3,4,5], S. Vrtala[1]

[1]*Medical University of Vienna, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Vienna, Austria*

[2]*University of Strasbourg, Faculty of Medicine, France.*

[3]*Sechenov First Moscow State Medical University, Laboratory of Immunopathology, Department of Clinical Immunology and Allergy, Moscow, Russia*

[4]*National Research Center, Institute of Immunology Federal Medico-Biological Agency of Russia, Moscow, Russia*

[5]*Karl Landsteiner University of Health Sciences, Krems, Austria*

Background: Around 30% of the general population is affected by house dust mite (HDM) allergy and it causes a wide range of allergic manifestations such as asthma, allergic rhinitis and atopic dermatitis. *Blomia tropicalis* (Blo t) is a house dust mite species of tropical and sub-tropical areas in South America, Africa and Asia which belongs to the superfamily of *Glycyphagidae*. Our goal was the isolation and characterization of allergens from this mite species to provide component resolved diagnosis in the form of a microarray chip.

Methods: To determine the clinical relevance of the individual *Blomia tropicalis* allergens, we produced wild-type-like recombinant allergens (Blo t 1, Blo t 2, Blo t 5, Blo t 8, Blo t 10, Blo t 13, Blo t 21) by expression in *Escherichia coli* BL21 (DE3) using the pET17b plasmid. Once expressed and purified, these recombinant allergens were characterized by SDS PAGE for purity and by circular dichroism for fold. Sera from patients sensitized to HDM and/or Blo t were tested on the microarray chip to determine the frequency of IgE recognition of the individual allergen molecules.

Results: Our results showed that Blo t 5, Blo t 21 and Blo t 2 are the most important allergens for Blo t sensitized patients and that Blo t sensitised patients also show IgE reactivity to Der p allergens, whereas Der p sensitised patients mainly react to Der p allergens.

Conclusion: With the panel of allergens produced, it is possible to achieve component resolved differential diagnosis between HDM (Der p and Der f) and *Blomia tropicalis*.

Funding: Supported by a research grant from WORG Pharmaceuticals, Hangzhou and by a research grant (Danube Allergy Research Cluster) of the country of Lower Austria.

A comprehensive analysis of the preventive application of allergen immunotherapy (pAIT) using respiratory allergens in children

Varsha Dwivedi[1], S. Kopanja[1], K. Schmidthaler[1], J. Sieber[1], C. Bannert[1], S. Gruber[1], E. Dehlink[1], Z. Szépfalusi[1]

[1]*Medical University of Vienna, Comprehensive Center of Pediatrics, Department of Pediatrics and Adolescent Medicine, Division of Pediatric Pulmonology, Allergy and Endocrinology, Vienna, Austria*

Background: An increasing number of studies have focused on the preventive application of allergen immunotherapy (pAIT), targeting different stages of the allergic march in children. Here, we present the current perspectives on the benefits of pAIT in children, focusing

particularly on the protection from new sensitisations, progression of existing allergic diseases and immunomodulatory outcomes.

Methods: PubMed, EMBASE and Cochrane central register were searched for clinical pediatric pAIT studies, followed by screening based on the defined inclusion criteria. Selected studies were reviewed for the above-mentioned outcomes addressing different levels of prevention (primary, secondary and tertiary), dose and routes of administration, treatment duration and study designs.

Results: After reviewing more than 700 articles, we identified 33 reports studying pAIT in 27 cohorts (N=2953 children). Safety and feasibility of primary and secondary prevention approaches has been shown in 3 pediatric cohorts. Secondary pAIT showed the potential to induce of T-cell mediated immune-tolerance. In tertiary pAIT studies, house dust mites (HDM) (n=13), grass/tree pollen (n=7), mold (n=1) and mixed-allergen formulations (n=4) were studied. While grass/tree pollen-pAIT seems to prevent the development of asthma in children suffering from allergic rhinitis, the efficacy of HDM-pAIT and other formulations are inconsistent (62% and 45% of favourable studies). Preventive outcomes of subcutaneous and sublingual routes were observed in 63% and 44% of the reporting studies, respectively. However, sublingual administration seems safer for delivering higher allergen concentrations over shorter treatment durations. Beneficial immunomodulation upon pAIT was reported in 8 out of 10 cohorts. Nine trials have studied pAIT in a placebo-controlled design.

Conclusion: Primary and secondary prevention of allergic disease remains wishful and plausible. The current strength of evidence is limited and warrants further investigation through well-designed studies. Though the prevention of new sensitisations awaits conformation, tertiary pAIT with grass/tree pollen appears potent for halting the progression of existing allergic disease(s) in children.

Funding: This work was supported by funding from the Government of Lower Austria (Land Niederösterrich)

The mold allergen Alt a 1 as a carrier of iron-quercetin complexes is hypoallergenic *in vitro* and *in vivo*

Aila Fakhimahmadi[1,2], K. Hufnagl[1,2], I. Hasanaj[1], N. Szepannek[1], G. Hofstetter[1], M. Wiederstein[3], M. Berger[1,2], S.A. Jensen[2], J. Bacqué[4], M. Gorfer[4], C. Pogner[4], Z. Dvorak[5], R. Bianchini[1,2], I. Pali-Schöll[1,2], E. Jensen-Jarolim[1,2,6], F. Roth-Walter[1,2]

[1]*Interuniversity Messerli Research Institute, Comparative Medicine, Vienna, Austria*

[2]*Medical University of Vienna, Institute of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Austria*

[3]*University of Salzburg, Department of Biosciences & Medical Biology, Salzburg, Austria.*

[4]*AIT Austrian Institute of Technology GmbH, Bioresources Unit, Center for Health & Bioresources, Tulln, Austria.*

[5]*Palacky University, Department of Cell Biology and Genetics, Faculty of Science, Olomouc, Czech Republic*

[6]*Biomedical International R+D GmbH, Vienna, Austria*

Background: *Alternaria alternata* is a common fungus, strongly related to severe allergic asthma. Here, we assessed the function of Alt a 1 the major allergen as an innate defense protein binding to micronutrients such as iron-quercetin complexes (FeQ2) and the impact of ligand-loading on its allergenicity.

Methods: The expression of Alt a 1 in the fungus *Alternaria* grown under iron-deprived or quercetin-enriched conditions was evaluated by ELISA and Immunoblot. Binding of Alt a 1 to iron-quercetin complexes (FeQ2) was analyzed via docking calculations. IgE-binding to Alt a 1 with ligands (holoAlt a 1) and without ligands (apoAlt a 1) was assessed. The impact of apoAlt a 1 and holoAlt a 1 on mast cell degranulation was examined with RBLsx38 cells. Human PBMCs were stimulated and examined for phenotypic changes in B- and T-cells using flow cytometry. The biological impact was investigated *in vivo* with BALB/c mice pretreated intranasally with apo/holoAlt a 1 before intraperitoneal sensitization with Alt a 1 absorbed to alum. Subsequently, mice were challenged intraperitoneally with Alt a 1, and anaphylactic symptoms and immune responses were assessed.

Results: *Alternaria* grown under iron-deficient conditions overexpresses Alt a 1 allergen, whereas reduced expression was observed upon addition of quercetin. *In silico* and spectral analysis confirmed the binding of iron-quercetin complexes to Alt a 1 close to IgE epitopes. Of the 4 described IgE-epitopes, FeQ2 completely masked the IgE epitope Y87-D96. This resulted in up to 80% less patients' IgE (n=10) binding to holoAlt a 1 than to apoAlt a 1 in ELISA and immunoblot. Compared to apoAlt a 1, holoAlt a 1 binding to iron-quercetin complexes decreased specific mast cell degranulation in RBLsx38 cells. HoloAlt a 1 stimulation of peripheral blood mononuclear cells resulted in reduced expression of $CD19^+$ B lymphocytes, and $CD3^+$ $CD4^+$ T helper cells. In mice, pretreatment with holoAlt a 1 significantly reduced allergic symptoms, splenic IFN-γ and IL-13 secretion, and antigen presenting cells, whereas it promoted Treg cells.

Conclusion: Iron-deficient conditions induce Alt a 1 expression in *Alternaria*. Alt a 1's high affinity to iron complexes suggests a role as micronutrient scavenger. Ligand binding strongly affected the allergenicity of Alt a 1 leading to reduced IgE binding, hampered mast cell degranulation *in vitro* and reduced symptoms *in vivo*, emphasizing that only apoAlt a 1 acts as an allergen, whereas in combination with iron-quercetin complexes, it appears rather immunosuppressive.

Funding: This study was funded by the Danube Allergy Research Cluster-DARC #08 of the Karl-Landsteiner University, Krems, Austria, to EJJ. FRW and EJJ are inventors on EP2894478, owned by Biomedical International R+D GmbH, of which EJJ is shareholder.

Transcriptional network of early B cells analyzed by targeted *in vivo* protein degradation

Anna Sales Fedl[1*], T. A. Schwickert[1*], S. Gruenbacher[1*], H. Tagoh[1], Q. Sun[1], R. Schenk[1], K. Froussios[1], M. Jaritz[1] and M. Busslinger[1]

[1]*Research Institute of Molecular Pathology, Vienna Biocenter, Vienna, Austria*

[*]*equal first authors*

Background: Early B cell development relies on the coordinated activity of key transcription factors, including Ikaros (*Ikzf1*), E2A (*Tcf3*), Ebf1 and Pax5. Current methods for studying their function involve differential gene expression analysis of control and mutant B cells after Cre-mediated transcription factor deletion. However, this approach has limitations as it fails to capture which genes are directly and indirectly regulated. Additionally, the scope of this method is restricted by the availability of cell-type-specific Cre lines.

Methods: In this study, we present an alternative approach using targeted protein degradation based on the auxin-inducible degron (AID) system. To implement this strategy, we generated mice with an AID-tag inserted at the C- or N-terminus of the *Ikzf1*, *Tcf3*, *Ebf1* and *Pax5* allele. Furthermore, we generated a mouse model expressing

a mutant version of the Tir1 adapter protein (Tir1-F74G) under the control of the ubiquitously expressed *Rosa26* locus (*Rosa26*$^{Tir1\text{-}F74G}$), enabling rapid proteasomal degradation of the AID-tagged transcription factors upon intraperitoneal injection of the auxin derivative 5-Ph-IAA.

Results: The Ikzf1$^{Aid/Aid}$, Tcf3$^{Aid/Aid}$, Ebf1$^{Aid/Aid}$ and Pax5$^{Aid/Aid}$ Rosa26Tir1-F74G mice were viable and generated all B cell subsets. Moreover, in a proof-of-principle experiment, continuous depletion of the corresponding transcription factors by means of daily 5-Ph-IAA treatment recapitulated previously described conditional knockout phenotypes.

By combining acute transcription factor degradation with nascent transcript analysis, we now identified the direct target genes of Ikaros, E2A and Pax5 in pro-B, pre-B, and immature B cells. Notably, our findings revealed that Ikaros functions primarily as a transcriptional repressor, while E2A and Pax5 can both activate and repress their target genes. Importantly, each transcription factor largely regulated distinct sets of genes in the different B cell subsets, highlighting their unique functions during early B cell development.

Conclusion: By adapting a targeted protein degradation approach, we have successfully identified direct transcription factor regulated genes *in vivo*. This methodology overcomes the limitations of previous techniques and will provide novel insights into the specific functions of Ikaros, E2A, Ebf1 and Pax5 during early B cell development.

Lower magnitude and faster waning of antibody responses to SARS-CoV-2 vaccination in anti-TNF-α-treated IBD patients are linked to lack of activation and expansion of cTfh1 cells and impaired B memory cell formation

Erika Garner-Spitzer*[1], Angelika Wagner[1], Venugopal Gudipati[2], Anna-Margarita Schoetta[2], Maria Orola-Taus[1], Michael Kundi[3], Renate Kunert[4], Patrick Mayrhofer[4], Johannes B. Huppa[2], Hannes Stockinger[2], Rita Carsetti[5], Pia Gattinger[6], Rudolf Valenta[6,7], Bernhard Kratzer[8], Al Nasar Ahmed Sehgal[8], Winfried F. Pickl[7,8], Walter Reinisch[9], Gottfried Novacek[9], Ursula Wiedermann*[1]

[1]*Medical University of Vienna, Center of Pathophysiology, Infectiology and Immunology, Institute of Specific Prophylaxis and Tropical Medicine, Vienna, Austria*

[2]*Medical University of Vienna, Center of Pathophysiology, Infectiology and Immunology, Institute for Hygiene and Applied Immunology, Vienna, Austria*

[3]*Medical University of Vienna, Center for Public Health, Vienna, Austria*

[4]*University of Natural Resources and Life Sciences Vienna, Department of Biotechnology, Vienna, Austria*

[5]*B Cell Research Unit, Immunology Research Area, Bambino Gesu Children's Hospital, IRCCS; Rome, Italy*

[6]*Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Department of Pathophysiology and Allergy Research, Division of Immunopathology, Vienna, Austria*

[7]*Karl Landsteiner University of Health Sciences, Krems, Austria*

[8]*Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Immunology, Vienna, Austria*

[9]*Medical University of Vienna, Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Vienna, Austria*

Background: Patients with inflammatory bowel disease (IBD) and healthy controls received primary SARS-CoV-2-mRNA vaccination and a booster after six months. Anti-TNF-α-treated patients showed significantly lower antibody (Ab) levels than α4β7-integrin-antagonist recipients and controls. This study aimed to elucidate the underlying mechanisms on the basis of circulating T-follicular helper cells (cTfh) and B memory cells.

Methods: We measured SARS-CoV-2-specific Abs against Wuhan and Omicron variants, B- and T-cell subsets at baseline and kinetics of Spike (S)-specific B memory cells along with distributions of activated cTfh cells before and after 2-dose primary and booster vaccination.

Results: Anti-TNF-α treated IBD patients showed lower levels and faster waning of Wuhan- and Omicron-specific IgG. This was associated with reduced numbers of total and naïve B cells versus expanded plasmablasts prior to vaccination. Along with low Ab levels in anti-TNF-α-treated IBD patients, reduced S-specific B memory cells were established after the 2nd dose which declined to non-detectable after 6 months. This was in contrast to high responder α4β7-integrin treated IBD patients and controls, who mounted high S1-specific antibody levels and B memory cells up to 6 months post primary vaccination. Booster vaccinations induced a strong increase of both Abs and S-specific B memory cells in these groups. This was not the case in anti-TNF-α treated IBD patients, who also showed reduced neutralizing capacity against Omicron variants. Of note, activation of cTfh1 cells significantly correlated with the induced Ab levels and S-specific memory B cells, particularly after booster vaccination.

Conclusions: The reduced magnitude, persistence, and neutralizing capacity of SARS-CoV-2 specific Abs after vaccination in anti-TNF-α-treated IBD patients were associated with impaired formation and maintenance of S-specific B memory cells due to absent activation of cTfh1 cells after primary vaccination. This most likely led to only short-lived extrafollicular immune responses and diminished B memory cell diversification. These observations have implications for patient-tailored vaccination schedules/vaccines in anti-TNF-α-treated patients, irrespective of their underlying disease.

Funding: The study was funded by third party funding of the Institute of Specific Prophylaxis and Tropical Medicine at the Medical University Vienna.

Obesity increases allergic airway inflammation that can be successfully treated by oral immunotherapy

Nora Geissler[1], M. Orola[1], M. Alinagh[2], A. Nardo[3], T.M. Stulnig[3,4], A.Schmid[1], E. Korb[1], T. Svoboda[2], E. Garner-Spitzer[1], M. Kundi[1], M. Ehling-Schulz[2], I. Schabussova[1], A. Inic-Kanada[1*], U. Wiedermann[1*]

[1]*Medical University of Vienna, Institute of Specific Prophylaxis and Tropical Medicine, Center for Pathophysiology, Infectiology and Immunology, Vienna, Austria*

[2]*University of Veterinary Medicine, Functional Microbiology, Institute of Microbiology, Department of Pathobiology, Vienna Austria*

[3]*Medical University of Vienna, Clinical Division of Endocrinology & Metabolism, Department of Medicine III, Vienna, Austria*

[4]*Karl Landsteiner Institute for Metabolic Diseases and Nephrology, Department of Medicine III, Clinic Hietzing, Vienna, Austria*

Background: Obesity and allergy have become significant health problems, particularly in the westernized world. It is unclear whether obesity enhances respiratory allergy and whether tolerance can be efficiently induced in obese.

Methods: C57BL/6 male mice fed with a high-fat diet (HFD) or standard chow diet (STD) for nine weeks were immunized/sensitized and then challenged with ovalbumin (OVA). To induce tolerance, mice were orally treated with OVA before sensitization. Metabolic parameters and allergen-specific antibodies were measured in serum. Dif-

ferential cell counts were performed in bronchoalveolar lavage and cytokine measurements, FACS analysis, and immunofluorescence staining in the lung. Fourier transform infrared spectroscopy was used to investigate the molecular composition of the gut.

Results: HFD-fed animals exhibited twice the body weight and significantly higher leptin levels than STD-fed animals. Sensitization and challenge with OVA resulted in upregulating allergic parameters, such as eosinophil counts, Th2 cytokines, and OVA-specific IgE levels, which were significantly higher in obese compared to lean animals. Oral tolerance induction led to a non-allergic phenotype. The respective diet, but not tolerization, significantly influenced the cellular biochemical components in all experimental groups. In obese, this was associated with maintaining the M1 polarized macrophages in the lungs of tolerized animals, indicating that this cell type might have a role in counteracting the allergic phenotype.

Conclusions: We demonstrated that obese and lean mice showed a robust allergic response, albeit more substantial allergic inflammation in obese. Interestingly, the oral tolerance was not impaired and efficiently attenuated the increased allergic reaction in the obese mice. We suggest that pulmonary M1 macrophages are at least partly responsible for this effect in tolerized obese mice.

Funding: This work was supported by the Austrian Science Fund (FWF) grant no. W 1248 and the Danube Allergy Research Cluster - DARC #017 of the Karl-Landsteiner University, Krems, Austria.

Alt a 1´s function as an iron scavenger in *Alternaria*

Ilir Hasanaj[1], Aila Fakhimahmadi[1,2], Gerlinde Hofstetter[1], Karin Hufnagl[1,2], Christian Radauer[2], Clara Pogner[3], Markus Gorfer[3], Erika Jensen-Jarolim[1,2], Franziska Roth-Walter[1,2]

[1]Medical University of Vienna and University of Vienna Comparative Medicine, The Interuniversity Messerli Research Institute of the University of Veterinary Medicine Vienna, Vienna, Austria

[2]Medical University of Vienna, Center of Pathophysiology, Infectiology and Immunology, Institute of Pathophysiology and Allergy Research, Vienna, Austria

[3]Austrian Institute of Technology GmbH Bioresources Unit, Center for Health & Bioresources, Tulln, Austria

Background: *Alternaria alternata* is a fungus present in the soil, the atmosphere, the plants or in indoor environments and is one of the main responsible fungi associated with allergic asthma. One major allergen of this fungus, named Alt a 1, has been identified in the extracts, to which over 80% of all asthma patients being allergic to *Alternaria* solely have IgE against. As such, we assessed the physical features and biological function of Alt a 1.

Methods: Firstly, we assessed the Alt a 1 content in *Alternaria* extracts grown under different conditions by ELISA and Western blot. The molecular features of recombinant Alt a 1 were characterized by UV-VIS spectroscopy, dynamic light scattering and circular dichroism. Lastly, the biological impact of recombinant Alt a 1 with and without ligands was investigated by conducting cellular uptake studies with human Caco-2/TC7 and THP-1 cells. Endocytic pathways were blocked to unravel uptake mechanism of Alt a 1.

Results: Under iron-deficient conditions *Alternaria* enhance Alt a 1 expression with apoAlt a 1 (ligand-free form) capable of binding to quercetin and iron-quercetin. Secondary and quaternary structure is affected by iron-quercetin binding. Uptake of apoAlt a 1 into THP-1 and Caco-2/TC7 cells was significantly higher, compared to holoAlt a 1 (ligand-bound form) with inhibitory studies indicating a dynamin and clathrin-dependent uptake of Alt a 1 into THP-1 cells.

Conclusion: In summary, Alt a 1 expression is increased under iron-deficient conditions in *Alternaria* with Alt a 1 binding strongly to iron-quercetin complexes suggesting a function as an iron scavenger. In human cells uptake of empty Alt a 1 is enhanced compared to holoAlt a 1 and clathrin-mediated. Uptake of apoAlt a 1 led to a decrease of the labile iron pool thereby potentially increasing the allergenicity of *Alternaria alternata*.

Funding: This study was funded by the Danube Allergy Research Cluster-DARC #08 of the Karl-Landsteiner University, Krems, Austria, to EJJ. FRW and EJJ are inventors on EP2894478, owned by Biomedical International R+D GmbH, of which EJJ is shareholder.

Silicon-based microarrays for allergy diagnosis

Huey-Jy. Huang[1,2], M. Tulaev[1], R. Campana[1], O. Akinfenwa[1], M. Curin[1], E. Sarzsinszky[1], A. Karsonova[3], K. Riabova[3], A. Karaulov[3], K. Niespodziana[1], O. Elisyutina[4], E. Fedenko[4], A. Litovkina[4], E. Smolnikov[4], M. Khaitov[4], S. Vrtala[1], T. Schlederer[1], R. Valenta[1,2,3,4]

[1]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Department of Pathophysiology and Allergy Research, Division of Immunopathology, Vienna, Austria

[2]Karl Landsteiner University of Health Sciences, Krems, Austria

[3]Sechenov First Moscow State Medical University, Department of Clinical Immunology and Allergology, Laboratory of Immunopathology, Moscow, Russia.

[4]NRC Institute of Immunology FMBA of Russia, Moscow, Russia.

Background: Allergy has increased in developed countries and more than 30% of people are suffering from allergic symptoms. Allergy can have a strong effect on quality of life lead to life-threatening systemic allergic reactions. Identification of disease-causing allergens by detecting allergen-specific IgE, which allows having personalized medicine for treatment and prevention of allergic diseases based on the IgE sensitization profiles of individual allergic patients. Therefore, a precise and sensitive microarray-based allergy diagnosis is in need. Highly sensitive and flexible silicon elements containing allergen microarrays can be considered as alternative to currently available array technologies.

Methods: A panel of well-characterized indoor and outdoor respiratory allergens has been spotted on silicon slides using a SciFlex array spotter. Thirty ml of serum samples were applied on each of microarrays. Fluorescence signals of allergen-specific IgE and IgG were detected by a scanner.

Results: A serum pool from allergic patients was diluted 3- to 729-fold for IgE detection and 27- to 19,683-fold for IgG detection. Silicon surfaces give 5–10-fold higher sensitivity as compared to glass, which allows detecting allergen-specific IgG and also low allergen-specific IgE levels. Sera from HDM and mite-, cat-, birch pollen-allergic patients and non-allergic subjects were applied on allergen microarrays on silicon and glass. Our results indicate that allergen microarray-based on silicon are superior to glass for IgE and IgG detection of allergens and none of the non-allergic subjects showed detectable allergen-specific IgE reactivity.

Conclusion: Silicon-based allergen microarrays provide a precise and highly sensitive tool for allergy diagnosis, offering a promising tool for the rapid serological testing of antigen-specific IgE/IgG levels with small volumes of serum. Flexible silicon elements with 150 well-characterized allergens can be assembled in different formats for different uses.

Funding: This study was supported by the Country of Lower Austrias Danube Allergy Research Cluster program and by HVD Biotech, Vienna, Austria

Poster Session 3 (Posters 19 - 26)

Chairs: Prof. Pascual and Prof. Weninger

Micro-arrayed PR10 allergens and peptides thereof

Tianchi Jiang[1], G. Brazhnikov[1], M. Focke-Tejkl[1,2], O. Akinfenwa[1], M. Tulaev[1], R. Kiss[2], R. Valenta[1,2,3,4], R. Campana[1]

[1]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Immunology, Infectiology and Immunology, Division of Immunopathology, Vienna, Austria

[2]Karl Landsteiner University of Health Sciences, Krems, Austria

[3]National Research Center, Institute of Immunology Federal Medico-Biological Agency of Russia, Moscow, Russia

[4]Sechenov First Moscow State Medical University, Laboratory of Immunopathology, Department of Clinical Immunology and Allergy, Moscow, Russia

Background: The major birch pollen allergen Bet v 1 and structurally related pathogenesis-related class 10 (PR10) proteins represent a family of cross-reactive allergens in pollen, plant food and spices. Aim of the study was to establish chips containing micro-arrayed PR10 allergens and peptides thereof for studying antibody reactivity profiles in patients allergic to PR10 proteins and in non-allergic subjects exposed to PR10 allergens.

Methods: Recombinant PR10 allergens from pollen of birch (Bet v 1), alder (Aln g 1), hazel (Cor a 1.01), oak (Que a 1) and plant-derived food from peach (Pru p 1), soy (Gly m 4), kiwi (Act d 8), carrot (Dau c 1), apple (Mal d 1), hazelnut (Cor a 1.04) and celery (Api g 1) were expressed as hexahistidine-tagged proteins in *Escherichia coli*, purified by Nickel affinity chromatography and studied for purity, fold and molecular mass by SDS-PAGE, circular dichroism (CD) spectroscopy and mass spectrometry, respectively. Two unfolded Bet v 1 fragments comprising the first and second half of Bet v 1 were also obtained by E. coli expression and six peptides derived from the corresponding regions of Bet v 1 and Mal d 1 were prepared using solid phase peptide synthesis, purified by HPLC and checked by mass spectrometry. Antigens were immobilized on pre-activated glass slides using a SciFlexArrayer S12 (Scienion AG, Berlin, Germany).

Results: We demonstrate the utility of the PR10 allergen micro-array to study the extent of IgE cross-reactivity between Bet v 1 and related PR10 allergens in birch pollen allergic patients, the natural PR10-specific antibody response in non-allergic subjects and to verify that IgE antibodies of Bet v 1-allergic patients react exclusively to folded PR10 allergens but not to unfolded Bet v 1 fragments or synthetic unfolded PR10 allergen-derived peptides. By contrast IgG antibodies form allergic patients and non-allergic subjects react with conformational as well as unfolded epitopes. The PR10 allergen micro-array is also useful to study the induction of PR10 allergen-specific IgG antibodies and their epitope specificity in allergen-specific immunotherapy (AIT). Furthermore, the PR10 allergen micro-array is capable of measuring the effect of AIT-induced blocking antibodies on IgE reactivity in AIT-treated patients.

Conclusion: Our results demonstrate that IgE and IgG antibodies from birch pollen allergic patients recognize unrelated epitopes and thus seem to origin from clonally unrelated B cells. Furthermore, the PR10 allergen micro-array is useful for diagnostic purposes to reveal IgE cross-reactivity in PR10-allergic patients and to monitor antibody responses and blocking effects of IgG on IgE binding during AIT.

Funding: This work was supported by the Austrian Science Fund (FWF) project P34472-B, the DANUBE Allergy Research program of the Country of Lower Austria and by HVD Biotech, Vienna, Austria.

Variable IgE reactivity of Austrian fish-allergic patients to parvalbumins from locally available freshwater fish species

Tanja Kalic[1,2], N. Lengger[2], S. Kamath[2], M. Domocos[2], S. Schmalz[2], A. Kuehn[3], M. Aumayr[4], G. Lamorte[4], P. Forstenlechner[4], W. Hemmer[5], C. Hafner[1], H. Breiteneder[2]

[1]University Hospital St. Pölten, Karl Landsteiner University of Health Sciences, Department of Dermatology, St. Pölten, Austria

[2]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Pathophysiology and Allergy Research, Vienna, Austria

[3]Luxembourg Institute of Health, Department of Infection and Immunity, Esch-sur-Alzette, Luxembourg

[4]MacroArray Diagnostics, Vienna, Austria

[5]Floridsdorf Allergy Center, Vienna, Austria

Background: Our recent research on a multinational fish-allergic patients' cohort indicated that up to 21% of the patients may tolerate specific bony fish species, while up to 90% may tolerate cartilaginous fish. The major fish allergen parvalbumin is the most relevant molecule for diagnosis for >80% of the patients. There is a constant effort to improve the current diagnostic methods to include relevant species for specific geographic regions, thereby enabling the efficient identification of allergenic versus tolerated species for each patient. This study investigated the IgE reactivity of Austrian fish-allergic individuals to parvalbumins from 12 freshwater bony fish species and two cartilaginous species commonly consumed in Europe.

Methods: Parvalbumins were purified from muscle tissue of 14 fish species and specific IgE quantified in sera of 59 Austrian fish-allergic patients. Basophil activation tests were performed to explore the capability of parvalbumins to induce IgE cross-linking. Parvalbumin cross-reactivity was investigated in a multiplex-based inhibition assay.

Results: Based on multiplex IgE quantification for the parvalbumins, the highest reactivity (median IgE >4 kUA/L) was found for brook trout, brown trout and Danube salmon, all belonging to the Salmonidae fish family. The lowest IgE reactivity was observed for parvalbumins from eel, tench, Wels, and cartilaginous fish. The percentage of patients positive to specific species followed a similar trend: ≥85% of the patients reacted to parvalbumins from the Salmonidae family, while <50% reacted to parvalbumins from eel, tench, Wels and cartilaginous fish. In IgE inhibition assay, parvalbumins from the Salmonidae family showed the highest potential to inhibit IgE binding to other tested parvalbumins. Between the parvalbumins from the two cartilaginous fish species, a higher reactivity to shark (44% of patients positive) than to ray (1.7% positive) was observed.

Conclusion: Parvalbumins from all 12 investigated freshwater fish species, as well as shark parvalbumin, are relevant allergens. Among Austrian fish-allergic individuals, IgE reactivity to parvalbumins from the Salmonidae family is more frequently observed than for other investigated fish families. Molecular allergy testing using the parvalbumins from various species relevant for local consumption may help identify tolerated versus allergenic species for individual patients.

Funding: This work was supported by the Danube Allergy Research Cluster P-06 funded by the Country of Lower Austria.

Deep immune profiling of peripheral blood mononuclear cells of allergic and non-allergic chronic rhinosinusitis patients

Fana Alem Kidane[1], M. Rocha-Hasler[1], M. Zghaebi[1], V. Stanek[1], A. Tu[1], L. Müller[2], T. Bartosik[1], N.J. Campion[1], A. Spittler[2], S. Schneider[1], J. Eckl-Dorna[1]

[1]Medical University of Vienna, Department of Otorhinolaryngology; Upper airway research lab Vienna, Vienna, Austria

[2]Medical University of Vienna, Core Facility Flow Cytometry & Department of Surgery, Research Lab, Vienna, Austria

Background: Chronic rhinosinusitis with (CRSwNP) and without (CRSsNP) nasal polyps is a chronic inflammatory disease of the upper airways with severe symptom burden. CRSwNP can also be associated with asthma and hypersensitivity to non-steroidal anti-inflammatory drugs in a syndrome called NSAID-exacerbated respiratory disease (N-ERD). As type 2 immune responses are key drivers of both CRSwNP and respiratory allergies, we aimed to characterize the differences in blood-derived immune cell subsets and immune marker expression in patients suffering from CRS stratified for presence or absence of respiratory allergies employing deep immunophenotyping by mass cytometry.

Methods: We determined 38 different surface markers in peripheral blood mononuclear cells (PBMCs) by mass cytometry and levels of 33 different nasal and serum cytokine levels by the MSD platform in patients with CRSsNP, CRSwNP, N-ERD and disease controls (n=6 per group with half of the patients in each group with respiratory allergies).

Results: Our analysis of mass cytometry data using a manual gating as well as unbiased clustering approach showed an increased percentage of Th2a cells in PBMCs of allergic as compared to non-allergic individuals but no difference among the CRS subgroups. Moreover, analysis of cell marker expression revealed a lower expression of the germinal center homing marker CXCR5 and a higher expression of the differentiation marker CD45RA in naïve B cells, as well as stages of NK cells with decreased levels of CD56 in patients with CRSwNP and N-ERD. Furthermore, N-ERD patients had the highest expression of type 2 nasal cytokine profile compared to the other groups, whereas no differences in nasal type 2 cytokine levels could be observed between allergic and non-allergic subjects.

Conclusions: Our results using deep immunophenotyping of PBMCs in different CRS disease revealed differential expression of homing, differentiation and activation markers in NK and B cell subsets. Moreover, blood-derived Th2a cells but not nasal cytokine levels are elevated in allergic subjects. Thus allergy and CRS despite being both type 2 driven diseases show differential effects on blood-derived immune cell subsets and nasal cytokine levels.

Investigation of mechanisms for binding, uptake and processing of virus-like nanoparticles (VNP) by antigen presenting cells (APC)

Armin Kraus[1], B. Kratzer[1], W. F. Pickl[1,2]

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

[2]Karl Landsteiner University, Krems, Austria

Background: Virus-like nanoparticles (VNP) are regarded as a safe vaccination platform and have proven to be suitable for allergen-specific immunotherapy in preclinical models. In fact, we showed previously that VNP derived from the Moloney murine leukaemia virus (MoMLV), and containing a shielded version of the major mugwort pollen allergen Art v 1 (MA::Art v 1 VNPs) were found to be hypoallergenic and to induce immunotolerance. In that study, uptake of VNP by lung APCs was revealed *in vivo*, however without elucidating the exact uptake mechanisms operative.

Methods: We here tested a collection of different inhibitors for their impact on VNP uptake by APC. Firstly, the uptake of MA::Art v 1 VNP was examined with murine DC 2.4 dendritic cells and human leukemic THP 1 monocytic cells by a split luciferase reporter system and flow cytometry. In parallel, classical fluid phase markers and fluorescently labelled VNPs were used for control purposes and to confirm results obtained within the split reporter system. Different fluorescently labelled VNP (FITC, cell-mask orange, mCherry) were used to study VNP uptake by primary APC types isolated directly from the lungs and the spleens of mice. These organs were selected to provide insight into the future prospects for success of i.t.- and i.v.-based application routes.

Results: The combined results of this study indicate that MA::Art v 1 VNPs are taken up into APC primarily by macropinocytosis, as confirmed by studies performed with *bona fide* APC-lines and primary APC. VNP uptake could be blocked with sucrose (300 - 500 mM) and Rottlerin (1 - 10 µM), both substances are well-established inhibitors of macropinocytosis.

Conclusions: We here established and validated a high-throughput split luciferase reporter-based system for the screening of a collection of uptake inhibitors and confirmed obtained results using fluorescently labelled VNP. We identified macropinocytosis as the main mode of VNP uptake by APC *in vitro* and *in vivo*, which may pave the way to further improve VNP-based applications in the future.

Funding: This work is supported by the Federal State of Lower Austria under the Danube Allergy Research Cluster (Danube ARC) grant no. FA624A0402 and the Medical University of Vienna.

Surface-exposed IL-12 efficiently enhances allergen-laden virus-like nanoparticles (VNP) to effectively modulate allergic immune responses in a preclinical model of mugwort allergy

Bernhard Kratzer[1], S. Hofer[1], P. Tauber[1], D. Trapin[1], W. Keller[2], G. Gadermaier[3] and W. F. Pickl[1,4]

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

[2]*University of Graz, Institute of Molecular Biosciences, BioTechMed, Division of Structural Biology, Graz, Austria*

[3]*University of Salzburg, Department of Biosciences, Division of Allergy and Immunology, Salzburg, Austria,*

[4]*Karl Landsteiner University, Krems, Austria*

Background: Virus-like nanoparticles (VNP) are a well-established platform for vaccination and immunomodulation. Recently, we have developed technology to decorate VNP with functionally active cytokines and to encase allergen molecules inside VNP. Here we have analyzed whether surface decoration with IL-12 of such VNP, which contain the allergen within their viral core in a form that is thoroughly shielded from the outside world, would improve their immunomodulatory properties.

Methods: The major mugwort pollen allergen Art v 1 was encased within VNP by N-terminal fusion of the Art v 1-coding sequence to the viral matrix protein MAp15 of MoMLV. The surface expression of single-chain (p35::p40) mIL-12 was achieved by its C-terminal fusion with the minimal CD16b GPI-anchor acceptor sequence. The immunomodulatory capacity of such VNP was analyzed with the help of a mugwort allergen-specific humanized mouse model *in vitro* and *in vivo*.

Results: IL-12$^+$ allergen-expressing VNP were able to induce high numbers of IFN-g$^+$CD4$^+$ T cells in sorted naïve allergen-specific T cells from humanized mice in the presence of BM-DCs, similar to classical Th1 polarizing conditions. Induced Th1 cells remained stable after re-stimulation with allergen in the presence of BM-DCs. In splenocyte cultures of humanized allergy mice, allergen-laden and IL-12 decorated VNP induced the secretion of high levels of IFN-g, moderate levels of IL-10, and very low levels of IL-4, IL-5, IL-13 and IL-17A. While IL-12$^+$ VNP moderately inhibited T cell proliferation, the proportion of IFN-g$^+$CD4$^+$ T cells was significantly increased. Among the CD3$^+$CD4$^+$IFN-g$^+$ T cells an additional population co-expressing clear-cut levels of IL-10 was induced, suggesting the induction of a Tr1 cell-like phenotype. Furthermore, IL-12$^+$ VNP strongly inhibited the expansion of allergen-specific, IL-13$^+$ Th2 cells of humanized allergy mice *in vitro*. *In vivo*, after prophylactic intranasal treatment with IL-12-decorated allergen-laden VNP and subsequent exposure of mice to allergen aerosol a reduced expansion of IL-4$^+$ and IL-13$^+$ CD4$^+$ T cells was observed compared to control allergen laden VNP, expressing an inactive form of IL-12.

Conclusion: Decoration of allergen-laden VNP with IL-12 improves their immunomodulatory capabilities and therefore could be a useful new tool for the treatment of allergies in the future.

Funding: This work is supported by the Federal State of Lower Austria under the Danube Allergy Research Cluster (Danube ARC) grant no. FA624A0402 and the Medical University of Vienna.

A novel wheat allergen chip for analysis of wheat allergen-specific antibody responses

Andrea Krisai[1], L.M. Call[1], H.-J. Huang[2], T. Schlederer[2], M. Tulaev[3], M. Focke-Tejkl[3], B. Linhart[2], R. Valenta[2, 3, 4, 5], H. Grausgruber[1]

[1]*University of Natural Resources and Life Sciences, Vienna, Department of Crop Sciences, Institute of Plant Breeding, Tulln, Austria*

[2]*Medical University of Vienna, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Division of Immunopathology, Vienna, Austria*

[3]*Karl Landsteiner University of Health Sciences, Krems, Austria*

[4]*MBA of Russia, NRC Institute of Immunology, Moscow, Russia*

[5]*Sechenov First Moscow State Medical University Laboratory for Immunopathology, Department of Clinical Immunology and Allergy, Moscow, Russia*

Background: Wheat (*Triticum aestivum*) is a major pillar of human diet, being widely grown and processed into a variety of food products. However, apart from its nutritional value, wheat has gained recognition as an important allergen source, with an increasing prevalence of wheat-related allergies worldwide. Patients suffer from wheat food allergy and respiratory allergy to wheat flour, which can also induce celiac disease. At present, 28 allergens from wheat have been characterized and listed in the International Union of Immunological Societies (IUIS) allergen nomenclature database. Aim of this study was to produce a chip containing a comprehensive panel of wheat allergen molecules for the measurement of allergen-specific IgE, IgA and IgG antibody responses.

Methods: Fourteen purified recombinant wheat allergens and 20 wheat allergen-derived synthetic peptides were prepared and spotted onto glass-slides using a sciFLEXARRAYER SX (Scienion, Berlin, Germany). The microarray contained the following allergens: Tri a 12, Tri a 15, Tri a 17, Tri a 20, Tri a 25, Tri a 26, Tri a 28, Tri a 29, Tri a 30, Tri a 32, Tri a 35, Tri a 37, Tri a 39 and avenin-like protein. In a first series of pilot experiments, sera from wheat allergic patients and non-allergic individuals were used to establish the measurement of allergen-specific IgE, IgA, and IgG antibody responses. For calibration purposes, fluorescence units obtained by chip measurements were related to the corresponding ImmunoCAP values.

Results: A wheat allergen chip containing a comprehensive collection of wheat allergens and wheat allergen-derived peptides was produced based on ISAC technology. In initial experiments using sera from wheat allergic and non-allergic individuals, we determined allergen-specific IgE, IgA, and IgG antibody responses to wheat allergens.

Conclusion: The wheat allergen chip is a useful tool to study allergen-specific antibody levels in patients suffering from different wheat-induced hypersensitivity diseases, as well as in healthy subjects for the investigation of normal antibody responses in relation to different forms of diet.

Funding: This work was supported by the DANUBE-ARC programme of the country of Lower Austria and by the Medical University of Vienna.

Modulation of human early B cell development by iberdomide-mediated degradation of Ikaros and Aiolos

Jakov Korzhenevich*[1], I. Janowska*[2], J. Staniek[2], R. Lorenzetti[2], F. Schmidt[1], L. Kostantinidis[3], M. Erlacher[4], J. Thiel[2,5], R. Voll[2], N. Venhoff[2], P. Schäfer[6], M. Rizzi[1,2,7]

*Contributed equally

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

[2]University Medical Center Freiburg, Department of Rheumatology and Clinical Immunology, Freiburg, Germany

[3]University Medical Center Freiburg, Department of Orthopedics and Trauma Surgery, Freiburg, Germany

[4]University Medical Center Freiburg, Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology and Oncology, Freiburg, Germany

[5]Medical University of Graz, Department of Internal Medicine, Division of Rheumatology and Immunology, Graz, Austria

[6]Bristol Myers Squibb, Translational Medicine, Princeton, New Jersey, Unites States of America

[7]University Medical Center Freiburg, Center for Chronic Immunodeficiency, Freiburg, Germany

Background: Immature B cells develop in the bone marrow (BM) from hematopoietic stem cells. Ikaros and Aiolos, members of the zinc-finger transcription factor family, regulate the development by activating or repressing gene expression. In humans, mutations in IKZF1, coding for Ikaros, or IKZF3, coding for Aiolos, are associated with impaired B differentiation in the bone marrow. Conversely, high Ikaros/Aiolos expression are associated with systemic lupus erythematosus (SLE) and multiple myeloma (MM). Targeted treatment with thalidomide analogues such as iberdomide to enhance degradation of Ikaros and Aiolos are used in MM patients and are under consideration in SLE. Our objective is to investigate the effect of iberdomide on human early B cell development simulated *in vitro*.

Methods: *In vitro* cultures over 3 days or 7 weeks based on bone marrow- or cord blood (CB)-derived lymphocytes, respectively, in presence of iberdomide. Spectral cytometry (17-color panel) allowing the discrimination of early B cell subpopulations.

Results: Iberdomide treatment led to enhanced degradation of Ikaros and Aiolos in BM- and CB-derived cultures. Addition of iberdomide early (day 7) to the CB-derived culture impaired the specification to the lymphoid lineage indicated by increased frequency and total numbers of $CD34^+$ progenitors, and decreased frequency of $CD10^+CD38^+$ lymphoid progenitors. Also, iberdomide delayed the commitment to the B cell lineage. These observations were confirmed by reduced *E2A* and *PAX5* gene expression, respectively.

Starting the iberdomide treatment from day 28 enhanced the proliferation of early progenitors resulting in increased total counts of $CD10^+CD38^+$ lymphoid-committed cells, however, had no effect on the development of immature B cells despite reduction of Ikaros and Aiolos protein levels by 50%.

Conclusions: Iberdomide impairs the specification to the lymphoid lineage and the commitment to the B cell lineage by enhancing Ikaros' degradation. In committed early B cells, reduction of Ikaros and Aiolos protein levels by 50% allows undisturbed development of immature B cells.

Funding: The research project was supported by Bristol-Myers Squibb (former Celgene) grant-DEU-131.

Fel d 2 is an important cross-reactive allergen for animal allergic patients: Mapping of IgE and T cell epitopes

Zicheng Liu[1], D. Trifonova[1], P. Gattinger[1], B. Linhart[1], M. Focke-Tejkl[1,2], R. Kiss[1], R. Valenta[1,2,3,4]

[1]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Pathophysiology and Allergy Research, Vienna, Austria

[2]Karl Landsteiner University of Health Sciences, Krems, Austria

[3]Sechenov First Moscow State Medical University, Laboratory for Immunopathology, Department of Clinical Immunology and Allergology, Moscow, Russia

[4]National Research Center, Institute of Immunology Federal Medico-Biological Agency of Russia, Moscow, Russia

Background: Serum albumins, in particular Fel d 2 from cat and Can f 3 from dog are important cross-reactive allergens for patients with allergy to animals. However, epitopes recognized by IgE of albumin allergic patients have not been investigated and albumin-specific T cell responses have not yet been studied.

To investigate Fel d 2-specific IgE epitopes, T cell responses in albumin allergic patients and to study cross-reactivity of antibodies induced in rabbits immunized with Fel d 2 and Fel d 2-derived peptides.

Methods: Sera and peripheral blood mononuclear cells (PBMCs) were obtained from Fel d 2-allergic patients. Thirteen peptides with a length of 29-45 amino acids spanning the surface-exposed areas of Fel d 2 were synthesized and purified. Fel d 2 and Fel d 2-derived peptides were studied for structural fold by circular dichroism (CD) spectroscopy. Patients IgE reactivity to complete folded Fel d 2, to albumins from 8 animal species as well as to the thirteen Fel d 2-derived peptides was tested by dot-blotting using ^{125}Iodine labelled anti-human IgE antibodies. Fel d 2-specific $CD4^+$ cell proliferation was investigated by FACS-based carboxifluorescein diacetate succinimidyl ester (CFSE) dilution assays in patients PBMCs. Furthermore, sera from rabbits immunized with natural Fel d 2 and KLH-coupled Fel d 2 peptides were tested for IgG reactivity to albumins from 14 animal species and human serum albumin (HSA).

Results: Results obtained so far show that Fel d 2 and Can f 3 are the most frequently recognized albumins recognized by patients IgE. Patients IgE antibodies recognize exclusively complete and structurally folded Fel d 2 but not unfolded Fel d 2-derived peptides. $CD4^+$ T cell proliferation specific for Fel d 2 and Fel d 2-derived peptides was detected in a Fel d 2-allergic patient. Rabbit IgG antibodies raised against Fel d 2 and certain C-terminal Fel d 2-derived peptides cross-reacted strongest with albumins with close sequence similarity to Fel d 2 (i.e., Can f 3 and HSA) but showed also auto-reactivity to rabbit serum albumin.

Conclusion: IgE epitopes of Fel d 2 are conformational but not sequential and Fel d 2-allergic patients contain Fel d 2-specific $CD4^+$ cells. Accordingly, it should be possible to engineer hypoallergenic immunotherapy vaccines based on Fel d 2 peptides for the treatment of albumin allergic patients. However, immunization with Fel d 2-derived peptides may induce auto-reactivity and it remains to be studied if Fel d 2-allergic patients exhibit IgE and T cell auto-reactivity to HSA.

Funding: This work was supported by the DANUBE-ARC program of the Country of Lower Austria.

Poster Session 4 (Posters 27 - 35)

Chairs: Prof. Batista and Prof. Vrtala

Optimisation of the labelling of extracellular vesicles to study their mechanism of uptake

Lukas Neuninger[1], M. Thaler[1], M.Wysmołek[1], A.Razim[1], A. Schmid[1], T. Weinmayer[1], A. Inic-Kanada[1], I. Schabussova[1]

[1]Medical University of Vienna, Institute of Specific Prophylaxis and Tropical Medicine, Centre for Pathophysiology, Infectiology and Immunology, Vienna, Austria

Background: Extracellular vesicles (EVs) are small bilayer membrane structures. Together with cytokines, chemokines and cell surface receptors, EVs play an important role in cell-cell communication. They are produced by prokaryotic and eukaryotic cells in different ways in response to external or internal stimuli. Depending on the stimulus and cellular source, EVs can contain different cargoes, including DNA, RNA, proteins and lipids. *E. coli O83* is a Gram-negative probiotic bacterium used in the clinic. We have previously shown that E. coli O83 releases outer membrane vesicles (OMVs) with immunomodulatory potential *in vitro* and *in vivo*. The mechanisms of uptake of *E. coli O83* OMVs (EcO83-OMVs) into mammalian cells have not yet been investigated.

Methods: To visualise the uptake of EcO83-OMVs into cells, vesicles were labelled with octadecyl-rhodamine B, a fluorescent dye with an alkyl tail that protrudes into the lipid membrane. EcO83-OMVs were labelled with three different concentrations of rhodamine. To remove unbound dye, vesicles were purified by a density gradient. The purified vesicles were characterised using Zetasizer a multi-angle dynamic light scattering device.

Results: We found that an amount of 30 µg of rhodamine for vesicles with a particle concentration of 10^{12} particles per ml was optimal to label them effectively. A lower amount of dye led to unlabelled vesicle in less dense fractions after the purification step and a higher rhodamine concentration led to additional unbound rhodamine within the fractions containing vesicles. Further could we show an improvement of labelling by loading the vesicle at the bottom of the density gradient.

Conclusion: We have optimised an effective method for labelling EcO83-OMVs to study their uptake into mammalian cells.

Antibodies specific for the membrane-proximal site of ICAM-1 inhibit rhinovirus infection

Katarzyna Niespodziana[1†], E.E. Waltl[2†], M. Bajic[1,3], I. Zettl[1], P. Pazderova[1], C. Weichwald[1], J. Kollarova[1], D. Ivanov[1], M. Focke-Tejkl[1], P. Errhalt[4], V. Niederberger[2*], R. Valenta[1,3,5,6*] & S. Flicker[1]

[1]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Department of Pathophysiology and Allergy Research, Vienna, Austria

[2]Medical University of Vienna, Department of Otorhinolaryngology, Vienna, Austria

[3]Karl Landsteiner University of Health Sciences, Krems, Austria

[4]University Hospital Krems and Karl Landsteiner University of Health Sciences, Department of Pneumology, Krems, Austria

[5]NRC Institute of Immunology FMBA of Russia, Moscow, Russia.

[6]Sechenov First Moscow State Medical University, Department of Clinical Immunology and Allergy, Laboratory for Immunopathology, Moscow, Russia.

[†]contributed equally and are listed in alphabetical order;

Background: Rhinovirus (RV) infections are major causes of common colds and trigger exacerbations of severe respiratory illnesses such as childhood wheeze, asthma and chronic obstructive pulmonary disease (COPD). Intracellular adhesion molecule 1 (ICAM-1) is important for regulating cellular responses in inflammation, injury resolution and tumorigenesis but also serves as receptor for major group RVs.

Objective: To identify parts of ICAM-1, which are involved in RV infection.

Methods: Eleven synthetic peptides including surface-exposed amino acids of the extracellular portion of ICAM-1 were synthesized and used to raise specific rabbit antisera. Titers of anti-peptide antibodies were calibrated and tested for cross-reactivity to each peptide. Their ability to inhibit RV infection was investigated in cellular virus-neutralization assays, in non-invasive electrical impedance-based measurements in RV-infected cultured respiratory epithelial cells and in molecular interaction assays based on purified ICAM-1 and RVs to identify ICAM-1 peptide sequences involved in RV binding and infection. Inhibition of viral genome replication by antibodies after infection of cultured HeLa cells was measured by rt-PCR.

Results: Anti-peptide10 antibodies and F(ab')2 fragments thereof were found to bind exclusively to peptide 10 from the membrane-proximal domain D5 of ICAM-1. Pepide10-specific antibodies were more effective in inhibiting cellular RV infection of HeLa cells and cultures of primary human nasal epithelial cells, RV-induced damage of respiratory epithelial cell layers and direct RV binding to ICAM-1 than antibodies raised against peptides from the so far known RV-binding site at the ICAM-1 N-terminus.

Conclusion: We identified a novel ICAM-1 peptide that is involved in RV binding to ICAM-1 and RV infection, and thus may serve as a novel target for treatment of RV infections and RV-triggered respiratory diseases.

Funding: This work was supported by grants F4605, F4607, by the project No. P29398, by the International PhD Program "Molecular, Cellular and Clinical Allergology" (MCCA) grant W-1248 of the Austrian Science Fund (FWF), by the DANUBE-ARC program of the Country of Lower Austria and by Viravaxx, Vienna, Austria.

The cytoskeletal protein profilin is a major allergen in saltwort (Salsola kali)

Ludmila Peterkova[1], P. Gattinger[1], D. Trifonova[1,2], M. Focke-Tejkl[1], V. Garib[1,3], N. Magbulova[4], G. Djambekova[4], N. E. Zakhidova[5], M. K. Ismatova[5], M. Tulaev[1], M. Kundi[6,7], A. V. Karaulov[2], R. Valenta[1,2,7,8]

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

[2]*Sechenov First Moscow State Medical University, Laboratory for Immunopathology, Department of Clinical Immunology and Allergy, Moscow, Russia*

[3]*Ministry of Higher Education, Science and Innovation, Tashkent, Uzbekistan*

[4]*International Center of Molecular Allergology, Ministry of Higher Education, Science and Innovation, Tashkent, Uzbekistan*

[5]*International Allergy Center, Tashkent, Uzbekistan*

[6]*Medical University of Vienna, Center for Public Health, Department of Environmental Health, Vienna, Austria*

[7]*Karl Landsteiner University, Krems an der Donau, Austria*

[8]*National Research Center – Institute of Immunology FMBA of Russia, Moscow, Russia*

Background: Pollen from *Salsola kali*, i.e., saltwort, Russian thistle, is a major allergen source in the coastal regions of Europe, in Turkey, Central Asia and Iran. *Salsola kali* allergic patients mainly suffer from hay-fever (i.e., rhinitis and conjunctivitis), asthma and allergic skin symptoms. The goal of this study was to investigate the possible clinical importance of individual *Salsola kali* allergen molecules.

Methods: *Salsola kali* allergen molecules rSal k 1, rSal k 2, rSal k 3, rSal k 5, rSal k 6 were expressed in *Escherichia coli* as recombinant proteins containing a C-terminal hexahistidin tag and purified by Nickel affinity chromatography. The purity of the recombinant allergens was analyzed by SDS-PAGE. Their molecular weight was determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and their fold and secondary structure was studied by circular dichroism (CD) spectroscopy. Sera from clinically well characterized *Salsola kali*-allergic patients (n=77) from Uzbekistan were used for IgE-reactivity and basophil activation experiments. *Salsola kali* allergen-specific IgE levels and IgE levels specific for timothy grass pollen profilin, Phl p 12 and the calcium-binding timothy grass pollen allergen Phl p 7, both of which contain the IgE epitopes of the corresponding *Salsola kali* profilin and calcium-binding allergen as determined by IgE inhibition experiments, were measured by ImmunoCAP. The allergenic activity of natural *Salsola kali* pollen allergens was studied in basophil activation experiments.

Results: Natural *Salsola kali* allergens induced strong and dose-dependent basophil degranulation indicating that *Salsola kali* allergens have allergenic activity in IgE-sensitized patients. Recombinant *Salsola kali* allergens were folded and exhibited the deduced molecular weight, when studied by CD analysis and mass spectrometry, respectively. The sum of recombinant allergen-specific IgE levels and allergen-extract-specific IgE levels were highly correlated. Sal k 1 and profilin were recognized by more than 50% of *Salsola kali*-allergic patients and therefore represent major allergens whereas the other *Salsola kali* allergens were less frequently recognized. Specific IgE levels were highest to profilin and profilin-specific IgE levels were higher in patients with more than one allergic symptom.

Conclusion: Profilin is a major allergen for *Salsola kali*-allergic patients and should be included in the diagnostic panel of allergen molecules and in molecular allergy vaccines for the treatment and prevention of *Salsola kali* allergy.

Funding: Funded by the Danube Allergy Research Cluster program of the Country of Lower Austria, by a grant of the Russian Science Foundation (the Project: No: 23-75-30016: „Allergen micro-array-based assessment of allergic sensitization profiles in the Russian Federation as basis for personalized treatment and prevention of allergy (AllergochipRUS)"), by a grant from the Ministry of Higher Education, Science, and Innovation, Republic of Uzbekistan (AL-482104522) and by HVD Biotech, Vienna, Austria.

Purification and characterization of poppy seed allergens

Aleksandra Podzhilkova[1], M. Bublin[1], C. Nagl[1], C. Bindslev-Jensen[2], E. Eller[2], C.G. Mortz[2], K. Hoffmann-Sommergruber[1]

[1]*Medical University of Vienna, Institute of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Vienna, Austria*

[2]*Odense University Hospital, Department of Dermatology and Allergy Center, Odense Research Center for Anaphylaxis (ORCA), Odense, Denmark*

Background: Poppy seeds (*Papaver somniferum L*) are used as ingredients and garnishing of cakes and bread. Poppy seeds contain 45–50% oil, which can be used as a source of high amount vitamin E and a moderate amount of phytosterols as compared to other plant oils. Poppy seed allergy is rare, however cases of poppy seed allergy were reported. It has been shown, poppy seed allergic patients are at risk to cross react with tree nuts. The aim of this study was to purify and characterize IgE-binding proteins from poppy seed.

Methods: Poppy seeds were obtained from a local store, ground, defatted and dried at room temperature. Afterwards, proteins were purified from poppy seed extract applying a combination of different chromatographical methods. Physiochemical characterization of purified proteins was performed by circular dichroism (CD) spectroscopy and mass spectrometry. Allergen specific IgE was evaluated by ELISA and Western blot using 43 sera from poppy seed allergic and sensitized patients.

Results: Proteins were purified according to established protocols for seed storage proteins and were checked for purity and molecular mass. CD spectrometry confirmed α-helix and β-sheet based structures that corresponding to legumin- and vicilin-like proteins. Mass spectrometry indicated theoretical molecular masses for cupin proteins: 46 and 49 kDa. In addition, an unknown protein was isolated from poppy seed extract with a molecular mass about 10-12 kDa. ELISA and Western blot confirmed IgE recognition of all three purified proteins. When testing 43 poppy seed sensitized patients' sera, 38 sera had specific IgE against poppy seed extract. Out of those, 34 and 30 were positive for legumin- and vicilin-like proteins, respectively. In addition, 23 sera showed IgE-binding to an unknown protein.

Conclusion: Our study provides novel data on poppy seed proteins and their IgE-binding capacity. In addition, the mass spectrometry data showed the similarity between poppy seed proteins and proteins from nuts, sesame and sunflower. Allergenicity and cross reactivity of poppy seed proteins will be further investigated.

Funding: Danube Allergy Research Cluster P07, FA 648AO111

Unleashing the Future of Rheumatoid Arthritis Therapy: Exploring Epigenetic Compounds

Teresa Preglej[1], M. Brinkmann[1], L. Göschl[1], A. Tosevska[1], T. Krausgruber[2], M. Bonelli[1]

[1]*Medical University of Vienna, Department of Medicine III, Division of Rheumatology, Vienna, Austria*

[2]*Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria*

Background: Rheumatoid arthritis (RA) is a systemic autoimmune disorder that, if left untreated, leads to irreversible joint destruction, disability, and chronic pain. Despite significant therapeutic advancements in the past two decades, a substantial number of patients do not respond to existing therapies, underscoring the necessity for new treatment targets. Recent research has implicated alterations in the epigenetic patterns of immune cells in the pathogenesis of autoimmune diseases. In this study, our objective is to provide additional evidence supporting the potential use of epigenetic compounds as a novel treatment strategy for RA patients.

Methods: We established an in vitro activation assay for Peripheral Blood Mononuclear Cells (PBMCs) to screen various epigenetic compounds. Activated PBMCs obtained from both RA patients and healthy controls were treated with 26 different epigenetic compounds, and the effects were assessed using a combination of RNA-sequencing and multi-color immunophenotyping. The resulting data were analyzed and integrated using state-of-the-art bioinformatic tools.

Results: Global analysis of the RNA-sequencing dataset revealed distinct clusters predominantly defined by specific compound classes. Detailed investigation of the transcriptional signatures unveiled an enrichment of genes associated with immunological processes, cytokine signaling, and autoimmune diseases, including RA. Consequently, the transcriptional overlaps with RA signature genes were utilized to rank the different compounds. The top-ranked compounds were further characterized using multi-color immunophenotyping. A custom-designed spectral flow cytometry panel enabled the identification of major PBMC subsets and assessment of the expression of 19 activation markers. The analysis revealed compound-, disease-, and cell type-specific effects, highlighting both activating and inhibitory signatures.

Conclusion: The drug screening assay employed in this study successfully identified compounds that directly target pathogenic signatures associated with RA. These compounds will undergo subsequent in vitro investigations using single-cell RNA-sequencing readout and in vivo models of RA. Ultimately, the generated data will lay the foundation for utilizing epigenetic compounds as a novel treatment strategy for RA.

Funding: This work was supported by the FWF Special Research Program F70 (F07003).

Transfer of allergen-expressing immune cells for tolerance induction in grass pollen allergy

Lisa Prickler[1], A.M. Weijler[1], V. Kainz[1], R. Steiner[1], K. Mengrelis[1], J. Mucha[1], B. Kratzer[2], U. Baranyi[3], N. Pilat[1], W.F. Pickl[2], R. Valenta[4,5,6,7], B. Linhart[4], T. Wekerle[1]

[1]*Medical University of Vienna, Department of General Surgery, Division of Transplantation, Vienna, Austria*

[2]*Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Immunology, Vienna, Austria*

[3]*Medical University of Vienna, Department of Cardiac Surgery, Vienna, Austria*

[4]*Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Pathophysiology and Allergy Research, Vienna, Austria*

[5]*National Research Center, Institute of Immunology Federal Medico-Biological Agency of Russia, Moscow, Russia*

[6]*Sechenov First Moscow State Medical University, Department of Clinical Immunology and Allergy, Laboratory for Immunopathology, Moscow, Russia*

[7]*Karl Landsteiner University of Health Sciences, Krems, Austria*

Background: Prophylactic strategies to prevent allergy development through establishing tolerance remain an unmet medical need. We have recently developed an adoptive cell transfer protocol inducing allergen-specific tolerance through transplantation of allergen-expressing bone marrow cells. In this study, we investigated whether the transfer of allergen-expressing lymphocyte subsets is able to induce allergen-specific tolerance.

Methods: $CD19^+$ B cells from Phl p 5-transgenic BALB/c mice were isolated and transferred to naive BALB/c mice, pretreated with a short course of rapamycin and anti-CD40L antibody. Mice were immunized s.c. 3 times in 4-week intervals with Phl p 5 and Bet v 1 as control antigen. Peripheral blood was taken to determine the induced molecular chimerism by FACS analysis and serum samples were analyzed for Phl p 5 and Bet v 1-specific IgE and IgG_1 levels by ELISA. For investigation of allergen-induced lung inflammation a whole body plethysmography (WBP) was performed.

Results: Transfer of $10*10^6$ Phl p 5-expressing purified $CD19^+$ B cells to naive BALB/c mice induced B cells chimerism for up to 2-3 months and prevented the development of a Phl p 5-specific IgE and IgG1 antibody response for the length of the so far follow-up period (15 weeks). Tolerance induction was specific for Phl p 5, as antibody responses to the control allergen, Bet v 1 were induced. $CD19^+$ B cell-treated mice showed preserved lung function upon intra nasal application of Phl p 5 in contrast to sensitized controls.

Conclusion: We demonstrated that the transfer of Phl p 5-expressing $CD19^+$ B cells induces allergen-specific tolerance. These findings underscore the potential of prophylactic adoptive cell transfer, for inducing tolerance in IgE mediated allergy.

Funding: The work was funded by the DANUBE-ARC grant of the country of Lower Austria

Characterization of the L. plantarum extracellular vesicles

Agnieszka Razim[1], D. Parthasarathy[1], E. Krčmářová[2], M. Wysmołek[1], A. Schmid[1], P. Migdał[3],C. Daniel[4], A. Inic-Kanada[1], U. Wiedermann[1], S. Górska[3], I. Schabussova[1]

[1]Medical University of Vienna, Institute of Specific Prophylaxis and Tropical Medicine, Vienna, Austria

[2]1st Faculty of Medicine Charles Univeristy, Institute of Immunology and Microbiology, Prague, Czech Republic

[3]Hirszfeld Institute of Immunology and Experimental Therapy PAS, Wroclaw, Poland

[4]University of Lille, CNRS, INSERM, CHU Lille, Institut Pasteur de Lille, U1019 - UMR 9017 – Center for Infection and Immunity of Lille, F-59000 Lille, France

Background: It has recently become known that bacteria release extracellular vesicles (bEVs) containing specific cargo such as lipids, proteins, lipopolysaccharides, lipoproteins, nucleic acids and peptidoglycan. The role of these vesicles is not yet fully understood. We also do not know how they interact with our body or whether the bEVs reflect the properties of the parent bacterium. This knowledge can be used to develop new carriers based on bacterial cell vesicles for vaccines or anti-allergy therapies. In our research, we characterize the vesicles of the probiotic *Lactobacillus plantarum* NCIMB8826 in the terms of their cargo and immunomodulatory properties.

Methods: *L. plantarum* NCIMB8826 was grown anaerobically in MRS medium (37 °C) to OD of 1.5. bEVs were isolated by centrifugation (4 000 x g, 20 min), filtration (0.22 µm filter), concentration by Amicon Stirred Cell (300 kDa filter) and ultracentrifugation (3 h, 150 000 x g). bEVs were purified with the IZON qEV original column and characterized by Zetasizer (size and particle numbers), TEM, SDS-PAGE (with MS protein identification), Bioanalyzer (RNA, DNA and protein content). The immunomodulatory properties and cytotoxicity of the bEVs have been tested *in vitro*. Stability studies are ongoing.

Results: We are able to isolate up to 2 x 10^{12} bEVs from a 5-litre bacterial culture. The produced EVs are less than 100 nm in size and contain diverse proteins and small RNA. Interestingly, we could not detect DNA. bEVs have different immunomodulatory properties than the parent bacterium and are not toxic when tested *in vitro*.

Conclusion: It has already been shown that *L. plantarum* species have an immunomodulatory effect. We have shown that this Gram-positive bacteria produces EVs and we have characterized them. This is the first step towards developing a platform for an intranasal vaccine or anti-allergy therapy based on probiotic bEVs.

Funding: This research was funded by HORIZON-MSCA-2021-PF (project no. 101066450), Danube Allergy Research Cluster and Foundation for Polish Science (FNP).

Differences in cross-reactive IgG responses after flavivirus infections

Lena D. Roßbacher[1], S. Malafa[1], K. Huber[2], J.H. Aberle[1], S.W. Aberle[1], F.X. Heinz[1], K. Stiasny[1]

[1]Medical University of Vienna, Center for Virology, Vienna, Austria

[2]Ludwig-Maximilians-University, Department of Infection and Tropical Medicine, Medical Clinic and Polyclinic IV, Munich, Germany

Background: Flaviviruses comprise important human arthropod-borne pathogens, including dengue virus (DENV), Zika virus (ZIKV) and tick-borne encephalitis virus (TBEV). Due to global warming flavivirus vectors continuously expand their habitats, resulting in increased co-circulation of different flaviviruses, for example, dengue and Zika viruses in tropical and sub-tropical regions. Furthermore, ticks whom transmit tick-borne encephalitis (TBE) constantly increase their territories in Europe. Hence, the importance of understanding the effect of pre-existing flavivirus exposure on secondary immune responses. In our study, we addressed this issue by analyzing human polyclonal serum samples from TBE and DEN patients with and without prior flavivirus immunity with respect to their binding and neutralizing ability against different flaviviruses.

Methods: Antibody binding efficiency of human sera to virus specific and broadly flavivirus cross-reactive antigens was evaluated by ELISA. To assess the antibody neutralizing ability, neutralization tests were executed. The contribution of cross-reactive antibodies to virus neutralization was determined by depletion experiments.

Results: Both homologous and heterologous pre-existing immunity had a strong impact on the extend of virus-specific IgG antibody response after TBEV infections, inducing a substantial TBE-specific antibody booster. Moreover, naïve and homologous pre-immune TBE patients had lower cross-reactive antibody titers. In contrast, high amounts of cross-reactive IgG antibodies could be found in heterologous pre-immune individuals. Interestingly, pre-immune dengue patients show differences in their immune responses, with a broader spectrum of virus-specific and cross-reactive antibody titers. Furthermore, broadly flavivirus cross-reactive antibodies play a major role in dengue virus neutralization. However, cross-reactive antibodies do not contribute to TBEV neutralization.

Conclusion: These findings suggest that different flaviviruses induce variable amounts of cross-reactive antibodies, which might be explained by distinctive structural properties of the respective viruses. The data obtained from this study highlights the influence of a pre-existing memory on antibody fine specificities but also provide valuable information for flavivirus vaccination efficacy.

Funding: This work was supported by the Austrian Science Fund (FWF) grant no. P29928-B30

Tropomyosin is the major allergen in patients with severe anaphylactic reactions to shrimp but in vitro immunological analysis cannot predict severity of symptoms

Eszter Sarzsinszky[1], H.-J. Huang[1,6], M. Tulaev[1], R. Kiss[1], M. Focke-Tejkl[1], T. Schlederer[1], W. Hemmer[2], N. Douladiris[3], V. Garib[1], R. Valenta[1,4,5,6], S. Vrtala[1]

[1]Medical University of Vienna, Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Vienna, Austria,

[2]Floridsdorf Allergy Center, Vienna, Austria,

[3]2nd Pediatric Clinic, National & Kapodistrian University of Athens, Department of Allergy and Clinical Immunology, Athens, Greece

[4]NRC Institute of Immunology FMBA of Russia, Moscow, Russian Federation,

[5]Sechenov First Moscow State Medical University, Laboratory of Immunopathology, Department of Clinical Immunology and Allergy, Moscow, Russian Federation

[6]Karl Landsteiner University for Healthcare Sciences, Krems, Austria

Background: Shrimp allergy is a growing health concern because it can lead to severe and life-threatening anaphylactic reactions. To investigate what allergen molecules are associated with severe shrimp allergy and if *in vitro* parameters such as allergen-specific IgE levels, IgE recognition of sequential epitopes and/or allergen-

specific basophil activation can serve as biomarkers for severe anaphylactic reactions upon ingestion of shrimp in sensitized patients.

Methods: Recombinant tropomyosins from shrimp *(Penaeus monodon)*, mites *(Dermatophagoides pteronyssinus, Blomia tropicalis)*, cockroach *(Blatella gemanica)*, worm *(Anisakis simplex)* and snail *(Helix aspersa)* as well as Pen m 2 and Pen m 4 were expressed and purified. Nine peptides spanning the Pen m 1 sequence were synthesized. Specific IgE levels were measured by ImmunoCAP, ImmunoCAP ISAC microarray, and using an in-house microarray chip (TM-chip) in shrimp allergic patients´ sera. Severity of clinical symptoms in patients were graded according to the World Allergy Organization Guidance 2020 (WAO). Allergenic activity was measured by basophil activation assays.

Results: All patients with relevant anaphylactic symptoms to shrimp (=/>WAO grade 3) showed IgE reactivity to tropomyosins. Only few patients were sensitized to Pen m 2 and/or Pen m 4 and Pen m 1 elicited basophil activation at lower concentrations than Pen m 2 and Pen m 4. There was no association between tropomyosin-specific IgE levels, IgE recognition of Pen m 1-derived peptides and basophil sensitivity to Pen m 1 with the severity of symptoms.

Conclusion: Tropomyosins from different species show IgE cross-reactivity and Pen m 1-specific IgE reactivity is a biomarker for shrimp allergy. However, the *in vitro* immunological parameters tested in our study cannot predict the severity of clinical reactions to shrimp ingestion.

Funding: Supported by a grant of the Danube Allergy Research Cluster from the Country of Lower Austria

Poster Session 5 (Posters 36 - 43)

Chairs: Prof. Carsetti and Prof. Radbruch

Immunological characterization of a novel preclinical TCR- and HLA-transgenic humanized model for birch pollen allergy

Mirjam Schaar[1], B. Kratzer[1], A.N.A. Sehgal[1], D. Trapin[1], B. Bohle[2], A. Neunkirchner[1], W.F. Pickl[1,3]

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

[2]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Department of Pathophysiology and Allergy Research, Vienna, Austria

[3]Karl Landsteiner University, Krems, Austria

Background: Sensitization to birch (*Betula verrucosa*) pollen is one of the main causes of pollinosis in the Northern hemisphere. Bet v 1 represents the major birch pollen allergen, to which over 95% of birch pollen allergic patients are sensitized. Approximately 70% of Bet v 1-sensitized individuals also suffer from oral allergy syndrome (OAS) caused by cross-reactivities of fruits, nuts and vegetable allergens with Bet v 1. Previous studies identified Bet v $1_{142\text{-}153}$ as the immunodominant T cell epitope in birch pollen allergic individuals and elucidated its contribution to Bet v 1-associated OAS. In addition, certain HLA molecules have been shown to be associated with a higher likelihood of developing allergy to birch pollen, as has already been demonstrated for other allergens, such as for the major mugwort pollen allergen Art v 1.

Methods: A double transgenic mouse model expressing a human Bet v $1_{142\text{-}153}$-specific T cell receptor (TCR) and the human leukocyte antigen (HLA)-DR7 was generated, to investigate the processes of allergic sensitization and specific organ manifestations upon natural allergen exposure *via* the airways.

Aims: The primary objective of this study is to systematically characterize the novel Bet v 1-specific mouse model. In a first step, the immunological phenotypes of the different, non-exposed mouse strains (wt, TCR-tg, HLA-DR7-tg, TCR/HLA-DR7-tg) will be characterized using multiparameter flow cytometry, immunohistochemistry and multiplex ELISA. Next, the impact of different allergen exposure regimen (acute, chronic, intermittent) will be evaluated regarding induction of airway hyperresponsiveness (Penh), allergic airway remodeling, induction of Bet v 1-specific IgE (and other antibody classes) and changes in systemic and local cytokine levels. Finally, the new preclinical model will be used to (re-)evaluate established and novel preventive and therapeutic strategies for allergic diseases using active and passive allergen-specific vaccination approaches, monoclonal antibodies targeting cytokines, TCR-signal modulators and small molecule inhibitors (SMI), among others.

Outlook: By establishing a preclinical model responsive to natural exposure to birch pollen allergens and using it to investigate the processes of allergic sensitization and disease, this study will contribute to our better understanding of the modalities to prevent and treat this highly prevalent disease.

Funding: This work is supported by the Federal State of Lower Austria under the Danube Allergy Research Cluster (Danube ARC) grant no. FA624A0402 and the Medical University of Vienna

Dysregulation of the B cell compartment and low BAFF-R in AAV patients

Franziska Schmidt*[1], N. Venhoff*[2], R. Lorenzetti*[2], S. Pfeiffer[2], L. Niessen[2], J. Korzhenevich[1], I. Janowska[2], J. Staniek[2], R. E. Voll[2], M.-T. Bott[2], J. Thiel*[2,3], M. Rizzi*[1,2]

*Contributed equally

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

[2]University of Freiburg, Faculty of Medicine, Medical Center - University of Freiburg, Department of Rheumatology and Clinical Immunology, Freiburg, Germany

[3]Medical University of Graz, Department of Internal Medicine, Division of Rheumatology and Immunology, Graz, Austria

Background: B cells are central in the pathogenesis of ANCA associated vasculitides (AAV). The efficacy of remission induction and maintenance by B cell depleting therapy strongly underlines the importance of B cells in the disease and the use of RTX has successfully been implemented in the treatment of AAV. However, B cell depletion after application of RTX is markedly prolonged in AAV compared to other autoimmune diseases, indicating an impairment of the B cell compartment.

Methods: We analyzed the B cell compartment in AAV patients treated with RTX or not and healthy controls (HD) using spectral cytometry. For analysis, patients were grouped according to therapy.

Furthermore, serum BAFF concentration was measured and BAFF-R expression of isolated B cells was determined by qPCR and Western Blot.

Results: In general, B-lymphocytopenia could be observed in most patients. Low transitional B cell numbers in untreated patients as well as in the RTX+azathioprine/mycophenolate mofetil group could indicate an impaired B cell development. Contrary, transitional B cell counts were significantly increased in both the RTX only and the RTX+leflunomide/methotrexate treated group, indicative of beginning or ongoing B cell repopulation. Interestingly, the increased amount of transitional B cells did not lead to replenishment of the later stages of peripheral B cell maturation in these groups, pointing to a maturation stop in peripheral B cell reconstitution.

In line, low BAFF-R expression caused by enhanced BAFF-R processing was detected in AAV B cells and might contribute to impaired B cell survival in the periphery of patients.

Conclusions: The prolonged depletion of B cells in AAV patients after RTX therapy might indicate an underlying problem in the B cells that is unmasked by treatment. Low transitional B cells and low BAFF-R expression could contribute to a delayed recovery of the B cell pool after RTX treatment in AAV.

The essential role of Ikaros and Aaiolos in plasma cell differentiation and homeostasis

Tanja Anke Schwickert[1], Q. Sun[1], K. Schindler[1], K. Froussois[1], M. Jaritz[1], M. Busslinger[1]

[1]Research Institute of Molecular Pathology (IMP), Vienna Biocenter (VBC), Campus-Vienna-Biocenter 1, Vienna, Austria

Background: The differentiation of B cells into long-lived plasma cells, crucial for antibody secretion in response to pathogenic invaders, involves significant changes in gene expression. While certain transcription factors such as Blimp1, Xbp1, and Irf4 have been recognized for their roles in plasma cell development, the specific functions of Ikaros and Aiolos, members of the Ikaros transcription factor family, remain unknown.

Methods: In this study, we employed conditional gene deletion and acute protein degradation techniques to investigate the transcriptional role of Ikaros and Aiolos in antibody-secreting cells. We introduced a novel plasma cell-specific Cre line, *Bhlha15*-Cre, and utilized the improved auxin-inducible degron (AID) system (Yesbolatova et al., 2020) for the targeted degradation of Ikaros and Aiolos both *in vitro* and *in vivo*.

Results: Our findings indicate that while plasmablast differentiation is dependent solely on Ikaros, both transcription factors are indispensable for the maintenance of long-lived plasma cells. Deletion of either Ikaros or Aiolos alone was tolerated by plasma cells but resulted in a defect in their longevity. The enhanced AID system facilitated the efficient degradation of Ikaros and Aiolos in both *in vitro* and *in vivo* antibody-secreting plasmablasts. Prolonged protein degradation successfully replicated expected phenotypes, confirming the essential role of Ikaros in plasmablast differentiation. Acute degradation, on the other hand, enabled the analysis of transcriptional activity prior to the emergence of secondary phenotypic defects.

Conclusion: Therefore, through RNA-sequencing experiments conducted after acute degradation, we identified direct target genes regulated by Ikaros in antibody-secreting plasmablasts. In contrast to previous reports, our findings demonstrate that Ikaros acts as a dedicated repressor. Ikaros downregulated many signal transducers and transcriptional regulators, especially members of the NfκB pathway. These results highlight the importance of Ikaros in suppressing excessive activation to facilitate plasmablast differentiation.

Funding: This work was supported by the Austrian Science Fund (FWF) grant no. P 34372.

Altered-peptide ligands and IL-15: An effort to improve virus-like nanoparticle-based hypoallergenic allergy vaccines

Al Nasar Ahmed Sehgal[1], J. Schmid[1], B. Kratzer[1], D. Trapin[1], W.F. Pickl[1,2]

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

[2]Karl Landsteiner University, Krems, Austria

Background: At least 95% of mugwort allergic patients are sensitized against the major mugwort pollen allergen Art v 1. In the context of allergen-specific immunotherapy (AIT), we harnessed a virus-like nanoparticle platform to encapsulate full-length Art v 1, the major mugwort pollen allergen, or its altered-peptide ligands (APLs) exhibiting single amino acid substitutions in the TCR recognition site, armed with surface-decorated mIL-15 cytokine aiming to direct differential TCR signaling and induce a Th1(/Treg) response.

Methods: To generate such VNPs, HEK293T cells were co-transfected with the expression constructs coding for MAp15 (Moloney murine leukemia virus, MoMLV, matrix protein) fused to his-tagged versions of Art v 1 (wt, APL derivatives) and mIL-15 linked to the CD16b GPI-anchor attachment sequence in addition to MoMLV original gap-pol plasmid for particle production. The presence of the surface-anchored mIL-15 and intracellular Art v 1-His proteins was validated with flow cytometry of the producer cell line and biochemical characterization of VNPs was performed with the Western blot. Through double sandwich ELISA, the amount of Art v 1-His inside the VNP cargos was quantified which allowed standardization

for functional analyses. Biological assessment of purified VNPs was evaluated with splenocytes and bone marrow-derived dendritic cells (BMDCs) from Art v 1-specific hTCR/DR1 double-transgenic allergy mice and cellular proliferation/activation as well as the profile of secreted cytokines was monitored.

Results: Surface-exposed mIL-15 VNPs upregulated activation markers (HLA-DR, CD40, CD80, CD86) on BMDCs after coincubation for 24 hours. In addition, MA::Art v 1-His+mIL-15::GPI VNPs showed robust splenocytes stimulation compared to the unchaperoned MA::Art v 1 expressing VNPs while 'empty' VNPs displaying only the respective cytokines entirely failed to activate Art v 1-specific T cells. Likewise, the ratio of Th1(Treg)/Th2 cytokine production determined through multiplexing was influenced by the encased Art v 1 APLs and mIL-15-decorated versions of VNPs.

Conclusion: The here presented VNP-based strategy of delivering shielded Art v 1 APLs together with membrane-bound interleukins may prove instrumental in optimizing particle-based prophylactic and/or therapeutic allergy vaccines.

Funding: This work is supported by the Federal State of Lower Austria under the Danube Allergy Research Cluster (Danube ARC) grant no. FA624A0402 and the Medical University of Vienna.

Affinity matters for IgE-blocking: Evidence from allergen-specific monoclonal IgG1 antibodies sourced from an individual after successful immunotherapy

Maria R. Strobl[1], H. Demir[1], G. Stadlmayr[2], F. Stracke[2], R. Hoelzl[2], B. Bohle[1], G. Wozniak-Knopp[2]

[1]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Pathophysiology and Allergy Research, Vienna, Austria

[2]BOKU University of Natural Resources and Life Sciences, Department of Biotechnology, Institute of Molecular Biotechnology, Vienna, Austria

Background: The induction of allergen-specific blocking IgG antibodies is a hallmark of successful allergen immunotherapy. Recently, passive immunotherapy with humanized allergen-specific IgE-blocking monoclonal antibodies (mAbs) successfully reduced respiratory symptoms of birch pollen and cat allergic individuals. Interestingly, cocktails of allergen-specific mAbs more efficiently prevented effector cell activation than isolated patient-derived polyclonal IgG antibodies, which displayed lower affinities than the engineered mAbs. This tempted us to assess the potential correlation of antibody binding strength and IgE-blocking capacity.

Methods: RNA was isolated from peripheral blood mononuclear cells of an individual who, after daily sublingual administration of the recombinant major apple allergen Mal d 1 for 16 weeks, displayed reduced allergic symptoms to apple. Clinical improvement was accompanied by Mal d 1-specific IgG1 blocking antibodies. Fab fragments were generated by yeast display technology and selected for Mal d 1-binding. Specific Fabs were reformatted to full IgG1 antibodies and expressed in mammalian cells. The allergen-specificity of these mAbs was confirmed by ELISA. Their kinetics of allergen binding were studied with surface plasmon resonance. Also, mAbs were added to Mal d 1 at molar ratios of 1:1, 10:1, and 100:1 and tested for their ability to inhibit allergen-induced activation of basophils from non-treated individuals with birch pollen-related apple allergy. Two clones were affinity matured by light chain shuffling and their characteristics were compared to the parental clones.

Results: Four engineered IgG1 mAbs displayed strong Mal d 1-binding and three of them inhibited Mal d 1-induced basophil activation at an antibody:allergen ratio of 100:1. Affinity maturation of two mAbs resulted in a total of five descendants with a 2- to 5-fold higher affinity than their parental antibody. These descendants displayed a stronger IgE-blocking activity than their parental clones in basophil inhibition tests at each tested molar ratio of antibody to allergen.

Conclusions: We demonstrate that a high affinity of allergen-specific antibodies is essential for efficient inhibition of IgE-mediated effector cell activation. Therefore, allergen-specific mAbs of highest affinities should be favored for optimal success of passive immunotherapy.

Funding: This work was supported by the Austrian Science Fund (FWF) grants no. P32953 and I4437, the Austrian Jubiläumsfond, project no. ÖNB17947, and by the Danube Allergy Research Cluster, Country of Lower Austria, and Medical University of Vienna, Austria. The project was also supported by EQ-BOKU VIBT GmbH and the BOKU Core Facility Biomolecular & Cellular Analysis. The Austrian Federal Ministry for Digital and Economic Affairs, the National Foundation for Research, Technology and Development, and the Christian Doppler Research Association are gratefully acknowledged.

Transcriptome analysis reveals the nasal provocation–associated cellular responses in birch pollen allergic and non-allergic subjects

Srinidhi Sudharson[1,2], A. Meshcheryakova[2], J. Eckl-Dorna[3], S. Derdak[4], N. Schweitzer[3], N. Lengger[2], T. Kalic[1,2], D. Mechtcheriakova[2], H. Breiteneder[2], C. Hafner[1]

[1]University Hospital St. Pölten, Karl Landsteiner University of Health Sciences, Department of Dermatology, St. Pölten, Austria.

[2]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Pathophysiology and Allergy Research, Vienna, Austria.

[3]Medical University of Vienna, Department of Otorhinolaryngology, Vienna, Austria.

[4]Medical University of Vienna, Core facilities, Vienna, Austria.

Background: Birch is one of the major elicitors of pollinosis in Europe. Interaction of birch pollen (BP) with airway epithelial cells results in an immune response, which may lead to allergic sensitization in certain individuals. In the current pilot study, we aim to characterize the cellular responses upon BP nasal provocation (NP) in BP-allergic and non-allergic individuals, using a systems biology approach.

Methods: BP-allergic (n=11) and non-allergic individuals (n=12) were recruited and informed consent was obtained. The individuals were subjected to NP with saline solution (N1) as baseline and an aqueous BP NP solution (N2), on two separate days. Nasal scrapings (NS) were obtained 15 minutes after N1. Participants were assigned to four different time points to obtain NS after N2. Total RNA was isolated from NS, sequencing was performed and gene expression profiles between N1 and N2 were compared. The differentially expressed genes (DEGs) were further analyzed.

Results: At time points 15, 30, 60 and 120 minutes after nasal provocation (N2), 8, 123, 13 and 21 DEGs in allergic patients and 13, 3, 21 and 7 DEGs in non-allergic subjects, respectively, were obtained. Preliminary gProfiler over-representation analysis with combined DEGs from all time points revealed significantly enriched cytokine signaling pathways in allergic patients and keratinization related pathways that are common in both allergic and non-allergic groups. Ingenuity pathway analysis with combined DEGs identified 92 significant Canonical Pathways and 3122 Upstream Regulators in allergic

patients. The Signature Tool from GENEVESTIGATOR dissected top 50 conditions that showed highest similarity with the herein defined gene expression signature.

Conclusion: Our results demonstrate activation of cellular pathways related to cell adhesion, innate immune responses and cell proliferation in allergic patients after NP. These dissected pathways open a new perspective to study the roles of novel targets and key players in BP allergy.

Funding: This work was supported by the Danube Allergy Research Cluster P-06 funded by the Country of Lower Austria.

From antibodies to the clinical development of several B-cell peptide-based vaccine candidates

Joshua Tobias[1], J. Wilkinson[1], A. Schmid[1], P. Steinberger[2], S. Högler[3], E. Garner-Spitzer[1], M. Kundi[4], C. C. Zielinski[5], U. Wiedermann[1]

[1]Medical University Vienna, ISPTM, Center for Pathophysiology, Infectiology and Immunology, Vienna, Austria

[2]Medical University Vienna, Institute of Immunology, Center for Pathophysiology, Infectiology and Immunology, Vienna, Austria

[3]University of Veterinary Medicine Vienna, Institute of Pathology, Unit of Laboratory Animal Pathology, Vienna, Austria

[4]Medical University Vienna, Department of Environmental Health, Center for Public Health, Vienna, Austria

[5]Vienna Cancer Center, Medical University Vienna, and Vienna Hospital Association

Background: The application of therapeutic monoclonal antibodies (mAb) by passive immunization/transfer has shown tremendous success in treating various types of cancers and infectious diseases. However, as a distinct advantage, active immunization/vaccination with B cell peptide/mimotope-based vaccines, in addition to being cost-effective, can induce immunological memory for the production of antibodies in the host with a similar therapeutic effect as the corresponding mAbs. In line with this approach, using a computer algorithm, we have previously formulated a Her-2/neu vaccine (HER-Vaxx), comprising B-cell peptide from the binding site of Trastuzumab®, inducing anti-tumor immune responses and immunological memory in preclinical and clinical investigations.

Methods: Using overlapping peptides derived from the sequence of the proteins associated with a respective disease, a platform, for systematic identification and characterization of mimotopes representing linear or conformational B cell peptides/mimotopes of therapeutic mAbs, has been established.

Results: The established platform has resulted in the identification of several B cell peptides/mimotopes from the immune checkpoint inhibitors and the Her-2/neu-targeting mAb Pertuzumab®, which after active immunization have shown a significant capacity in inducing an anti-tumor effect in syngeneic mouse models for Her-2/neu-expressing solid tumor and lung metastases. The application of the platform has also recently resulted in the identification of B-cell peptides as potential vaccine candidates against SARS-CoV-2 or other human coronaviruses.

Conclusion: The platform has demonstrated a proven capacity in identifying several immunogenic B cell-peptide/mimotope-based vaccine candidates, with potential translation into the clinic, reflecting a paradigm change from passive immunization to vaccination-based therapies.

Funding: The Medical University of Vienna, and a research grant from Imugene Ltd (until 31.10.2020) to the Medical University of Vienna.

Novel recombinant hypoallergenic cat allergy vaccine for immunotherapy

Daria Trifonova[1], M. Curin[1], M. Focke-Tejkl[1,2], M. van Hage[3], J. Konradsen[4,5], U. Käck[6,7], R. Kiss[2], H-J. Huang[1], S. Vrtala[1], K. Riabova[8], A. Karsonova[8], A. Karaulov[8], R. Valenta[1,2,8,9]

[1]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Department of Pathophysiology and Allergy Research, Division of Immunopathology, Vienna, Austria

[2]Karl Landsteiner University of Health Sciences, Krems, Austria

[3]Karolinska Institute and University Hospital, Department of Medicine Solna, Immunology and Allergy Unit, Stockholm, Sweden

[4]Karolinska University Hospital, Astrid Lindgren Children's Hospital, Stockholm, Sweden

[5]Karolinska Institutet, Department of Women's and Children's Health, Stockholm, Sweden

[6]Karolinska Institutet, Södersjukhuset, Department of Clinical Science and Education, Stockholm Sweden

[7] Sachs' Children and Youth Hospital, Södersjukhuset, Stockholm, Sweden

[8]Sechenov First Moscow State Medical University, Department of Clinical Immunology and Allergy, Laboratory of Immunopathology, Moscow, Russian Federation

[9]NRC Institute of Immunology FMBA of Russia, Moscow, Russia

Background: More than 200 million people worldwide are allergic to cats. Cat allergic patients often suffer from severe allergic symptoms, especially allergic asthma. The most effective treatment for allergy is allergen-specific immunotherapy (AIT) but current allergen extract-based vaccines can induce severe side effects. We report the engineering and characterization of a recombinant cat allergy vaccine based on a fusion protein consisting of hypoallergenic peptides derived from the three most important cat allergens Fel d 1, Fel d 4 and Fel d 7 fused with the hepatitis B virus-derived PreS surface antigen as an immunological carrier protein.

Methods: Five recombinant fusion proteins containing differently arranged allergen-derived peptides, tentatively named SuperCat 1-5 were expressed in Escherichia coli, purified, characterized for physicochemical properties and investigated regarding their allergenic activity (i.e., IgE reactivity and induction of effector cell activation in cat allergic patients). Their ability of inducing protective IgG responses was evaluated by immunization of rabbits in comparison with currently registered allergen extract-based vaccines.

Results: SuperCats 1-5 lacked relevant IgE reactivity and did not induce basophil activation demonstrating lack of allergenic activity. Allergen-specific IgG antibodies induced by immunization of rabbits with only two injections, especially with SuperCat 1 and 5, but not with registered extract-based vaccines, were able to block cat allergic patients IgE binding to all three important cat allergens, Fel d 1, Fel d 4 and Fel d 7, and even to cross-reactive major dog and horse allergens.

Conclusion: SuperCats can now be evaluated in clinical trials for safety and efficacy for treating patients suffering from cat allergy.

Funding: Supported by the Danube Allergy Research Cluster funded by the country of Lower Austria, by a grant from Worg Pharmaceutical, Hangzhou, China, and by the grant from the Russian Science Foundation (project no.: 23-75-30016: "Allergen micro-array-based assessment of allergic sensitization profiles in the Russian Federation as basis for personalized treatment and prevention of allergy (AllergochipRUS)")

Poster Session 6 (Posters 44 - 52)

Chairs: Prof. Busslinger and Prof. Rizzi

Generation of caninized checkpoint inhibitors anti-PD1 and anti-PD-L1 mAbs with specific IgG1 and IgG4 canine constant regions for cancer treatment in dogs

Alexandra Tsal-Tsalko[1,2], P. Drakopoulou[1,2], G. Hofstetter[1], M. Matz[2], S. N. Karagiannis[3,4], E. Jensen-Jarolim[1,2], R. Bianchini[1,2]

[1]Medical University of Vienna, University of Veterinary Medicine Vienna, University of Vienna, the Interuniversity Messerli Research Institute, Department of Comparative Medicine, Vienna, Austria

[2]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Immunology, Vienna, Austria

[3]King's College London, Guy's Hospital, School of Basic & Medical Biosciences, St. John's Institute of Dermatology, 9th Floor Tower Wing, London SE1 9RT, United Kingdom.

[4]King's College London, Guy's Cancer Centre, School of Cancer & Pharmaceutical Sciences, Breast Cancer Now Research Unit, London SE1 9RT, United Kingdom.

Background: Although dogs and humans have similar cancer types, cancer-related morbidity, and mortality, anti-cancer checkpoint inhibitors against PD-1 and PD-L are unavailable for canine cancer. However, PD-1 and PD-L1 are expressed in dogs, with 66.2% and 75.7% amino acid similarities between humans and dogs, respectively. This study aimed to describe caninized IgG1 or IgG4 anti-PD1 and anti-PDL1 checkpoint inhibitors and investigate their effects on canine cancer cell lines.

Methods: To express canine antibodies, Expi293F and ExpiCHO cells were transfected with vectors containing canine IgG1 or IgG4 constant regions and the variable regions of humanized monoclonal antibodies pembrolizumab (anti-PD-1) and atezolizumab (anti-PD-L1). Using affinity chromatography, recombinant canine antibodies were purified from supernatants and analyzed by SDS-PAGE. AF647 antibody labeling kit labeled pembrolizumab, atezolizumab, and their canine counterparts. The labeled antibodies, as well as unlabeled antibodies plus a secondary antibody rabbit anti-dog IgG F(ab')2 PE, were applied in flow cytometry to stain dog cancer cell lines D17, CF33, and CF41.

Results: Expi293F and ExpiCHO cells produced atezolizumab IgG1 with a yield of 0.4 mg/ml, and the antibody had been correctly assembled, as determined by SDS-PAGE. In all tested cancer cells D17, CF33, and CF41, humanized pembrolizumab and atezolizumab recognized canine homologous PD-1 and PD-L1. Additionally, purified recombinant caninized atezolizumab IgG1 detected PD-L1 on D17, CF33, and CF41.

Conclusions: Humanized monoclonal antibodies pembrolizumab and atezolizumab recognize PD-1 and PD-L1 molecules expressed by canine cancer cell lines. Moreover, our newly developed caninized atezolizumab IgG1 is correctly assembled and, like humanized atezolizumab, can bind PD-L1 expressed by canine cancer cells. Further investigation of atezolizumab IgG1's functional properties will be conducted. Our results suggest that checkpoint inhibitors can be an innovative treatment for dogs with cancer.

Funding: This work was supported by the Messerli Foundation, Sörenberg, Switzerland, partly by Danube Allergy Research Cluster (DARC), project #08 of the Karl Landsteiner University, Krems, Austria.

Vaccination with recombinant grass pollen allergy vaccine BM325 is safe and induces protective Phl p 5-specific immune response in non-allergic subjects

Inna Tulaeva[1,2], D. Trifonova[1,2], P. Gattinger[1], M. Tulaev[1], M. Weber[1], M. Focke-Tejkl[1], U. Wiedermann-Schmidt[3], R. Valenta[1,2,4,5]

[1]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Pathophysiology and Allergy Research, Vienna, Austria

[2]Sechenov First Moscow State Medical University, Department of Clinical Immunology and Allergology, Laboratory of Immunopathology, Moscow, Russia

[3]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Specific Prophylaxis and Tropical Medicine, Vienna, Austria

[4]NRC Institute of Immunology FMBA of Russia, Moscow, Russia

[5]Karl Landsteiner University for Health Sciences, Krems, Austria

Background: Allergy is the most common hypersensitivity disease affecting more than 30% of the world population and no specific prophylactic measures have been introduced so far. The recombinant peptide-carrier fusion vaccine BM32 has substantially alleviated seasonal symptoms in grass pollen allergic patients in the course of four clinical trials. Here we characterize the immune response to BM325, a component of BM32 containing B cell epitopes of timothy grass pollen allergen Phl p 5, in non-allergic subjects in order to investigate the vaccine's prophylactic potential.

Methods: Thirty-two subjects with no serological and clinical signs of grass pollen allergy have been immunized with 5 x 20 ug BM325 or placebo in a monthly interval, serum samples had been collected monthly up to one year from baseline (ClinicalTrials.gov ID: NCT03625934, the assessment of allergen-specific immune responses is an academic substudy). The levels of Phl p 5-specific immunoglobulins were assessed by ELISA. The inhibition of the binding of grass pollen allergic patients' IgE to Phl p 5 was determined with a competitive IgE-inhibition ELISA. The potential of the induced antibodies to inhibit the basophil β-hexosaminidase release was investigated using humanized rat basophil leukemia cells which had been loaded with serum IgE from grass pollen allergic patients. The sensitization profile of treated subjects was determined by ImmunoCAP ISAC allergen microarrays.

Results: We found that BM325 induced the increase of Phl p 5-specific IgG levels in sera of BM325-vaccinated subjects but not in placebo group. The subclass analysis has shown that the Phl p 5-specific antibody response was composed mainly of IgG1 but also IgG4 has been induced. Importantly, we observed no induction of Phl p 5-specific IgE in the course of immunization as well as no changes in the sensitization profile before and after treatment. The induced antibodies were capable of inhibiting allergic patients' IgE binding to Phl p 5 as well as blocking Phl p 5-induced basophil degranulation.

Conclusion: Here we demonstrate that BM325 can be safely applied to non-allergic patients. The vaccination elicited the Phl p 5-specific antibody response which is able to block allergic immune reactions. These results pave the road to further studies of allergen-specific prophylactic immunization and developing strategies for early prevention of allergy.

Funding: This work was supported by the Danube Allergy Research Cluster (DARC) and Viravaxx AG research grants.

Diphtheria cases in Austrian asylum centers collide with an insufficiently seroprotected resident population

Angelika Wagner[1], J. Jasinska[1], D. Schmid[2], M. Kundi[3], U. Wiedermann[1]

[1]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Specific Prophylaxis and Tropical Medicine, Vienna, Austria

[2]Medical University of Vienna, Center of Pathophysiology, Infectiology and Immunology, Division of Infection Diagnosis & Infectious Disease Epidemiology, Vienna, Austria

[3]Center of Public Health, Institute of Environmental Hygiene; Medical University Vienna, Vienna, Austria

Background: Diphtheria cases increased in several European countries - including Austria - since the beginning of 2022. So far, cases occurred in migrant communities, but the question arose on how well the population is protected to avoid transmission.

Methods: Therefore, we aimed to analyze retrospectively seroprotection rates and antibody waning against diphtheria toxoid (DT) across all age groups from samples tested at the Austrian reference laboratory for diphtheria, tetanus and pertussis serology at the ISPTM between March 1, 2010 and January 31, 2022. In addition, we compared results with tetanus toxoid (TT) antibody concentrations, if available, since generally combined diphtheria and tetanus vaccines are used. Anti-DT and -TT antibody concentrations were assessed by commercial ELISA according to the manufacturer´s instructions (Binding site) and antibody concentrations ≥0.1 IU/mL considered as seroprotective.

Results: We detected an overall prevalence of seroprotection against DT of 63.96% (95%CI: 62.82%; 65.09%; 6,554/10,247) and against TT of 95.99% (95%CI: 95.43%; 96.48%; 7,712/8,034) between 2018 and 2022. DT seroprotection differed between age groups (p<0.0001) with lowest seroprotection prevalence in ≥60 years-old (51.39%; 95%CI: 47.75%; 55.01) and with the highest prevalence of seroprotection in the ≥15-<60 year-olds (66.43%; 95% CI: 65.19%; 67.65%). Concordantly, the geometric mean antibody concentration (GMC) against DT was lowest in the ≥60 years-old (0.9 IU/mL; 95%CI 0.08;0.10). In contrast, the GMC against TT was 7.9-fold higher compared to that for DT. From 89 individuals, antibody concentration results from multiple time points between 2010 and 2022 were available to determine antibody waning. The annual percentage change of TT antibody concentrations was higher (6.9%) than for DT (2.9%).

Conclusion: The observed and considerable lower seroprotection against DT compared to TT is most likely attributable to missed booster vaccinations and lower immunogenicity of the reduced DT dose in booster vaccine formulations. From public health perspective raising awareness for regular booster vaccinations against diphtheria in combination with tetanus and pertussis throughout a lifetime is important to close gaps in seroprotection.

Funding: Third party funding (diagnostic research) from the Institute of Specific Prophylaxis and Tropical Medicine, Medical University Vienna.

Two-year follow-up of immune responses to primary and booster vaccination with COVID-19 mRNA vaccines in immunocompromised individuals.

Angelika Wagner[1], P. Gattinger[2], **C. Auer[1],** E. Garner-Spitzer[1], A. Wessely[1], P. Pichler[1], I. Zwazl[1], M. Orola-Taus[1], S. Tobudic[3], M. Kundi[4], H. Stockinger[5], R. Valenta[2], G. Novacek[6], C. Zielinski[7], W. Reinisch[6], U. Wiedermann[1]

[1]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Specific Prophylaxis and Tropical Medicine, Vienna, Austria

[2]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Department of Pathophysiology and Allergy Research, Division of Immunopathology, Vienna, Austria

[3]Medical University of Vienna, Department of Internal Medicine I, Division of Infectious Diseases and Tropical Medicine, Vienna, Austria

[4]Medical University of Vienna, Center for Public Health, Vienna, Austria

[5]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute for Hygiene and Applied Immunology, Vienna, Austria

[6]Medical University of Vienna, Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Vienna, Austria

[7]Central European Cancer Center, Wiener Privatklinik, Vienna, Austria, and Central European Cooperative Oncology Group, HQ: Vienna, Austria

Background: Patients with immunocompromising conditions are at increased risk for severe COVID-19. Since, COVID-19 mRNA primary vaccination was less immunogenic in immunocompromised patients, recommendations for additional doses and shorter booster intervals after primary vaccination followed. Yet, the effect of breakthrough infections on antibody levels as well as the response to virus variants is less well established in immunocompromised individuals.

Methods: Therefore, we investigated immune responses to primary and booster vaccinations in patients with different immunocompromising conditions using monovalent SARS-CoV-2 mRNA vaccines. We included patients with solid tumors of the lung or breast (SoTu, n=63), multiple myeloma (MM, n=70) as well as inflammatory bowel disease (IBD, n=130) and healthy controls (n=66). Binding S1-specific antibody levels were measured to the ancestral virus hu-1 before and after each vaccination up to the fourth dose. Additionally, Omicron BA.4/5 RBD-specific binding and inhibitory antibodies were analyzed before and after booster vacciantions. In a subgroup of participants, we further determined cellular responses before and after booster doses (results pending). Moreover, breakthrough infections were recorded.

Results: Although the majority of SoTu and MM patients seroconverted after primary COVID-19 vaccination, 7% of SoTu and 17% of MM showed early antibody waning and became seronegative already before six months after primary vaccination. Similar findings were obtained in IBD patients on anti-TNF treatment. Of note, in these patients spike-specific B memory cells also declined six months after the second dose. While after the third vaccine dose antibody concentrations increased in all immunocompromised patients, breakthrough infections (28% SoTu, 26% MM, 39% IBD and 24% controls), though with mild course, occurred during the Omicron wave. Following breakthrough infection, antibody levels increased until the next booster dose. Omicron variant BA.4/5 binding and inhibitory antibodies were diminished after the third and fourth dose in the immunocompromised groups.

Conclusion: Immunocompromised patients showed earlier antibody waning to mRNA COVID-19 vaccination, possibly associated with reduced long-term protection. Particularly the immunocompromised may benefit from adapted vaccines. These findings may also indicate for other vaccine-preventable diseases that vaccine responsiveness should be verified after primary vaccination in immunocompromised.

MHC-specific IgE augments allo-specific T cell proliferation and donor-specific B cell activation in a CD23-dependent manner in mice

Anna M. Weijler[1], M. Muckenhuber[1], M. Buresch[1], B. Linhart[2], T. Wekerle[1]

[1]Medical University of Vienna, Department of General Surgery, Division of Transplantation, Vienna, Austria

[2]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Department of Pathophysiology and Allergy Research, Division of Immunopathology, Vienna, Austria

Background: Donor-specific IgE was demonstrated in murine and human transplant recipients. While the role of IgE in allergy and other TH2 type diseases is well described, the question of a possible pathomechanism of this antibody isotype in transplant rejection remains unanswered. IgE immune complex formation and subsequent binding to CD23 (FcεRII) on B cells is connected to increased T cell activation and elevated allergen-specific antibody levels in allergy. Here we investigate a possible similar role of MHC-specific IgE in transplant rejection.

Methods: Serum isolated from C57BL/6 mice after rejection of a fully mismatched BALB/c cardiac allograft was incubated with donor MHC monomers in order to form MHC-IgE immune complexes. Heat inactivation of serum was used to inactivate IgE in control samples. Hindlimb footpads of C57BL/6 mice were injected with monomer-incubated serum samples followed by isolation of draining lymph nodes (dLN) after 7 days and analysis via Flow Cytometry. Additionally, a group of mice was treated systemically with an anti-CD23 antibody (clone B3B4), specifically inhibiting the binding of IgE, before footpad injection.

Results: Injection of heat-inactivated serum as well as blocking of CD23 in recipient mice significantly decreased CD4+ and CD8+ T cell activation as well as CD8+ T cell proliferation in the dLN. Decreased levels of CD4+ T cell proliferation were seen in dLN injected with heat-inactivated serum but not in αCD23 treated mice. Levels of MHC class I alloreactive germinal center B cells were significantly decreased when heat-inactivated serum was injected or CD23 was blocked.

Conclusion: These results suggest that MHC-IgE complexes augment T cell activation as well as levels of donor-specific B cells. Donor-specific IgE might play a role in transplant rejection via formation of MHC-IgE immune complexes and binding to CD23.

Funding: Weijler AM is a recipient of a DOC fellowship of the Austrian Academy of Sciences (DOC/25556). The work was funded by the DANUBE-ARC grant of the country of lower Austria.

γδ T cells in Allergy and its Cross-linking to Obesity and Oral Tolerance Development

Tamara Weinmayer[1], N. Geissler[1], M. Haas[1], A. Schmid[1], I. Schabussova[1], U. Wiedermann[1], A. Inic-Kanada[1]

[1]Medical University of Vienna, Institute of Specific Prophylaxis and Tropical Medicine, Center for Pathophysiology, Infectiology and Immunology, Vienna, Austria

Background: Obesity impairs the function of the lung mucosal tissue, resulting in increased susceptibility to allergies. Evidence shows that γδ T cells promote allergic airway hyper-reactivity by producing Th2-type cytokines and regulating inflammatory responses. Here, we hypothesize that γδ T cells could play a role in allergy development and immune tolerance, and these processes are dependent on the used diet.

Methods: Male C57BL/6 mice were fed high-fat- (HFD) or standard chow diet (STD) for nine weeks, followed by immunization and challenge with OVA. Tolerance was induced orally or intranasally with OVA before sensitization. Lung and gut tissue were taken and used for analysis. The γδ T cell receptor gene expression was determined by RNA isolation, reverse transcription into cDNA, and quantitative polymerase chain reaction (PCR). Hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) staining were used for histological analysis. Immunohistochemistry was performed to localize γδ T cells in the lung and gut samples.

Results: We observed the infiltration of immune cells in the lung in allergic obese and lean mice, which indicated lung tissue inflammation. The gene expression of γδ T cells in the lung of allergic obese mice was significantly reduced compared to lean mice. Interestingly, the intranasal treatment with OVA increased γδ T cell mRNA irrespective of the used diet. Trendwise, the increase of γδ T cell mRNA was observed in both diet groups, but this result was statistically significant only in lean mice compared to orally tolerized lean mice and lean sham control.

In the gut tissue, we observed no significant differences in γδ T cell gene expression between the diet groups. Immunohistochemistry staining confirmed γδ T cell populations in the lung and gut tissue in obese and lean mice.

Conclusion: Further experiments on deciphering the role of γδ T cells in oral tolerance in obesity are ongoing. Understanding the mechanisms that mediate a cross-talk between obesity, allergy, and tolerance may lead to the identification of novel treatments for morbidities associated with a Westernized lifestyle.

Funding: This work was supported by the Austrian Science Fund (FWF) grant no. W 1248 and the Danube Allergy Research Cluster - DARC #017 of the Karl-Landsteiner University, Krems, Austria.

Characterizion of mono- and polyclonal antibodies for the detection of soy in foods

Michael Wiederstein[1], K. Lauter[1], S. Baumgartner[1]

[1]University of Natural Resources and Life Sciences, Vienna, Department of Agrobiotechnology IFA-Tulln, Institute of Bioanalytics and Agro-Metabolomics, Tulln, Austria

Background: Soybean (Glycine max) is the most used vegetarian protein source and a common ingredient of industrially produced foods. In addition to several nutritional and health benefits, soybean is classified as an allergenic source affecting 0.2-0.4% allergic individuals worldwide, even causing severe allergic reactions such as anaphylaxis. Therefore, the detection of soybean allergens in food is a crucial step in allergen avoidance and contributes to allergy risk management.

Methods: Soybean specific monoclonal antibodies were produced by immunizing Balb/c mice with defined soybean extracts containing high amounts of the respective soybean allergen. Additionally, soybean-specific polyclonal antibodies were collected from the serum of soybean immunized rabbits. The antibodies were characterized with different immunological methods including Western Blot and different formats of ELISA. Cross-reactivity tests were performed with extracts of peanut, lentil, bean, hazelnut wheat, lupine, milk to exclude false-positive results and to confirm the specificity of the soybean-specific antibodies. Further, soybean containing and soy-free foods were analyzed.

Results: Four monoclonal antibodies were obtained after successful immunization of Balb/c mice with soybean extracts and the following cell fusion and cell cultivation. Western Blotting and ELISA using well-characterized soybean extracts confirmed the specificity of the monoclonal antibody to soybean allergens. As expected, the polyclonal soybean-specific antibodies recognized several soybean allergens. The applied soybean-specific antibodies recognized soybean allergens in different soybean extracts and soybean-containing foods.

Conclusion: Antibody-based immunoassays are a suitable, state-of-the-art method, to specifically detect food allergens in different extracts and food samples. These soybean-specific antibodies will be further used in the ELISA and LFD development to improve allergen screening of food material.

Funding: This work was supported by the Government of Lower Austria under grant agreement K3-T-74/001-2019.

Epitope mapping of SARS-CoV-2 RBD for the identification of B-cell peptide candidates towards vaccine development

Joy Wilkinson[1], Joshua Tobias[1], Ursula Wiedermann-Schmidt[1]

[1]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Specific Prophylaxis and Tropical Medicine, Vienna, Austria

Background: With the onset of SARS-CoV-2 variants and subsequent infections, multiple means of acquiring immunity and mitigating infection have been developed, in particular by passive immunization-based therapies with monoclonal antibodies (mAbs). Unlike vaccines, however, mAbs do not provide long-term acquired immunity to patients. Thus, by applying a platform for the identification of binding epitope/mimotope of the therapeutic mAbs (Tobias et al, 2020, 2022), we aimed to identify B-cell peptide candidates representing the binding epitopes of the therapeutic SARS-CoV-2 mAbs Cilgavimab, Tixagevimab and Sotrovimab targeting the RBD of the virus. Vaccines constructed based on such B-cell/mimotopes would not only induce antibodies with similar functionality as the respective mAbs in the host, but also induce long-term immunological memory against SARS-CoV-2.

Methods: Based on the established platform, colony blot, dot blot, and Western blot assays were used as preliminary screenings to identify conformational epitopes of Cilgavimab, Tixagevimab and Sotrovimab on the original (Wuhan) SARS-CoV-2 RBD, using 50-mer amino acid overlapping peptides spanning the domain. Biotinylated Peptide ELISA using 15-mer peptides spanning the RBD were tested to identify the linear epitopes of the above-mentioned mAbs, as well as plasmablast-based mAbs and serum samples from mRNA–based individuals vaccinated with Pfizer mRNA vaccine, and serum samples from infected individuals. Synthesized unconjugated peptides were then used in inhibition ELISAs to evaluate the capacity of the peptides to obstruct the binding of therapeutic mAbs to the SARS-CoV-2 RBD (Wuhan).

Results: Two peptides and their adjacent regions were identified using the platform, as being major linear binding epitopes overlapping with conformational epitopes, across tested mAbs. These peptides were consistently dominantly present in multiple assays with therapeutic mAbs and the examined sera from post-vaccinated and post-infected individuals. These peptides also showed a capacity in inhibiting the binding of other human coronavirus peptides to the mAbs.

Conclusion: Peptides identified using the established platform may be formulated in B-cell peptide-based prophylactic vaccines to induce long-term immunological protection for not just SARS-CoV-2, but as possible pan-coronavirus vaccines.

Funding: The Medical University of Vienna.

Controlled nasal allergen exposure in allergic patients expands IgE$^+$ plasmablasts expressing pre-existing IgE VH regions

Mohammed Zghaebi[1], A. Neumann[2], V. Stanek[1], N. J. Campion[1], N. Schweitzer[1], A. Tu[1], V. Garib[3], I. Tulaeva[3], S. Schneider[1], L. Müller[4], K. Gangl[1], T. Bartosik[1], S. Vopava[5], S. Flicker[3], A. Spittler[4], T. Perkmann[5], R. Valenta[3,6], J. Eckl-Dorna[1]

[1]Medical University of Vienna, Department of Otorhinolaryngology, Vienna, Austria

[2]Omiqa Bioinformatics, Berlin, Germany

[3]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Department for Pathophysiology and Allergy Research, Division of Immunopathology, Vienna, Austria

[4]Medical University of Vienna, Department of Surgery and Core Facility Flow Cytometry, Vienna, Austria.

[5]Medical University of Vienna, Department of Laboratory Medicine, Vienna, Austria.

[6]Karl Landsteiner University of Health Sciences, Krems, Austria

Background: IgE plays a crucial role in orchestrating allergic inflammation in atopic subjects by mediating effector cell activation as well as promoting T-cell proliferation through IgE-mediated facilitated antigen presentation. The boosting of secondary allergen-specific IgE responses in allergic patients is not yet completely understood. Here we aimed to characterize the secondary allergen-specific IgE responses in birch pollen allergic patients following controlled intranasal allergen provocation.

Methods: Four birch pollen allergic subjects were intranasally provoked for three consecutive days with birch pollen extract. Blood samples were obtained 1, 2 and 3 weeks after provocation. Nasal and serum allergen-specific immunoglobulins (Ig) levels were measured by ELISA and ImmunoCAP. Analysis of allergen-specific B-cells was conducted by flow cytometry. Immunophenotyping of 30 different immune cell populations was performed by mass cytometry. IgE Next-Generation Sequencing was used for the characterization of IgE repertoire.

Results: We found a clonal expansion of a rare population of allergen-specific IgE^+ plasmablasts as early as one week after allergen provocation, coinciding with an increase in serum Bet v1-specific IgE. IgE clonal lineages and heavy chain variable region (VH) gene families identified at baseline persisted and expanded during the observation period.

Conclusion: Our pilot study in birch pollen allergic patients indicates that nasal respiratory allergen contact expands a pre-existing population of clonally related/identical IgE sequences suggesting a direct activation of allergen-specific IgE memory B-cells located in or close to the nasal mucosa, which give rise to IgE^+ plasmablasts and rises in secondary IgE production.

Funding: Supported by the Country of Lower Austria's funded Danube Allergy research cluster (DARC).

IRC/RPT Poster Sessions

Poster Session 1 (Posters 2 - 9)

Chairs: Dr. Vietzen and Assoc. Prof. Bond

MacDeath: A cell death screen identifies novel macrophage-depleting agents for the therapy of macrophage activation syndrome and cancer.

Paul Ettel[1*], Stephan Forisch[1*], Mario Mazic[1], Andrea Vogel[1], Roko Sango[1,2], Velina S Atanasova[1], Anna Gschwendtner[1], Helmut Dolznig[1], Markus Hengstschläger[1], Thomas Rattei[2], Markus Kubicek[3], Mario Mikula[1], Thomas Weichhart[1,*]

[1]*Medical University of Vienna, Center for Pathobiochemsitry & Genetics, Vienna, Austria*

[2]*University of Vienna, Centre for Microbiology and Environmental Systems Science, Vienna, Austria*

[3]*CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria*

[*]*Contributed equally*

Background: Tissue resident macrophages are key players in fighting pathogens and maintaining tissue homeostasis. Yet, in diseases like cancer or autoinflammatory diseases macrophages have been shown to profoundly worsen disease progression. However, there is a lack of drugs that target macrophages for therapeutic benefit in these diseases. Here, we aimed to identify drugs that deplete macrophages and used these as novel therapeutic approaches in mouse models of macrophage activation syndrome and melanoma.

Methods: A drug screen was carried out measuring ATP to assess cell viability. Cell viability of bone-marrow derived macrophages *in vitro* was investigated by using a PrestoBlue Cell Viability assay. To determine peritoneal macrophage populations after treatment with the drugs of interest, peritoneal macrophages were isolated and analyzed by flow cytometry. Therapeutic potential of these drugs was tested by using established *in vivo* models of macrophage activation syndrome and melanoma in wildtype C57BL6/J mice.

Results: A large drug screen of 1943 compounds identified 228 drugs that were able to kill bone-marrow derived macrophages (BMDMs) *in vitro*. Further *in vitro* studies showed that out of these candidate drugs 3 specifically were able to kill macrophages without affecting other cell types. To evaluate whether these drugs are also able to kill macrophages *in vivo*, we injected these drugs *i.p.* and analyzed macrophage populations in the peritoneum. This led to a significant reduction of tissue resident macrophages. Additionally, we investigated if the candidate drugs could be used as a treatment in a mouse model of macrophage activation syndrome (MAS) and melanoma. Indeed, in the MAS model, treatment with 2 of the drugs significantly decreased splenomegaly – a hallmark of disease progression – and all 3 drugs impaired hepatic inflammation significantly, another key symptom. Moreover, all three candidate drugs significantly decreased tumor size in the melanoma mouse model.

Conclusion: In this study we identified drugs that can kill macrophages *in vitro* and *in vivo*. Additionally, we were able to show that these drugs can improve the outcome in preclinical models of macrophage activation syndrome and melanoma.

The impact of maternal SARS-CoV-2 infection on offspring immunity

Alina Fokina[1], R. Gawish[1], A. Hladik[1], K. Lakovits[1], S. Knapp[1]

[1]*Medical University of Vienna, Department of Medicine I, Research Division Infection Biology*

Background: Physiologically, the immune system undergoes several alterations during pregnancy, which can influence the ability to defend against an infection. The COVID-19 pandemic posed a recent challenge for pregnant women, who were at higher risk of developing severe disease and pregnancy complications. The precise mechanisms underlying the reportedly altered disease severity during pregnancy as well as the impact of maternal infections on the immune response of the offspring is not well understood.

Methods: We induced murine COVID-19 using a mouse-adapted SARS-CoV-2 virus (maVie16) and infected timed-pregnant dams (E14.5) to analyze the immune response. In a second set of experiments, we tested the impact of maternal infection on the offspring's immune response to an unrelated pathogen and infected young adult mice from naïve or post-COVID mothers with *S. pneumonia* intranasally.

Results: SARS-CoV-2 infection during pregnancy resulted in a dampened inflammatory response and decreased lung inflammation that came at the cost of a delayed viral clearance. Although the infection was maternally restricted, pregnant dams showed some placental alterations.

When we infected the offspring of dams that experienced COVID-19 during pregnancy with *S. pneumonia*, we discovered a faster induction of pro-inflammatory mediators in the lung, which resulted in an augmented early neutrophil recruitment to the site of infection. This response ultimately resulted in significantly improved survival from pneumococcal pneumonia.

Conclusion: The immune response to SARS-CoV-2 infection during pregnancy is dampened, and a maternal infection imprints on the immune response of the offspring.

Funding: This work was supported by the Austrian Science Fund (FWF).

Mitochondrial oxidative stress promotes an IL-4 producing phenotype in human T cells

Tobias Frey[1], K. Schaffer[1], L.Kojic[1], M. Hollenstein[1], R. Schmidt[1], K.G. Schmetterer[1]

[1]*Medical University of Vienna, Department of Laboratory Medicine, Vienna, Austria*

Background: Numerous studies have indicated that the production of reactive oxygen species (ROS) influences intracellular signaling pathways and thereby shapes effector functions of T cells. One major source of ROS is the superoxide production by mitochondria. Yet, the exact contributions of different ROS levels on immune cell function are only incompletely understood.

Methods: Human CD4+ and CD8+ T cells were activated with anti-CD3/anti-CD28 antibodies in the presence of the Coenzyme Q10 analogue Mitoquinone-mesylate (MitoQ), which increases superoxide levels in a dose-dependent fashion. In parallel, CRISPR/Cas9-mediated knockout of Superoxide Dismutase 1 (SOD1) in primary human T cells was performed, leading to disabled ROS scavenging and ensuing higher superoxide levels. Subsequently, in both mod-

els, effector functions were measured by flow cytometry, multiplex ELISA and qPCR. Similarly, RNAseq from human CD4+ T cells activated in the presence of MitoQ was performed.

Results: Activation of CD4+ and CD8+ T cells in the presence of MitoQ led to a marked decrease of most effector functions including proliferation, expression of activation markers and cytokine production. Of note, IL-4 production was initially also decreased but showed a marked rebound at later time points of activation. This was observed at the protein level as well as the transcription level, indicating that superoxide stress leads to an altered expression dynamic of IL-4. This observation was replicated in the SOD1 knockout Supernatants from MitoQ treated T cells led to a strong Th2 polarization when transferred to naïve CD4+ T cells. As first insights into the mechanism, RNAseq data revealed gene signatures relating to the ER stress response thus potentially indicating a link between oxidative stress and IL-4 production.

Conclusion: Our data strongly indicate that oxidative stress induces a distinct IL-4 producing phenotype. This may especially be of interest as novel mechanism triggering Th2 polarization and allergic sensitization.

Funding: This work was supported by the Austrian Science Fund (FWF) grant no. P 34728-B.

The adaptor protein TRAT1 modulates Th17 and Treg effector functions

Tobias Frey[1], K. Schaffer[1], L.Kojic[1], M. Hollenstein[1], R. Schmidt[1], **K.G. Schmetterer[1]**

[1]Medical University of Vienna, Department of Laboratory Medicine, Vienna, Austria

Background: T Cell Receptor Associated Transmembrane Adaptor 1 (TRAT1) is an adaptor protein with multiple intracellular signal domains which is associated with the TCR. Yet, the role of TRAT1 in CD4+ effector and regulatory T-cell responses is only incompletely understood.

Methods: Expression of TRAT1 was measured in resting and anti-CD/anti-CD28 activated Teff and Treg specimen by intracellular flow cytometry. For functional testing we performed CRISPR/Cas9-mediated knockout of TRAT1 or retroviral overexpression in primary human CD4+ T cells. Subsequently, bulk RNAseq from TRAT1-KO CD4+ T cells and functional testing of TRAT1-KO and TRAT1-tg Teff and Treg was performed.

Results: Expression analysis using qPCR and flow cytometry showed low expression levels in Treg compared to Teff, which further induced TRAT1 expression following anti-CD3/anti-CD28 activation. RNAseq analyses revealed gene signatures of hyper-activated PI3K signaling in TRAT1-KO T cells which was supported by increased proliferation, surface marker expression and phosphorylation of STAT transcription factors. In contrast, a marked down-regulation of IL-17 was observed, which was mechanistically related to the hyperactivation of STAT6. Retroviral overexpression of TRAT1 into Treg led to enhanced suppressive capacity in both iTreg and tTreg was associated with the up-regulation of the TGFb-associated functional markers LAP and GARP. Activation of tTreg in the presence of IL-2 led to increased TRAT1 expression in these cells. Accordingly, TRAT1-KO tTreg showed less suppressive capacity in response to IL-2. Mechanistically, this was related to the down-regulation of STAT3 signaling in TRAT1-tg Treg.

Conclusion: Our data support crucial roles for TRAT1 signaling in the function of Th17 and regulatory human CD4+ T cells.

Funding: This work was supported by the Austrian Science Fund (FWF) grant no. P 34728-B.

Better induction of broadly neutralizing antibodies with recombinant fusion protein combining HBV PreS with wildtype and omicron-derived RBD than with strain-specific fusion proteins or mixes thereof

Pia Gattinger[1], B. Kratzer[2], A. Ohradanova-Repic[3], A.A. Sehgal[2], L. Gebetsberger[3], G. Tajti[3], H. Stockinger[3], W. F. Pickl[2,4], R. Valenta[1,4]

[1]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Department of Pathophysiology and Allergy Research, Vienna, Austria

[2]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Immunology, Vienna, Austria

[3]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute for Hygiene and Applied Immunology, Vienna, Austria

[4]Karl Landsteiner University of Health Sciences, Krems, Austria

Background: The global COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has slowed down due to natural and vaccine-induced immunity as well as the current dominance of less pathogenic strains. Nevertheless there is a not yet met need for safe vaccines capable of boosting cross-protective antibody responses to former, current and future virus variants in low- or non-responders and in persons who remain at risk to develop severe forms of disease.

Objectives: To design, produce and characterize strain-specific (i.e., wildtype or omicron), bivalent (i.e., mix of wildtype and omicron) and chimeric (i.e., combination of wildtype and omicron) SARS-CoV-2 protein subunit vaccines (PreS-RBD) based on recombinant fusion proteins consisting of two RBDs fused to hepatitis B virus (HBV) surface antigen PreS.

Methods: *In vitro* characterization of strain-specific, bivalent and chimeric vaccines by protein chemical, immunological methods and immunization of BALB/c mice with aluminium-hydroxide adsorbed proteins and aluminium hydroxide alone (i.e., placebo) to study specific antibody and cytokine responses, safety and cross-protective virus neutralization.

Results: All subunit vaccines could be easily produced, were stable at 4°C and safe. Antibodies induced after vaccination with different doses of strain-specific, bivalent and chimeric PreS-RBD fusion constructs reacted with wild type and omicron RBD in a dose-dependent manner. RBD-specific IgG levels induced by the different vaccines were comparable but the chimeric PreS-RBD induced virus neutralization titres against omicron (median VNT50: 5000), which were 2-7-fold higher than the strain-specific and 6-fold higher than the bivalent vaccine.

Conclusion: The chimeric PreS-RBD subunit vaccine, which can be easily produced by adsorption to aluminium hydroxide, demonstrates stability at 4°C, enables the administration of precise doses, and exhibits a favourable safety profile. It was superior regarding induction of neutralizing antibodies as compared to strain-specific and bivalent subunit vaccines of the same type. Accordingly, the chimeric PreS-RBD subunit vaccine is an excellent candidate for further evaluation in *in vivo* protection models and clinical studies.

Funding: Supported in part by the Danube Allergy Research Cluster program funded by the Country of Lower Austria and the Austrian Science Fund, Grant numbers: DK-W1248 and P 34253-B.

The role of host cellular proteins in SARS-CoV-2 innate immune evasion

Laura Gebetsberger[1], G. Tajti[1], F. Fontaine[2], T. Hannich[2], N. Marella[2], A. Mueller[2], H. Stockinger[1], A. Ohradanova-Repic[1]

[1]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute for Hygiene and Applied Immunology, Vienna, Austria

[2]CeMM - Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

Background: Many enveloped viruses incorporate biologically active host cellular proteins into their virions during the assembly or budding process from infected cells. The functional significance of these virion-associated cellular proteins remains largely uncharacterized. Here, we aimed to identify host cellular proteins present in the virions of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and elucidate their putative roles in the viral life cycle, particularly in the context of the host immune response.

Methods: SARS-CoV-2 virions produced by a human cell line were purified by size exclusion and subjected to mass spectrometry analysis. The presence of candidate human proteins in the virions was verified by Western blotting. Functional assays were conducted by the use of specific blocking antibodies and/or enzymatic cleavage.

Results: We show that SARS-CoV-2 virions hijack a particular subset of host cell proteins to evade humoral innate immune responses. Blockage of the biological functions of the host-derived proteins restored the sensitivity of SARS-CoV-2 to innate immunity. In the future, we will continue to investigate the effects of SARS-CoV-2 humoral immune evasion on cellular immune responses, with particular emphasis on human blood monocytes and monocyte-derived macrophages.

Conclusions: Our results reveal an intriguing immune escape mechanism of SARS-CoV-2 with possible implications in the immunopathology of COVID-19.

Funding: This work is supported by the Austrian Science Fund (FWF) grant P 34253-B.

The Impact of Obesity on Vaccination to SARS-CoV-2 in C57BL/6 Mice

Nora Geissler[1], M. Orola[1], A. Schmid[1], T. Weinmayer[1], I. Schabussova[1], A. Inic-Kanada[1], U. Wiedermann

[1]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Specific Prophylaxis and Tropical Medicine

Background: Obesity is a severe health problem, and its prevalence is increasing worldwide. Previous studies have shown that obesity alters the immune response to certain vaccines and impairs the production and maintenance of antigen-specific antibodies. However, the effect of obesity on the Covid-19 vaccine response is not clear yet.

Methods: We fed male C57BL/6 mice a high-fat diet (HFD) or standard diet (STD) for 18 weeks. After ten weeks, we intramuscularly vaccinated the mice with Spikevax (mRNA-1273) vaccine, and they received a second dose four weeks later. Seven weeks after the second dose, we administered a booster mRNA-1273 dose. We measured the levels of spike-specific antibodies in bronchoalveolar lavage (BAL) and sera of HFD and STD-fed mice. We also conducted the FACS analysis on the spleen and the lung to determine T-cell and B-cell subpopulations in both vaccinated and control mice.

Results: We found that spike-specific antibodies were induced in mRNA-1273-treated mice. However, the spike-specific IgG and IgG1 levels in the BAL and sera of HFD-fed mice were lower after receiving the booster vaccine than in STD-fed mice. Interestingly, FACS analysis revealed a higher percentage of B cells in lymphoid organs and a lower T cell percentage in the spleen and lung of vaccinated obese mice than in vaccinated lean mice. Moreover, when we gated on T cell subpopulations, we observed a significant increase in effector CD4+ and CD8+ T cells in the spleen of vaccinated obese compared to the respective lean animals.

Conclusion: Our study suggests that obese mice exhibit a decreased spike-specific immune response to mRNA-1273 compared to lean animals. Specifically, we observed a higher percentage of B cells in vaccinated obese compared to lean mice. We suggest that the function of B cells in obese mice is altered, and they do not produce appropriate levels of spike-specific antibodies comparable to those of lean mice.

Funding: This work was supported by the Austrian Science Fund (FWF) grant no. W 1248 and the Danube Allergy Research Cluster - DARC #017 of the Karl-Landsteiner University, Krems, Austria.

Changes of the TCRβ repertoire after CMV infection in CMV naive kidney transplant recipients

Andreas Heinzel[1], R. Reindl-Schwaighofer[1], V. Mühlgrabner[2], K. Hu[1], H. S. Chen[1], J. Holler[2], J. Huppa[2], R. Oberbauer[1]

[1]Medical University of Vienna, Department of Internal Medicine III, Division of Nephrology and Dialysis Vienna, Austria

[2]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute for Hygiene and Applied Immunology, Vienna, Austria

Background: CMV is a common complication after kidney transplantation. CMV naive kidney transplant recipients (KTRs) receiving a transplant from a CMV positive donor almost always exhibit CMV viremia after the stop of CMV prophylaxis. We sought to elucidate whether immunosuppressed KTRs can mount a CMV specific immune response as evidenced by the changes in the TCRβ repertoire.

Methods: Ten KTR who were either CMV immunized or received a transplant from a CMV negative donor and two CMV naive KTR who experienced a first CMV infection following transplantation were included in this study. CMV pp65 peptide NLVPMVATV (HLA*A2:01) specific CD8+ T-cells from the two CMV naive KTRs were obtained using tetramer-based fluorescence activated cell sorting before and at multiple timepoints after CMV infection and subjected to 10x Genomics 5' VDJ single cell sequencing. The bulk T-cell receptor beta (TCRβ) repertoires of CD8+ tetramer negative T-cell fractions from the two CMV naive KTRs and all CD8+ T-cells from the remaining KTRs were determined using NGS based TCRβ immune cell sequencing.

Results: CMV pp65 peptide specific CD8+ T-cells were undetectable in PBMCs from the two CMV naive KTRs, but increased respectively to 1.4% and 4.6% of CD8+ T-cells following CMV infection. In both individuals a single, but not shared, dominant TCRβ clonotype accounting for 96% and 70% of all pp65 peptide specific CD8+ T-cells was observed. The CMV pp65 peptide specific CD8+ TCR

repertoire remained stable throughout the observation period of up to 21 months post CMV infection. Bulk TCRβ repertoire analysis of CD8+ T-cells revealed a strong perturbation of the TCRβ repertoire following first time CMV infection. Both individuals had an influx of previously undetectable clonotypes surpassing any clonotypes observed prior to infection in terms of clonotype frequency. At the first timepoint analyzed after viral clearance (negative PCR) the top clonotype from one of the two patients accounted for 18% of all CD8+ T-cells and its complement determining region 3 had been described as CMV reactive in a previous study (Chen et al. Cell Press, 2017). While the top clonotype from the second patient had not previously been described as CMV-reactive, two of the patients' new high frequency clonotypes were identified as CMV reactive in previous studies. No comparable perturbations of the TCRβ repertoire were observed in the ten KTR who were either CMV immunized or received a transplant from a CMV negative donor.

Conclusion: Results from this pilot study showed that CMV naive immunosuppressed KTRs are able to mount a CMV specific cellular immune response after infection as evidenced by the appearance of HLA-A2/pp65 tetramer positive T-cells and further previously undetectable TCRβ clones with known CMV reactivity.

Funding: This work was supported by a grant from the Christine Vranitzky Stiftung zur Förderung der Organtransplantation awarded to Roman Reindl-Schwaighofer.

Poster Session 2 (Posters 10 - 17)

Chairs: Univ. Prof. Barisani and Univ. Prof. Wekerle

Single-cell profiling reveals the heterogeneity, signatures, and regulation of pathogenic Th2 cells in allergic asthma

Matarr Khan[1], M. Alteneder[1], W. Reiter[2,3], M. Madern[1], T. Krausgruber[4,5], L. Dobnikar[4], M. Waldherr[1], C. Bock[4,5], M. Hartl[2,3], W. Ellmeier[1], J. Henriksson[6,7], N. Boucheron[1]

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

[2]*University of Vienna, Department of Biochemistry and Cell Biology, Max Perutz Labs, Vienna BioCenter, Vienna, Austria*

[3]*Max Perutz Labs, Mass Spectrometry Core Facility, Vienna BioCenter, Vienna, Austria*

[4]*CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria*

[5]*Medical University of Vienna, Institute of Artificial Intelligence, Center for Medical Data Science, Vienna, Austria*

[6]*Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå, Sweden*

[7]*Umeå University, Umeå Centre for Microbial Research, Department of Molecular Biology, Umeå, Sweden*

Background: Lung pathogenic T helper type 2 (pTh2) cells have been identified as the main drivers of allergic asthma, but fundamental questions remain regarding their heterogeneity, signatures, and molecular regulation. A major limitation to our understanding of the molecular mechanisms regulating pTh2 cells is the lack of an appropriate system to generate and investigate them *in vitro*. It is well known that the differentiation and effector functions of immune cells are tightly regulated by epigenetic processes such as reversible lysine acetylation orchestrated by histone acetyltransferases and histone deacetylases (HDACs). HDAC1, a class I histone deacetylase, is an important epigenetic regulator of T cells, but its role in pTh2 cells is yet to be determined.

Methods: Here, using a house dust mite model of allergic asthma, single-cell RNA sequencing (scRNA-seq) of lung CD4$^+$ T cells, and mice with a T cell-specific deletion of HDAC1, we uncover the heterogeneity and signatures of murine pTh2 cells, and the role of HDAC1 in these cells.

Results: Our analyses reveal two distinct subsets of lung pTh2 cells: pathogenic effector Th2 (peTh2) and Th2 tissue-resident memory (Th2 Trm) cells. Both pTh2 cell subsets are highly proinflammatory and exhibit distinct transcriptional and phenotypic signatures as compared with other lung Th subsets. From our scRNA-seq analysis, we identify conditions to generate pTh2 cells *in vitro* and confirm that these *in vitro* generated pTh2 cells have a similar transcriptional profile as lung peTh2 cells. Leveraging our new *in vitro* system, we demonstrate that the p38 mitogen-activated protein kinase pathway is critical for interleukin-5 (IL-5) and IL-13 expression in pTh2 cells. Our data further illustrate the importance of HDAC1 in limiting the pathogenicity of lung and *in vitro* pTh2 cells, particularly IL-5, as well as the formation of lung Th2 Trm cells.

Conclusion: Collectively, we have generated novel insights into pTh2 cell biology. Notably, our new *in vitro* strategy for generating pTh2 cells will be useful in uncovering the molecular mechanisms involved in pTh2-mediated allergic asthma.

Funding: This work was supported by the Austrian Science Fund (FWF) grant no. F 7004-B30.

Small extracellular vesicles (sEV) are released ex vivo from residual leukocytes and platelets remaining in plasma

Małgorzata S Małys[1], Maximilian C Köller[1], Kristin Papp[1], Christof Aigner[1,2], Daffodil Dioso[3], Patrick Mucher[4], Helga Schachner[1], Michael Bonelli[3], Helmuth Haslacher[4], Andrew J Rees[1], Renate Kain[1]*

[1]*Medical University of Vienna, Department of Pathology, Vienna, Austria*

[2]*Medical University of Vienna, Division of Nephrology and Dialysis, Department of Medicine III, Vienna, Austria*

[3]*Medical University of Vienna, Department of Internal Medicine III, Division of Rheumatology, Vienna, Austria*

[4]*Medical University of Vienna, Department of Laboratory Medicine, Vienna, Austria*

Background: Biomarker discovery studies are most efficiently conducted using archived samples from well-characterized clinical cohorts. Here we determined the effect of pre-analytics on the use of sEV as biomarkers for chronic autoimmune inflammatory diseases and used the results as the foundation for a proof-of-concept study of sEV in rheumatoid arthritis (RA) and anti-neutrophil cytoplasmic antibody-associated vasculitis (AAV).

Methods: Plasma, platelet poor plasma (PPP) and serum prepared from freshly drawn blood from four healthy individuals, nine with RA (four active; five in remission) and five with AAV were used to isolate sEV (either immediately or after storage) using differential ultracentrifugation: 30,000xg for 2 hours to remove large vesicles; and then at twice 100,000xg for 2 hours, first to pellet the sEV and then to wash the re-suspended pellet. The cellular origin sEV surface protein expression was characterized using antibody-coated beads (MACSPlex) and quantified by FACS.

Results: The sEV prepared from fresh and stored PPP and fresh plasma were similar in number and cellular origin, whereas there were significantly more in stored plasma, indicating ex vivo generation predom-

inantly by platelets but also from leukocytes; both fresh and stored serum contained ex vivo generated sEV, but only from platelets. By activating residual cells with Ca^{2+} we confirmed the potential for ex vivo release of sEV which was the least for PPP, thus PPP became a primary sample for sEV biomarker discovery. sEV expressing SSEA-4, CD44, and CD81 were more abundant in PPP from patients than from healthy controls, whereas those expressing CD31 expression were significantly less abundant. The signature was also visible in sEV isolated stored plasma and serum, presumptively because CD31 was the only protein in the signature that was expressed on ex vivo generated sEV, and its abundance was decreased.

Conclusions: Our proof-of-concept sEV biomarker study identified a four-protein signature which supports the promise of sEV as biomarkers in autoimmune diseases, whilst emphasizing the complications for analysis posed by ex vivo sEV generation, especially originating from platelets and leukocyte; these difficulties can be minimized using sEV isolated from biobanked PPP providing the foundation for large-scale studies.

Funding: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 813545.

Bone marrow resident long-lived plasma cells as source of chronic DSA

Moritz Muckenhuber[1], K. Mengrelis[1], T. Kreuzbauer[1], V. Kainz[1], AM. Weijler[1], T. Wekerle[1]

[1]Medical University of Vienna, Dept. of General Surgery, Div. of Transplantation, Vienna, Austria

Background: The importance of bone marrow resident plasma cells for long-lasting humoral immunity has been highlighted in several models for vaccination and infectious diseases. To which extent this cell population is also responsible for upholding a sustained humoral response against (donor)-HLA antigens in the transplant setting remains unclear. We therefore sought to identify and characterize DSA-secreting plasma cells to provide insight into their underlying biology.

Methods: C57BL/6 were grafted with a fully mismatched balb/c cardiac allograft without any immunosuppressive treatment. Serum DSA were assessed regularly via flow crossmatch. Spleen and bone marrow cells of each cardiac allograft recipient were harvested 20 weeks post transplantation und cultured separately for 48h. DSA within the cell culture supernatants were measured via flow crossmatch. Splenic and bone marrow plasma cells were quantified and characterized using flow cytometry in transplant recipients and age-matched naïve individuals. To directly identify DSA-secreting plasma cells, fluorophore conjugated donor MHC-tetramers were used for intracellular staining of fixed and permeabilized plasma cells.

Results: Fully mismatched balb/c heart allografts were rapidly rejected within 12 days in untreated BL6 recipients and elicited a substantial humoral allo-immune response. Bone marrow and spleen cells harvested from each individual cardiac allograft recipient 20 weeks post transplantation were cultured separately. A flow crossmatch of the supernatant demonstrated that this chronic DSA response was almost exclusively carried by the bone marrow rather than the spleen (mean in-vitro IgG DSA MFI; bone marrow: 441 vs. spleen: 59; p=0.0002). Within their bone marrow, transplant recipients carried a substantially greater proportion and absolute number of long-lived plasma cells (LLPC; B220- CD138+ TACI+ CD19-) (% LLPC within all plasma cells; naïve (n=7): 15.26 +/- 4.88% vs. HTX recipients (n=12): 33.60 +/- 5.04%; p=0.002) (x106; naïve (n=7): 0.18 +/- 0.07 vs. HTX recipients (n=12): 0.46 +/- 0.04; p=0.0004). Cultures of purified bone marrow plasma cell populations revealed that donor MHC-specific antibodies, in this setting, were exclusively secreted by LLPC.

Conclusion: Bone marrow resident long-lived plasma cells represent a crucial source of late donor-specific antibodies.

How differential TCR-engagement triggers differential T-cell activation – a molecular imaging approach

Vanessa Mühlgrabner[1], R. Platzer[1], A. Plach[1], V. Gudipati[1], L. Dupré[2] & J. B. Huppa[1]

[1]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute for Hygiene and Applied Immunology, Vienna, Austria

[2]Université Toulouse III Paul Sabatier, Centre de Physiopathologie de Toulouse Purpan, IINSERM, UMR 1043, France; CNRS, UMR 5282, Toulouse, France.

Background: T-cells can detect the presence of even a single stimulatory antigenic peptide fragment presented in the context of major histocompatibility complex molecules (pMHC) on the surface of antigen presenting cells (APCs). This exquisite sensitivity is conferred by their T-cell antigen receptors (TCRs) and initiates a down-stream signaling cascade mediated by TCR-proximal signaling molecules, such as Lck and ZAP-70. Different affinities / "qualities" of TCR-pMHC interactions (such as those of virus-specific vs. self-specific T-cells) result in differences in TCR- proximal signaling dynamics and T cell activation, however, the underlying molecular mechanisms are still insufficiently understood.

Methods: We employ live-cell microscopy with single molecule resolution, multiplexed calcium imaging and immunofluorescence stainings in CRISPR-Cas9 TCR-exchanged T cell populations which are lentivirally transduced with a fluorescent-protein-tagged ZAP-70 construct.

Results: We previously showed that T-cells featuring a cytomegalovirus (CMV)-specific TCR respond to single stimulatory ligands with robust activation of ZAP-70 via phosphorylation. In contrast and for unknown reasons, recognition of self-pMHCs by auto-reactive T-cells as described in the context of Type 1 diabetes, requires the presence of at least 1000-times more antigen for T-cell activation with considerably lower levels of ZAP-70 activation. The use of the photo-switchable fluorescent protein mEos3.2 allows us to precisely time the visualization of individual ZAP-70 molecules within the synapse. This single-molecule tracking approach revealed a mobile and immobile fraction of ZAP-70, the latter likely due to TCR/CD3-association, under stimulatory conditions. We observed less ZAP-70 immobilization in the absence of antigen and shorter dwell times of ZAP-70 within the synapse for self-specific, low-quality TCR-pMHC interactions than for high quality, antiviral T-cell responses.

Conclusion: Correlating in situ TCR-pMHC binding lifetimes with the signaling dynamics of ZAP-70 and downstream signaling effectors will allow us to decipher how ligand quality drives TCR-proximal signal transduction and T-cell activation in settings of antiviral and autoimmunity.

Funding: This work was supported by the Austrian Science Fund (FWF) grant no. P341118B.

Deciphering human antibody repertoires against the microbiome in health and disease

Thomas Vogl[1]

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

Background: Our current conception of antibody repertoires is mostly based on DNA sequencing of the respective antibody genes (BCRseq) whereas the actual antigens recognized are vastly unknown. We have developed and applied a novel high throughput method to unravel the functional capacity of these enormous immune repertoires targeting bacteria and viruses, shedding light on their role in modulating human health.

Methods: We have leveraged phage immunoprecipitation sequencing (PhIP-Seq) to measure antibody responses against 360,000 variants of bacterial, viral, and human origin in approximately 2,500 individuals. As chemical synthesis of peptide antigens is limited by short lengths and high costs, PhIP-Seq relies on antigen libraries encoded by synthetic DNA oligonucleotides. These libraries are cloned into, and displayed on the surface of, T7 phages. Antibody-bound phages are enriched by immunoprecipitation and identified by next-generation sequencing.

Results: Beyond baseline antibody responses in healthy individuals, we have studied different diseases such as ME/CFS, genetic and environmental determinants of antibody repertoires, as well as different antigen sets.

Conclusion: This platform technology can be leveraged in a plethora of ways to study the interplay of the human immune system and microbiota in cancer, ageing, and immune mediates diseases.

Funding: This work was supported by the Austrian Science Fund (FWF) grant no. J-4256 (Schrödinger Fellowship) and an ongoing ERC Start Grant (2022 – EarlyMicroAbs).

SARS-CoV-2 sensing by circulating monocytes: The role of Toll-like receptors

Gabor Tajti[1], L. Gebetsberger[1], R. Klasinc[1], J. Leitner[2], P. Steinberger[2], H. Stockinger[1], A. Ohradanova-Repic[1]

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

[2]*Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Immunology, Vienna, Austria*

Background: The innate immune system is considered as the first line defense against viral infections, such as SARS-CoV-2. Monocytes, as important cellular components of the innate immune system, play an inevitable role in the antiviral immune response, however, the mechanism how these cells sense SARS-CoV-2 is not yet clearly understood. Because Toll-like-receptors (TLRs) are important cell surface and intracellular sensors of monocytes and other innate immune cells, we conducted experiments to investigate the TLR-mediated activation of circulating monocytes by SARS-CoV-2. As virus uptake or infection of target cells is also a prerequisite to stimulate intracellular TLRs, we also studied the entry and subsequent stimulatory effect of SARS-CoV-2 on intracellular TLRs expressed in monocytes.

Methods: Monocytes of healthy volunteers were isolated via immunomagnetic positive separation, and were infected with SARS-CoV-2 in the presence or absence of specific TLR modulators or specific blocking antibodies. The uptake of virions was monitored by flow cytometry and fluorescent microscopy. The activation of monocytes was determined via RT-qPCR method. In some experiments, cells of the T cell line Jurkat and the monocytic cell line THP-1, expressing an NF-kB-EGFP reporter system and specific sets of TLRs were used to further elucidate the role of certain TLRs.

Results: Our results yielded three key findings: Firstly, we observed that circulating monocytes undergo activation in the presence of SARS-CoV-2 through a mechanism independent of TLRs expressed on the cell surface. Secondly, we found that the virus is capable to enter and infect monocytes through a yet unknown mechanism. Lastly, our results suggest the involvement of intracellular TLRs that sense single-stranded RNA in the innate immune response of monocytes to SARS-CoV-2.

Conclusion: Our results reveal a novel insight into the sensing of SARS-CoV-2 by monocytes emphasizing the role of single-stranded RNA sensing intracellular TLRs.

Funding: This work is supported by the Austrian Science Fund (FWF) grant P 34253-B.

The role of Schistosoma mansoni cysteine proteases in the modulation of the immune system.

Michael Thaler[1], L. Neuninger[1], M.Wysmołek[1], A. Razim[1], J.Dvořák[2], M. Horn[3], A. Leontovyč[3], M. Mareš[3], A. Inic-Kanada[1], A. Schmid[1], U. Wieder-mann[1] and I. Schabussova[1]

[1]*Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Specific Prophylaxis and Tropical Medicine, Vienna, Austria*

[2]*Czech University of Life Sciences Prague. Prague, Czech Republic*

[3]*Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic*

Background: Schistosomiasis is a chronic infectious disease caused by the parasitic trematode, *Schistosoma mansoni. S. mansoni* infection affects over 200 million people worldwide. The parasite invades the host through the skin and migrates through the lungs. In order to achieve invasion the parasites release proteases that digest host tissues and blood. As such, proteases are potential targets for new treatments and vaccines.

Methods: Firstly, the proteolytic activity of SmCB1 was assessed by the activity assay. The potential of SmCB1 to induce a vesicle release by adenocarcinomic human alveolar basal epithelial cells A549 48h post-treatment was analysed using Zetasizer. Finally, the effects of SmCB1 and the SmCB1-dependent vesicles from A549 cells were assessed in an *in vivo* experiment using a mouse model. The mice were treated intranasally with active and inactive SmCB1 or with vesicles from A549 cells for three consecutive days. Then mice were sacrificed and the lung cells were analysed by fluorescence activated cell sorting.

Results: The Zetasizer data showed that only active SmCB1 induce a vesicle release by A549 cells. Preliminary data suggest that short-term exposure to a small amount of SmCB1 does not elicit an immune response in the mouse lungs, while vesicles produced in response to SmCB1 show an immunosuppressive effect.

Conclusion: SmCB1 induces the release of vesicles from A549 cells. These vesicles reduce the number of immune cells in the mouse lung. SmCB1 showed a similar, but smaller effect with the tested amount.

Funding: This work was supported by the Austrian Science Fund (FWF) grant no. P 34867

SPECIAL THANKS TO OUR FUNDING ORGANIZATIONS!

DANUBE ALLERGY RESEARCH CLUSTER

KARL LANDSTEINER UNIVERSITY OF HEALTH SCIENCES

MEDICAL UNIVERSITY OF VIENNA

cellular molecular MCCA clinical allergology

University of Natural Resources
and Life Sciences, Vienna