CONGRESS PROGRAMME

Annual Meeting

Österreichische Gesellschaft für Allergologie & Immunologie

November 16th - 19th 2016

VILLA BLANKA | TYROL | AUSTRIA

www.oegai-2016.com

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Dear friends and colleagues,

on behalf of the Local Organising Committee (LOC) and the Austrian Society for Allergology and Immunology (ÖGAI) we would like to invite you to the ÖGAI 2016 Annual Meeting in Innsbruck.

The scientific programme of the Annual Meeting in Innsbruck will cover broad aspects of immunological and allergological topics, which will be addressed by outstanding national and international keynote speakers as well as short oral presentations of Austrian early-stage researchers. In addition to oral presentations, young scientists will have the opportunity to present and discuss their findings during poster sessions. This years´ session topics include Immunity of Infectious Diseases, Innate Immunity, Immune Cell Signaling, Adaptive Immune Regulation, Autoimmunity/ Immunodeficiencies, Clinical Allergology and Tumor Immunology. The meeting will be traditionally completed with the 'Clinical Day' held in German, which will focus this year on Clinical Immunology and is therefore especially tailored for practitioners and resident doctors.

The meeting takes place in the exclusive and newly built convention hall of the Villa Blanka over the rooftop of Innsbruck.

We would also like to thank all the sponsors and exhibitors for their valuable contribution and support!

We are looking forward to your active participation in this attractive meeting at a stimulating location and hope that this years´ Annual Meeting of the ÖGAI in Innsbruck will provide inspiring discussions in the fields of Immunology and Allergology!

With kind regards,

Assoc.-Prof. Dr. Wilflingseder Doris
Congress President
On behalf of the LOC

Prof. Dr. Günter Weiss
Congress President
On behalf of the LOC

Sehr geehrte Kolleginnen und Kollegen!

Im Anschluss an die Jahrestagung der Österreichischen Gesellschaft für Allergologie und Immunologie (ÖGAI), die diesmal vom 16.-18. November 2016 in Innsbruck stattfindet, möchten wir Sie herzlich zum Satellitensymposium über das Thema „Klinische Immunologie für Ärzte" am Samstag, den 19. November 2016, einladen.

Wie Sie aus dem Programm ersehen, werden wir unter Mitwirkung hervorragender, international ausgewiesener ExpertInnen versuchen, Ihnen die Prinzipien der normalen und pathologisch veränderten Immunreaktion in einem Tag nahezubringen. Die Veranstaltung wird in deutscher Sprache abgehalten.

Wir glauben, dass für ein derartiges Symposium grosser Bedarf besteht, hoffen auf Ihr Interesse und freuen uns auf Ihr Kommen.

Em. Prof. Dr. Med. Georg Wick
Congress President
On behalf of the LOC

Prof. Dr. Reinhold Schmidt
Congress President
On behalf of the LOC

Dear participants of the ÖGAI Annual Conference,

hay fever and pollen allergies are more common in spring and in the summer months than at any other time of year and at these times in particular many people place very big hopes in you. By means of your research, you contribute to making these otherwise so beautiful months bearable again for many allergy sufferers.

However, it is not just for people with ´pollen plague´, but also for patients with severe immune disorders that you manage to increase quality of life despite illness. Particularly through the dialogue that undoubtedly takes place at events such as the Annual Meeting of the Austrian Allergology and Immunology Society, you contribute to the continuous increase of knowledge.

I am pleased, dear participants, to be able to welcome you soon to the state capital of Tyrol. To note, high importance is placed in the field of health in Innsbruck. Therefore, the Medical University of Innsbruck is amongst the most popular universities of all to study at.

I wish you a pleasant and interesting stay in our Alpine city while you attend your Annual Meeting in November and I hope that the new knowledge you will undoubtedly acquire is accompanied by a lot of nice memories.

Mag.a Christine Oppitz-Plörer
Mayoresse of the State Capital Innsbruck

INNS' BRUCK · tirol Unser Land · HOROS · Medizinische Universität Innsbruck · Universität Innsbruck

GOLD SPONSORS

affymetrix eBioscience · BioLegend The path to legendary discovery · Biozym Science is our business · Beckman Coulter Life Sciences · Stallergenes Greer Life beyond allergy

SPONSORS

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DATE
November 16th-19th 2016

VENUE
Seminarzentrum | Villa Blanka
Weiherburggasse 8 | 6020 Innsbruck | Österreich
www.villablanka.com

ORGANISER
Österreichische Gesellschaft für
Allergologie und Immunologie
www.oegai.org

CONGRESS PRESIDENTS
Assoz.-Prof. Dr. Doris Wilflingseder
Prof. Dr. Günter Weiss
Prof. Dr. Georg Wick
Prof. Dr. Reinhold Schmidt

LOCAL ORGANISING COMMITTEE
Prof. Dr. Gottfried Baier
Prof. Dr. Beatrix Grubeck-Loebenstein
Prof. Dr. Cornelia Lass-Flörl
Prof. Dr. Nikolaus Romani
Prof. Dr. Matthias Schmuth
Assoz.-Prof. Dr. Patrizia Stoitzner
Prof. Dr. Andreas Villunger
Prof. Dr. Reinhard Würzner

YOUNG LOC
Dr. Wilfried Posch
Dr. Andrea Schroll

CONGRESS WEBSITE
www.oegai-2016.com

CONGRESS ORGANISATION
EVENT SERVICE RS GmbH (ESRS)
Maximilianstraße 9/3, 6020 Innsbruck
Tel: +43 (0) 512 / 56 35 98
Fax: +43 (0) 512 / 56 35 98 – 10
E-Mail: office@event-service.cc

ACCOMMODATION
Karin Werth | Sektion für Hygiene und Medizinische Mikrobiologie
Tel: +43 (0) 512 / 9003 - 70702
Fax: +43 (0) 512 / 9003 - 73700
E-Mail: karin.werth@i-med.ac.at

SPONSORSHIP | EXHIBITION
Nathalie Zoller
Tel: +43 (0) 512 / 56 35 98
Fax: +43 (0) 512 / 56 35 98 – 10
E-Mail: nathalie.zoller@event-service.cc

REGISTRATION | OPENING HOURS
Foyer | 1st floor

Wednesday	Nov 16th	17:00 - 19:30
Thursday	Nov 17th	08:00 - 18:00
Friday	Nov 18th	08:00 - 17:00
Saturday	Nov 19th	08:00 - 15:00

CONGRESS LANGUAGE
English
no simultaneous translation

PRESENTATION
Please deliver your presentation latest 2 hours before the session starts at the Speakers Lounge.

SPEAKERS LOUNGE
Restaurant No. 8 | 1st floor

Wednesday	Nov 16th	17:00 - 18:00
Thursday	Nov 17th	08:00 - 16:00
Friday	Nov 18th	08:00 - 16:00
Saturday	Nov 19th	08:00 - 15:00

INTERNET | WIRELESS LAN
Free Wi-Fi is available throughout the building for the period of the ÖGAI Annual Meeting.

MEDICAL HELP
Please contact the registration.

CERTIFICATION
For attending the ÖGAI Annual Meeting 2016 in Innsbruck from November 16th to November 18th you will get **22 DFP educational points** | ID education program: **555637**.

For attending the ÖGAI Annual Meeting - Allergietag 2016 on November 19th you will get **8 DFP educational points** | ID education program: **555639**.

Austrian doctors are asked to sign the "certification sheet", which you will find at the registration desk on the 1st floor. Please have your "ÖÄK-Arztnummer" ready. The congress organization will supply the educational points to your account.

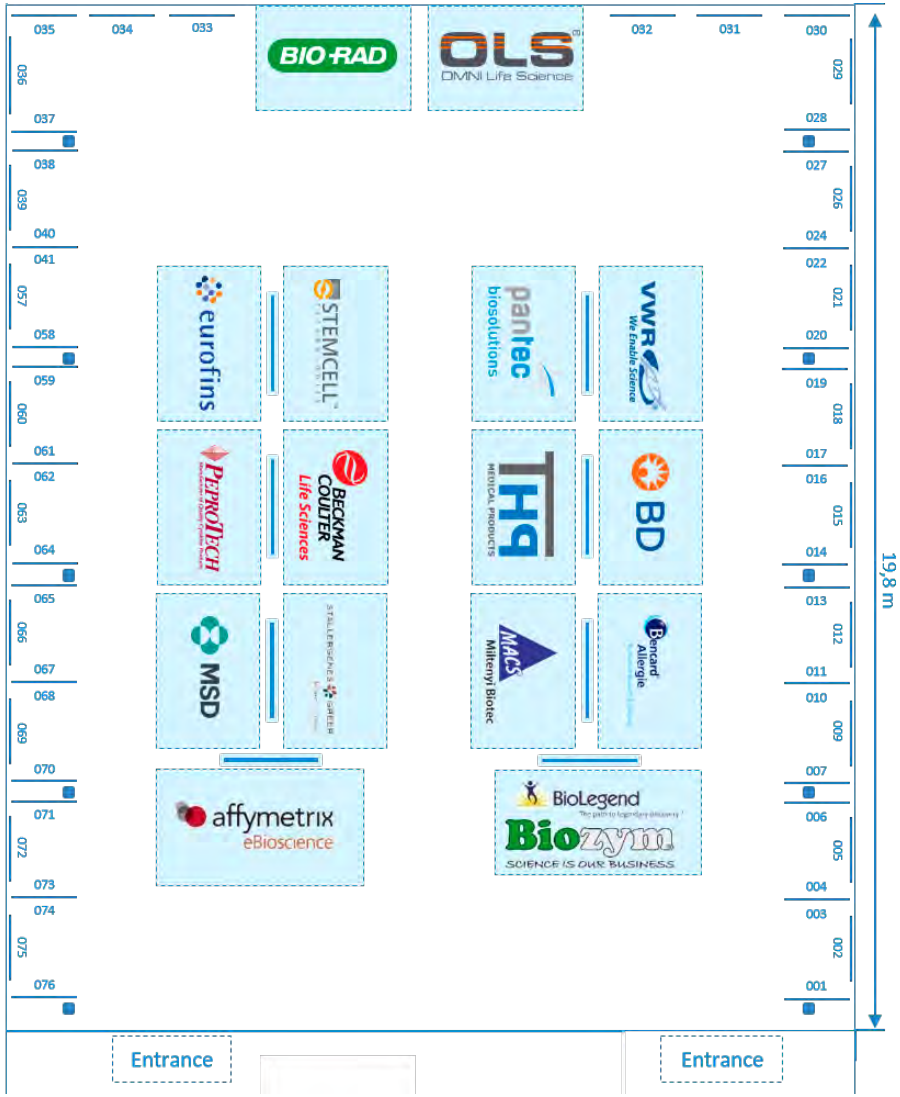
German doctors have to submit their certificate of attendance at the General Medical Council which will credit the CME-points.

Therefore Germany and Austria have an agreement, that the DFP-points will be credited one-to-one. Please find your certificate of attendance in your conference bag.

INDUSTRIAL- | POSTER EXHIBITION

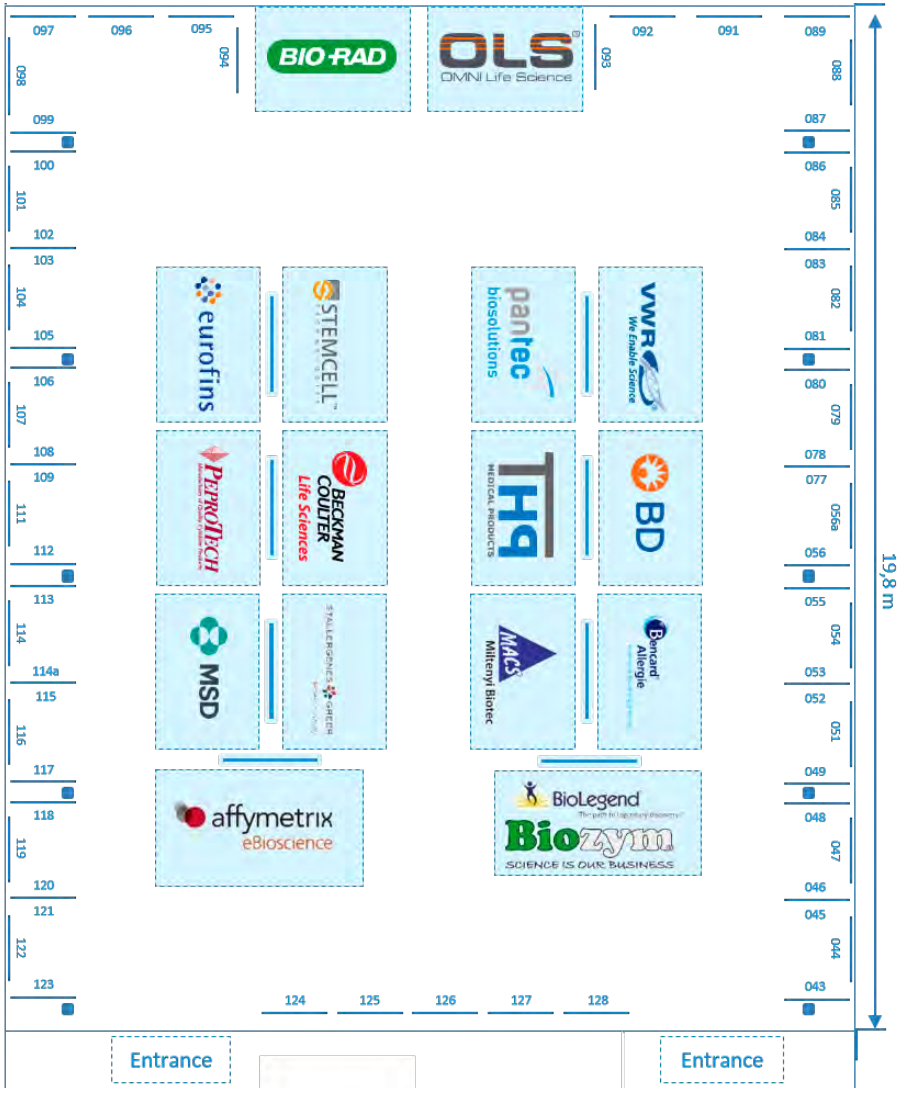
POSTER SESSION 1 | THURSDAY, NOVEMBER 17TH 2016

SESSION 1 SESSION 2 SESSION 4



POSTER SESSION 2 | FRIDAY, NOVEMBER 18TH 2016

SESSION 3 SESSION 5 SESSION 6 SESSION 7



	Wednesday | Nov 16th	Thursday | Nov 17th	Friday | Nov 18th	Saturday | Nov 19th
8:00		08:00 – 09:00 Registration	08:00 – 08:45 Registration	08:00 – 09:00 Registration
9:00		09:00 – 10:40 Session 1 Immunity to Infections	08:45 – 09:15 | Breakfast Session 09:15 – 10:40 Session 5 Immunodeficiencies | Autoimmunity	09:00 – 09:45 Crash Course physiologische (normale) Immunität
10:00		10:40 – 11:00 | Coffee Break	10:40 – 11:00 | Coffee Break	09:45 – 10:30 Immunopathologie - Übersicht 10:30 – 10:45 | Kaffeepause
11:00		11:00 – 12:30 Session 2 Innate Immunity	11:00 – 12:30 Session 6 Clinical Allergology	10:45 – 11:30 Immunmangel angeboren im Kindesalter
12:00		12:30 – 13:30 Lunch	12:30 – 13:30 Lunch	11:30 – 12:15 Immunmangel im Erwachsenenalter 12:15 – 13:00 Mittagspause
13:00		13:30 – 15:30 Poster Session 1 [Session 1 | 2 | 4]	13:30 – 15:30 Poster Session 2 [Session 3 | 5 | 6 | 7]	13:00 – 13:30 Überempfindlichkeitsreaktionen Typ I 13:30 – 14:00 Überempfindlichkeitsreaktionen Typ II
14:00		15:30 – 17:00 Session 3 Immune Cell Signaling	15:30 – 17:30 Session 7 Tumor Immunology	14:00 – 14:30 Überempfindlichkeitsreaktionen Typ III 14:30 – 15:00 | Kaffeepause
15:00		17:00 – 17:20 Coffee Break		15:00 – 15:30 Überempfindlichkeitsreaktionen Typ IV
16:00		17:20 – 18:30 Session 4 Adaptive Immune Regulation		15:30 – 16:00 Impfungen 16:00 – 16:30 Immunsystem im Alter
17:00	17:15 – 17:45 Registration	18:30 – 20:30 ÖGAI Assembly		
18:00	17:45 – 19:00 Opening Ceremony Opening Keynote Lecture Mike Malim			
19:00	19:00 – 22:00 Welcome Reception Poster- | Industrial exhibition		19:30 – 00:00 Social Evening Award Ceremony	
20:00		21:00 – 00:00 Young ÖGAI Party @ Weekender Upstairs		
21:00				

WEDNESDAY, NOVEMBER 16TH 2016

OPENING

Opening Ceremony **17:45 - 18:15**

Cell-autonomous mechanisms of HIV suppression **18:15 - 19:00**

Chair: Günter Weiss, Doris Wilflingseder
Speaker: Mike Malim

Meetingroom 2nd floor

WELCOME RECEPTION

19:00 - 22:00

Industrial Exhibition

THURSDAY, NOVEMBER 17TH 2016

SESSION 1

IMMUNITY TO INFECTIONS **09:00 - 10:40**

Chair: Cornelia Lass-Flörl, Winfried Pickl

Meetingroom 2nd floor

09:00 - 09:30 **Inflammasomes and the microbiota-partners in the control of fungal infections**
L. Romani

09:30 - 09:43 **Establishment of a 3D respiratory system to study fungal infections**
P. Chandorkar

09:43 - 09:56 **Myeloid STAT1 signaling protects from murine CMV infection and promotes inflammation-induced extramedullary hematopoiesis**
R. Gawish

09:56 - 10:10 **Homeostatically driven type-2 pathways shape and maintain the pulmonary immune environment.**
S. Saluzzo

10:10 - 10:40 **Modulators of macrophage function in bacterial infections**
S. Knapp

SESSION 2

INNATE IMMUNITY **11:00 - 12:30**

Chair: Florian Sparber, Reinhard Würzner

Meetingroom 2nd floor

11:00 - 11:30 **Innate lymphoid cells control organ homeostasis**
A. Diefenbach

11:30 - 11:41 **Human skin dendritic cell fate is differentially regulated by the monocyte identity factor KLF4 during steady state and inflammation**
C. Krump

11:41 - 11:53 **Cooperation of Langerhans cells and NK cells in the immunosurveillance of the epidermis during chemical carcinogenesis**
D. Ortner-Tobider

11:53 - 12:05 **Novel role for the SAA1-FPR2 axis in the initiation of type 2 immune responses**
U, Smole

12:05 - 12:40 **Fine-tuning of dendritic cell functions by complement-opsonized HIV**
W. Posch

SESSION 3

IMMUNE CELL SIGNALING **15:30 - 17:00**

Chair: Gottfried Baier, Hannes Stockinger

Meetingroom 2nd floor

15:30 - 16:00 **The nuclear orphan receptor NR2F6 as cancer immune checkpoint**
N. Kleiter

16:00 - 16:11 **Bim is a key target of miR-17~92 in B cells undergoing stress responses**
V. Labi

16:11 - 16:23 **Analysis of expression and function of CD39 on iTreg generated by different protocols**
M. Gerner

16:23 - 16:35 **The miR-15 family reinforces the transition from proliferation to differentiation in pre-B cells**
S. Herzog

16:35 - 17:00 **The Sin3A transcriptional regulator controls primary and transformed T cell behavior**
A. Mondino

SESSION 4

ADAPTIVE IMMUNCE REGULATION **17:20 - 18:30**

Chair: Beatrix Grubeck-Loebenstein, Barbara Bohle

Meetingroom 2nd floor

17:20 - 17:50 **Adoptive T cell immunotherapy: from single cells to immunity**
D. Busch

17:50 - 18:00 **The Bcl-2 prosurvival protein A1 is dispensable for T cell homeostasis upon viral infection**
S. Tuzlak

18:00 - 18:10 **The AMP analog AICAR modulates the Treg/Th17 axis through enhancement of fatty acid oxidation**
K.A. Mayer

18:10 - 18:20 **Oxidative stress and age-related impairments in the maintenance of immunological memory**
L. Pangrazzi

18:20 - 18:30 **MAZR: Modulating regulatory T Cell development and function**
L. Andersen

ÖGAI ASSEMBLY

18:30 - 20:30

Meetingroom 2nd floor

YOUNG ÖGAI PARTY

21:00 - 00:00

Weekender Upstairs

FRIDAY, NOVEMBER 18TH 2016

BREAKFAST SESSION

Chair: Winfried Pickl, Rudi Valenta — Meetingroom 2nd floor

08:45 - 09:15 **Tips for publishing – an editor's perspective**
C. Livingstone

SESSION 5

AUTOIMMUNITY | IMMUNODEFICIENCIES — **08:45 - 10:40**
Chair: Georg Wick, Igor Theurl — Meetingroom 2nd floor

09:05 - 10:00 **The Mosaic of autoimmunity –why do we develop autoimmune diseases**
Y. Shoenfeld

10:00 - 10:13 **Progressive Lung Disease is a Key Feature of Late Onset RAG Deficiency**
C.B. Geier

10:13 - 10:26 **In vivo generated peripheral regulatory T cells in skin grafting**
I.K. Gratz

10:26 - 10:40 **Active mTORC1 signaling induces macrophage granuloma formation and sarcoidosis progression**
M. Linke

SESSION 6

CLINICAL ALLERGOLOGY — **11:00 - 12:30**
Chair: Norbert Reider, Heinz Kofler — Meetingroom 2nd floor

11:00 - 11:30 **Paving the way for prophylactic allergy gene vaccines**
J. Thalhamer

11:30 - 11:40 **Human Dendritic Cells induce divergent Immune Responses according to the allergenic Potential of two homologous Lipocalins**
B. Posch

11:40 - 11:50 **Development and characterization of a ragweed allergy vaccine based on the peptide carrier principle**
M. Hochradl

11:50 - 12:00 **Modulatory capacities and possible implications of soluble Fc-epsilon-RI in the IgE-mediated immune response**
S. Monino-Romero

12:00 - 12:30 **Drug allergy is no allergy**
W. Pichler

FRIDAY, NOVEMBER 18TH 2016

SESSION 7

TUMOR IMMUNOLOGY — **15:30 - 17:30**
Chair: Patrizia Stoitzner, Andreas Villunger — Meetingroom 2nd floor

15:30 - 16:05 **Enlisting the immune system to fight cancer**
P. Romero

16:05 - 16:18 **Combination of oncolytic virotherapy and DC-based immunotherapy for the treatment of melanoma**
L. Koske

16:18 - 16:31 **MAPK-activated protein kinase MK2 exerts immune regulatory functions in the myeloid tumor microenvironment**
K. Soukup

16:31 - 16:45 **T cells from myeloma patients display features of both exhaustion and senescence**
C. Rieser-Zelle

16:45 - 17:30 **Mechanism of action of conventional and targeted anticancer therapies: reinstating immunosurveillance**
G. Kroemer

SOCIAL EVENING | AWARD CEREMONY

19:30 - 00:00
Restaurant Villa Blanka

AWARDS

EFIS-IL Lecture Award to GUIDO KROEMER

This year the EFIS-IL Lecture Award for an outstanding European scientist goes to GUIDO KROEMER. At this occasion a bronze medal with depictions of Paul Ehrlich and Elie Metchnikoff, designed by the Hungarian artist Gábor Szabo and a diploma will be handed over to GUIDO KROEMER at the Social Evening of the ÖGAI Annual Meeting 2016.

7 Poster and 7 Short talk awards (one per session)

7 poster and 7 short talk awards will be chosen from the submitted contributions. The awards are chosen by the chairs of the individual sessions and go to the best presenters in both categories, posters and short talks.

Clemens von Pirquet Award for research in allergology to ANNA GIERAS

The ÖGAI awards Dr. Anna Gieras for her outstanding works in the field of allergologic research.

Landsteiner Award for basic research in immunology to MARTINA MINNICH

The ÖGAI awards Dr. Martina Minnich for her outstanding works in the field of basic immunologic research.

Two PhD Awards for excellent works in the field of immunology and allergology are handed over to:

VICTORIA KLEPSCH - U&F Melchers PhD Award & REGINA SELB - ÖGAI PhD Award

SATELLITENSYMPOSIUM

SESSION 1

CRASH COURSE PHYSIOLOGISCHE (NORMALE) IMMUNITÄT — **09:00 - 09:45 Uhr**, Meetingraum 2 .Stock

09:00 - 09:45 **Entwicklung, Struktur und Funktion des menschlichen Immunsystems**
Herbert Strobl

SESSION 2

IMMUNOPATHOLOGIE — **09:45 - 10:30 Uhr**, Meetingraum 2 .Stock

09:45 - 10:30 **Einleitung und Übersicht**
Georg Wick

KAFFEEPAUSE — 10:30 - 10:45 Uhr

SESSION 3

IMMUNMANGELERKRANKUNGEN — **10:45 - 12:15 Uhr**, Meetingraum 2 .Stock

10:45 - 11:30 **Angeborene Immundefekte im Kindesalter**
Kaan Boztug

11:30 - 12:15 **Immundefekte im Erwachsenenalter**
Reinhold E. Schmidt

MITTAGSPAUSE — 12:15 - 13:00 Uhr

SESSION 4

ERKRANKUNGEN DURCH IMMUNOLOGISCHE ÜBEREMPFINDLICHKEITSREAKTIONEN — **13:00 - 15:30 Uhr**, Meetingraum 2 .Stock

13:00 - 13:30 **Typ I Erkrankungen (IgE-mediierte Allergien)**
Erika Jensen-Jarolim

13:30 - 14:00 **Typ II Erkrankungen (zytotoxische Antikörper und ADCC-mediierte Erkrankungen)**
Gerhard Zlabinger

14:00 - 14:30 **Typ III Erkrankungen (Immunkomplex-mediierte Erkrankungen)**
Georg Schett

KAFFEEPAUSE — 14:30 - 15:00 Uhr

15:00 - 15:30 **Typ IV Erkrankungen (durch zelluläre Immunreaktionen bedingte Erkrankungen)**
Hans Lassmann

SESSION 5

IMPFUNGEN — **15:30 - 16:00 Uhr**, Meetingraum 2 .Stock

15:30 - 16:00 **Eine Übersicht**
Martina Prelog

SESSION 6

DAS IMMUNSYSTEM IM ALTER — **16:00 - 16:30 Uhr**, Meetingraum 2 .Stock

16:00 - 16:30 **Herausforderung durch Altern**
Beatrix Grubeck-Loebenstein

VENUE

Seminarzentrum | Villa Blanka

Weiherburggasse 8 | 6020 Innsbruck | Österreich

www.villablanka.com

① ARRIVAL BY CAR

Innsbruck is located in the heart of Europe and the Alps. Its central location and excellent infrastructure makes it an easy destination to visit by car. Exceptional flexibility and ideal links with Central Europe's main transport routes and well developed road networks make driving to Innsbruck a particularly pleasant experience.

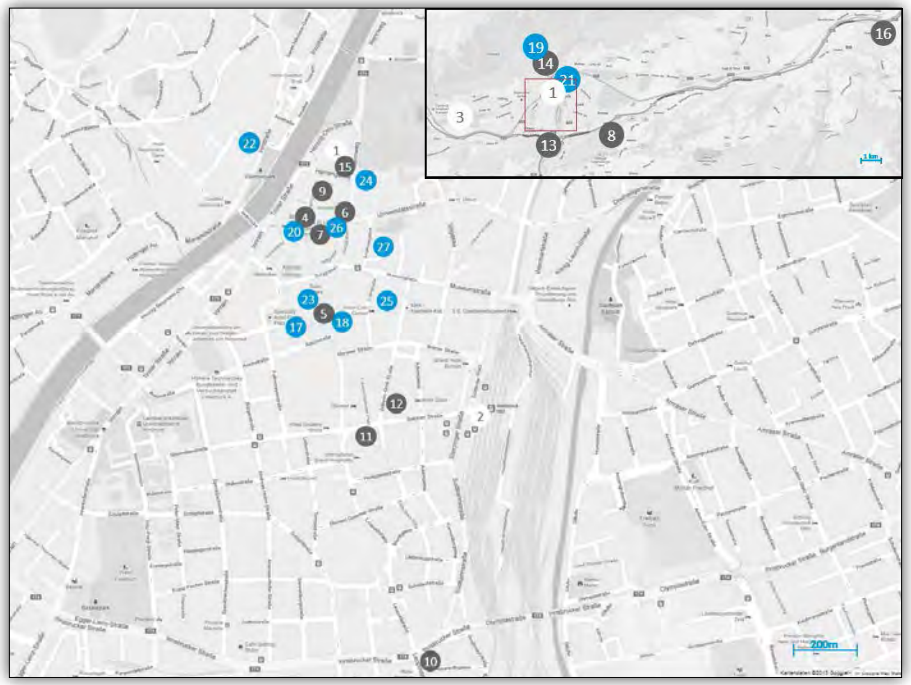
From Salzburg, Munich	about 2 hrs. driving time
From Vienna	about 5 hrs. driving time
From Zurich	about 3 hrs. driving time
From Milano	about 4 hrs. driving time

② ARRIVAL BY TRAIN

Relaxing, comfortable and safe – enjoy the stunning scenery in Innsbruck while taking advantage of the numerous international rail connections and perfectly scheduled train links to all European capitals.

③ ARRIVAL BY PLANE

Austrian Airlines as well as different Low-Cost Carriers offer daily direct connections from the most important European cities to Innsbruck.



SIGHT SEEING

4 GOLDEN ROOF („Goldenes Dachl")

Herzog-Friedrich-Straße 15, 6020 Innsbruck

A small, but magnificent sight decorates the gothic historic center of Innsbruck: the golden roof, which is entirely covered with fire-gilded copper shingles.

5 MARIA-THERESIEN STREET (pedestrian zone)

Innsbruck's main street. Extensive pedestrian area surrounded by gorgeous old houses and modern shopping malls. Enjoy a coffee break in one of the outdoor cafés next to the historical sights: St. Anne's Column and Triumphal Arch.

6 IMPERIAL HOFBURG („Kaiserliche Hofburg")

Rennweg 1, 6020 Innsbruck

www.hofburg-innsbruck.at

The state rooms of Hofburg Innsbruck – one of Austria's most important cultural highlight – are open again after an extensive and gentle general renovation.

7 CITY TOWER

Herzog-Friedrich-Straße, 6020 Innsbruck

Over 148 steps lead up to the 31-metre-high viewing platform, which overlooks the medieval streets of Innsbruck and offers stunning views of Bergisel, Patscherkofel mountain, the River Inn and the Nordkette mountain range. The City Tower is a good 50 years older than the Golden Roof. It was completed in 1450 on the side of the old town hall.

8 AMBRAS CASTLE

Schlossstraße 20, 6020 Innsbruck

www.schlossambras-innsbruck.at

Ambras Castle is one of the main attractions in Innsbruck, the state capital of Tyrol. Its cultural and historic significance is inseparable from the personality of Archduke Ferdinand II (1529-1595), who promoted the arts and sciences as a true Renaissance prince. He established the magnificent Ambras collections and had a museum facility built in the lower castle to house them, designed according to modern criteria from the time.

9 CATHEDRAL ST. JAKOB („Dom zu St.Jakob")

Domplatz, 6020 Innsbruck

Innsbrucks Cathedral is one of the most beautiful church architectures of the high Baroque era. It was built from 1717 until 1724 and replaced an older church of the Gothic era.

10 GLOCKENMUSEUM

Leopoldstraße 53, 6020 Innsbruck

www.grassmayr.at

Worldwide – in almost 100 countries – you can hear the sound of Grassmayrs' Tyrolean bells. Ever since 1599, bells and art works of bronze have been made at Grassmayr Bell Foundry. The mystical effects of the music of bells and secrets of the artistic craftsmanship in making them have left a deep imprint on Grassmayr, Austria's oldest family company, for more than 400 years.

11 CASINO INNSBRUCK

Salurner Straße 15, 6020 Innsbruck

www.casinos.at

Justifiably, Casino Innsbruck is one of the most beautiful Casinos of the world because of its impressive architecture and tasteful decoration surrounded by impressing scenery of Tyrolean Mountains.

12 AUDIOVERSUM

Wilhelm-Greil-Straße 23, 6020 Innsbruck

www.audioversum.at

Understand listening…

Twelve different locations invite you to see, hear and marvel to impressing exhibits and state-of-art installations.

13 BERGISEL SKI JUMP („Skischanze") & TYROL PANORAMA („Tirol Panorama")

Bergiselweg 3, 6020 Innsbruck

www.bergisel.info

The public ski jump can be described as a majestic building on the steeped in history Bergisel because of its location above the Olympic city Innsbruck. Only a few steps away is Tyrol Panorama located, which will take you on an exciting journey through time and Tyrol's most important stages of history.

Enjoy the breathtaking view above Innsbruck!

Opening hours:

Bergisel Ski jump: daily 09.00 a.m. – 06.00 p.m. | day of rest Tuesday

Tyrol Panorama: 09.00 a.m. – 05.00 p.m. | day of rest Tuesday

14 ALPENZOO

Weilherburggasse 37, 6020 Innsbruck

www.alpenzoo.at

Europes highest zoo on Innsbruck's sunny side (750m) inhabits more than 2.000 animals. An unique experience with animals for people of all ages.

5 minutes walking distance from Villa Blanka

Opening hours: 09.00 a.m. – 06.00 p.m.

SIGHTSEEING TOURS & EXCURSIONS

15 CABS („FIAKER")

Located directly in front of the Congress, the local cab drivers are waiting for you to drive you through the city and show you Innsbruck's sights romantically.

15 THE SIGHTSEER

Enjoy Innsbruck's sights in a compact way: with „The Sightseer". Get to the city's most interesting places and stay, where you like it the most. With daily passes you have the opportunity and the freedom to hop-on and hop-off at every "Sightseer"-station.

Departure Times at Congress/Hofburg:

09.32 a.m. | 10.12 a.m. | 10.52 a.m. | 11.32 a.m. | 12.12 p.m.| 12.52 p.m. | 01.32 p.m. | 02.12 p.m. | 02.52 p.m. | 03.32 p.m. | 04.12 p.m. | 04.52 p.m. | 05.32 p.m.

16 SWAROVSKY CRYSTAL WORLDS

Kristallweltenstraße 1, 6112 Wattens

www.kristallwelten.swarovski.com

Swarovski Crystal Worlds in Wattens – a museum about the subject of crystals and jewelry … simply magic! The multi-faceted play of crystalline colors and forms fascinates in the subterranean Chambers of Wonder. Under the direction of André Heller, innovative interpretations of renowned international artists, the world of special scent and sound merge here into a kaleidoscope for all the senses, in order to create new space for dreaming. The Crystal World-Shuttle is departing daily every second hours from 09.00 a.m. until 03.00 p.m. from Innsbruck to Wattens and back.

Opening hours: daily 08.30 a.m. – 07.30 p.m. | last entry 06.30 p.m.

Departure times Congress/Hofburg:

08.44 a.m. | 10.24 a.m. | 12.44 p.m. | 02.44 p.m. | 04.44 p.m. | 06.24 p.m.

BARS & RESTAURANTS

17 360° & RESTAURANT LICHTBLICK

Maria Theresienstraße 18, 6020 Innsbruck

Tel.: +43 664 840 65 70 50 | www.360-grad.at

Enjoy Restaurant Lichtblick´s delicious cuisine and the breathtaking 360 degree view above Innsbruck in the identically named bar close by. Because Lichtblick stands for openness and transparency and invites you to stay.

18 BAR CENTRALE & DAS SCHINDLER

Maria-Theresien-Strasse 31, 6020 Innsbruck

Tel.: +43 512 56 69 69 | www.dasschindler.com

Austrian cuisine is being interpreted in a modern way, variety and openness, under the influences of other countries and regions: Graceful Schindler. Underneath, you can enjoy tasteful long drinks until the early morning hours in Bar Centrale.

19 HITT & SÖHNE

Höhenstraße 147, Hungerburg, 6020 Innsbruck

Tel.: +43 664 5277565 | www.hittundsoehne.at

After a few minutes by bus or the modern Hungerburgbahn, which departs directly at the Congress Innsbruck, you are far away from hectic city center. Enjoy your food and drinks with the view over Innsbruck from Hitt und Söhne, the new modern bar above Innsbruck.

20 KATZUNG

Herzog-Friedrich Straße 16, Altstadt, 6020 Innsbruck

Tel.: +43 512 586 183 | www.cafe-katzung.at

Café Katzung - a coffeehouse with tradition that tends the coffee culture in a modern way: no matter what kind of table you get hold of!

21 LÖWENHAUS

Rennweg 5, 6020 Innsbruck

Tel.: +43 676 88 44 77 06 | www.loewenhaus.at

Being part of Innsbruck's traditional guesthouse, the Löwenhaus team pursuits consequently new trends, but never forget their origin. They try to breathe new life into the original Austrian dishes – and succeed!

22 M+M BAR

Innstraße 45, 6020 Innsbruck

Tel.: +43 699 15220139 | www.mm-bar.at

THE cocktail bar in Innsbruck offers you a huge selection of cocktails, longdrinks and liquors.

23 ORANGERIE

Maria-Theresien-Straße 10, 6020 Innsbruck

Tel.: +43 512 58 16 39 | www.orangerie-innsbruck.at

The stylish Orangerie offers a menu that sends you on a journey around all culinary delights.

24 PAVILLON

Rennweg 4, 6020 Innsbruck

Tel.: +43 512 25 70 00 | www.der-pavillon.at

A glass tube invites you to enjoy the mood lighting that creates a modern atmosphere – at the Landhausplatz in front of the congress.

25 SITZWOHL

Stadtforum, 6020 Innsbruck

Tel.: +43 512 562 888 | www.restaurantsitzwohl.at

Harmonious and elegant. But simultaneously comfortable rounds off the description of well-being and creates a place that please heart and soul.

26 STIFTSKELLER

Stiftgasse 1, 6020 Innsbruck

Tel.: +43 512 570 706 | www.stiftskeller.eu

Close to the old town you can find the Stifskeller. The restaurant and beer garden is located in the heard of Innsbruck's city and gives you a cozy get-together.

27 TREIBHAUS

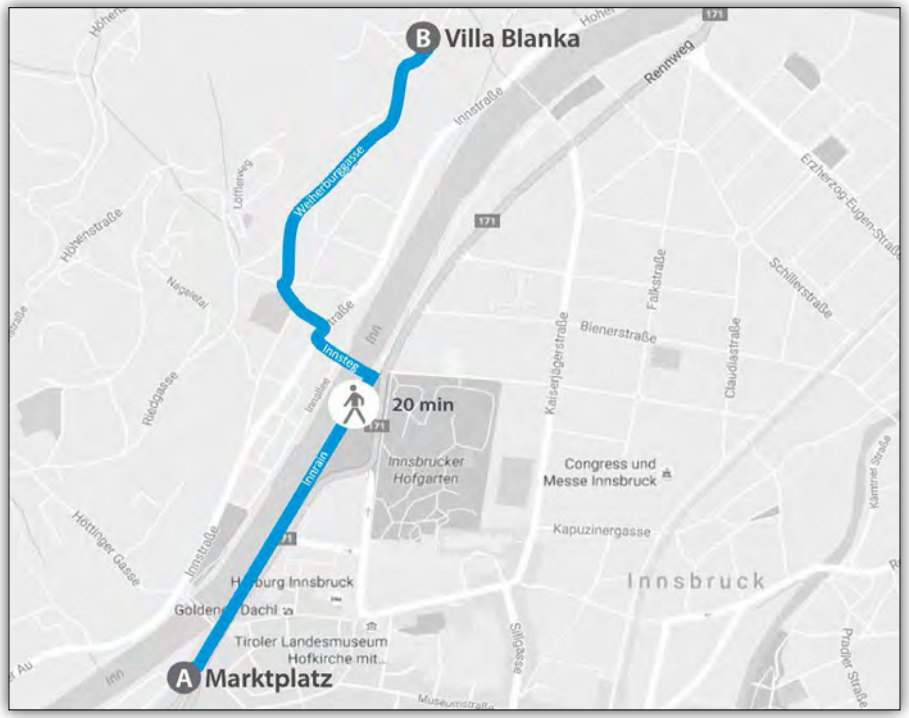
Angerzellgasse 8, am Volksgarten, 6020 Innsbruck

Tel.: +43 512 572 000 | www.treibhaus.at

Comparable to all beautiful places, you have to search the greenhouse. The lucky one, who finds it, is rewarded with a place for conversation with everyone. A cozy atmosphere brings together especially artists and „locals". While restaurant and café at daytime, artists of all types entertain at night.

WALKING MAP

The indicated pedestrian way in-between downtown Innsbruck and Villa Blanka takes 20 min.



BUS SCHEDULE

WEDNESDAY | November 16th

From	Departure	To	Arrival	Shuttles
Markthalle	16:46	Villa Blanka	16:51	2
Markthalle	17:01	Villa Blanka	17:06	2
Markthalle	17:16	Villa Blanka	17:21	2
Markthalle	17:31	Villa Blanka	17:36	2
Villa Blanka	21:08	Markthalle	21:14	1
Villa Blanka	21:38	Markthalle	21:45	1
Villa Blanka	22:08	Markthalle	22:14	2
Villa Blanka	22:38	Markthalle	22:44	2

BUS SCHEDULE

THURSDAY | November 17th

From	Departure	To	Arrival	Shuttles
Markthalle	08:16	Villa Blanka	08:21	2
Markthalle	08:31	Villa Blanka	08:36	2
Markthalle	08:46	Villa Blanka	08:51	2
Villa Blanka	18:38	Markthalle	18:44	1
Villa Blanka	19:08	Markthalle	19:18	1
Villa Blanka	20:38	Markthalle	20:44	1
Villa Blanka	20:53	Markthalle	20:59	2
Villa Blanka	21:08	Markthalle	21:18	2

FRIDAY | November 18th

From	Departure	To	Arrival	Shuttles
Markthalle	08:16	Villa Blanka	08:21	2
Markthalle	08:31	Villa Blanka	08:36	2
Markthalle	08:46	Villa Blanka	08:51	2
Villa Blanka	17:38	Markthalle	17:44	1
Villa Blanka	17:53	Markthalle	17:59	2
Villa Blanka	18:08	Markthalle	18:14	2
Markthalle	18:46	Villa Blanka	18:52	2
Markthalle	19:00	Villa Blanka	19:06	2
Markthalle	19:16	Villa Blanka	19:22	2
Villa Blanka	23:10	Markthalle	23:16	1
Villa Blanka	23:30	Markthalle	23:40	1
Villa Blanka	23:50	Markthalle	00:00	1
Villa Blanka	00:10	Markthalle	00:15	1
Villa Blanka	00:25	Markthalle	00:30	1

SATURDAY | November 19th

From	Departure	To	Arrival	Shuttles
Markthalle	08:16	Villa Blanka	08:21	1
Markthalle	08:31	Villa Blanka	08:36	1
Markthalle	08:46	Villa Blanka	08:51	1

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ABSTRACT BAND

Annual Meeting

Österreichische Gesellschaft für Allergologie & Immunologie

November 16th - 19th 2016

VILLA BLANKA | TYROL | AUSTRIA

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Oral Presentation-Nr. 001

Abstract-ID: O1 | Oral Presentation 17.11.2016, Session 1, Immunity to Infections 9.00-10.40

Establishment Of a 3D respiratory system to study fungal infections

P. Chandorkar[1], W. Posch[1], M. Steger[1], M. Blatzer[1], C. Ammann[2], M. Hermann[3], C. Lass-Flörl[1], D. Wilflingseder[1]

[1]Division of Hygiene & Medical Microbiology, [2]Department of Experimental Orthopaedics, [3]Department of Anaesthesiology & Critical Care Medicine, Medical University Innsbruck, Austria

Aspergillus (A.) fumigatus causes life-threatening pulmonary infections in patients with a challanged immune system. Current studies to better understand the fungal invasion process are primarily performed in animal models and cell lines, both afflicted with drawbacks. Thus, we set up a perfused 3-dimensional *in vitro* cell culture model with primary, differentiated human bronchial epithelial cells and immune cells important to sense fungal pathogens, i.e. alveolar macrophages or dendritic cells (DCs). Our highly sophisticated cell culture model is suited to study fungal-epithelial-immune interactions at the entry sites of the pathogen. Development, differentiation and interactions with *A. fumigatus* of epithelial - immune cell co-cultures under static or perfused conditions were studied using confocal, scanning electron and live cell microscopy. Cytokine analyses from *Aspergillus*-exposed tissues and TEER measurements were performed. Analysis over time by confocal and live cell microscopy showed that respiratory cells differentiated in an air-liquid interface to form tight junctions, produced mucus and developed cilia - this process was significantly accelerated under perfused compared to static conditions. Upon fungal infection, *A.fumigatus* was trapped in the muco-ciliary layer up to 3h before internalization by epithelial cells. In epithelial-immune cell co-cultures, migration of DCs from basolateral to apical side was detected upon fungal infection, where DCs co-localized with fungal hyphae. Our model will provide novel immunologic and mechanistic insights into *Aspergillus*-infection processes within 3D space. Furthermore, this model provides a vast array of applications with respect to respiratory challenges and diseases and can be exploited in terms of translational goals. Additionally, animal experimentation can be significantly reduced by use of this highly developed human system, thereby contributing to ethical considerations and higher biological relevance in terms of avoiding interspecies differences.

The study is supported by the FWF (W-1253) and the CD Laboratory for Invasive Fungal Infections.

SESSION 1 | IMMUNITY TO INFECTIONS

Oral Presentation-Nr. 002

Abstract-ID: O2 | Oral Presentation 17.11.2016, Session 1, Immunity to Infections 9.00-10.40

Myeloid STAT1 signaling protects from murine CMV infection and promotes inflammation-induced extramedullary hematopoiesis

*R. Gawish[*1], M. Biaggo[*1], C. Lassnig[1,2], Z. Bago-Horvath[3], R. Rom[1], L. Amenitsch[1], J. Kornoff[1], A. Krmpotic[4], S. Jonjic[4], B. Strobl[1] and M. Müller[1,2]*

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Cytomegalovirus (CMV) infection represents a major health care problem as it causes congenital birth defects and is often fatal for immune-compromised patients. It is well established that interferon (IFN) responses protect against various pathogens, including CMV, but little is known about the specific contribution of myeloid IFN-induced antiviral immunity. Using mice lacking the signal transducer and activator of transcription 1 (STAT1) in lysozyme expressing, myeloid cells (Stat1$^{\Delta MonoMac}$), i.e. harboring macrophages and monocytes that are unresponsive to all types of IFN, we investigated the role of myeloid STAT1 signaling during murine CMV (MCMV) infection and CpG-induced sterile inflammation. Myeloid STAT1 suppressed MCMV propagation and profoundly protected from infection-associated immunopathology in the spleen. In contrast, myeloid STAT1 did not significantly impact MCMV replication in the liver and associated hepatitis. Surprisingly, despite the fact that Stat1$^{\Delta MonoMac}$ mice exhibited more tissue damage in the spleen, they developed less severe splenomegaly as compared to littermate controls. This was at first counterintuitive but turned out to be the result of an impaired induction of extramedullary hematopoiesis (EMH) during MCMV infection. Using CpG, a well established trigger of EMH, we could further show that this effect not restricted to MCMV infections, as Stat1$^{\Delta MonoMac}$ mice also showed a failure in EMH triggered by CpG-induced sterile inflammation. In both models the total number of B cells, NK cells and erythroid cells was significantly lower in Stat1$^{\Delta MonoMac}$ animals as compared to littermate controls, while T cell and neutrophil numbers were unaffected. Taken together our data show that STAT1 signaling in macrophages on the one hand protects from CMV infection and on the other hand crucially mediates inflammation-induced EMH.

Oral Presentation-Nr. 003

Abstract-ID: O3 | Oral presentation: 17.11.2016, Session 1, Immunity to Infections 9.00-10.40

Homeostatically driven type-2 pathways shape and maintain the pulmonary immune environment

S. Saluzzo[1,2,3], A.D. Gorki[1,2] , B. Rana[3], S. Scanlon[3], R. Martins[1,2], O. Sharif[1,2], J.W. Warszwaska[1,2], K. Lakovits[1,2], A. Hladik[1,2], H. Jolin[3], I. Mesteri[4], A.N.J. McKenzie[3] & S. Knapp[1,2]

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At birth the lung epithelium is exposed to external environment and ventilation associated damage and at the same time needs to develop a protective immunity to infection. We investigated the homeostatic role of the epithelium-derived alarmin IL-33 in newborns IL-33 citrine reporter mice and the potential consequences of IL-33 downstream pathways in shaping the early immune environment of the lung. We discovered the immediate upregulation of IL-33 from the first day of life, closely followed by a wave of IL-13-producing type-2 innate lymphoid cells (ILC2s) and eosinophils influx. This physiological type 2 response to birth coincided with the appearance of alveolar macrophages (AMs) and their early in tissue polarization to an IL-13-dependent anti-inflammatory M2 phenotype. ILC2s contributed to post-natal lung quiescence at homeostasis by secreting low constant amounts of IL-13 maintaining the polarization of tissue resident AMs. In absence of ILC2s, transplanted monocytes progenitors presented a more inflammatory M1 phenotype at steady state. Finally, ILC2s and IL-13 derived polarization of AMs comes at the cost of a delayed response to *in vivo* and *in vitro* Streptococcus pneumoniae challenge. These data highlight the homeostatic role of ILC2s in setting the activation threshold in the lung, and underline their implications in anti-bacterial defenses.

SESSION 1 | IMMUNITY TO INFECTIONS

Poster-Nr. 001

Abstract-ID: P1 | Poster presentation: 17.11.2016

Non-hematopoietic cells in memory inflation

Z. Bánki[1,2], J. Colston[2], L.N. Lee[2], C. Willberg[2], J. Holzki[3], A.E. Denton[4], H. Stoiber[1], D. von Laer[1], D.T. Fearon[4], L. Cicin-Sain[3], B. Ludewig[5], P. Klenerman[2]

[1]Division of Virology, Medical University of Innsbruck, Innsbruck, Austria; [2]Peter Medawar Building for Pathogen Research, University of Oxford, Oxford, UK; [5]Immune Aging and Chronic Infections, Helmholtz Centre for Infection Research, Braunschweig, Germany; [3]Department of Medicine and Cancer Research UK Cambridge Institute, University of Cambridge, Cambridge, UK; [4]Institute of Immunobiology, Kantonsspital St. Gallen, St. Gallen, Switzerland

In recent years accumulation of specific CD8 memory T cells - termed memory inflation - has appeared to be one of the most important aspects of cytomegalovirus (CMV) immunobiology. Present studies indicate that DCs are responsible for the induction of primary CMV-specific CD8 T cells responses, whereas latently infected non-hematopoietic cells are thought to drive memory CD8 T cell inflation. In our study we took advantage of a novel model of memory inflation based on replication-deficient adenovirus (HuAd5), which recapitulates the phenotype, function, kinetics and distribution of memory inflation, without viral replication. First we investigated HuAd5 infection of non-hematopoietic stromal cell subsets in the secondary lymphoid tissues (LT) as well as liver sinusoidal endothelial cells (LSEC) using HuAd5 encoding GFP. We found that in the secondary LTs fibroblastic reticular cells (FRC) and lymphoid endothelial cells (LEC), but not blood endothelial cells (BEC) are susceptible for HuAd5 infection *in vivo*. Similarly, HuAd5 targeted LSECs in the liver. We further flow sorted and cultured individual cell populations and confirmed these findings *in vitro* . Furthermore, HuAd5-lacZ infected FRCs, LECs and LSECs were able to activate bgal-specific inflating, but not non-inflating CD8 T cells *in vitro* . Depletion of FRCs *in vivo*, however did not influence the maintenance of memory inflation. Since the intravenous infection route, which preferentially infected liver/spleen non-hematopoietic cells, triggered memory inflation, the antigen presentation by LSECs rather than FRCs and LECs drives memory inflation. These results further support that latently infected non-hematopoietic cells may critically contribute to memory inflation.

The study is supported by the FWF (an Erwin Schrödinger Fellowship J-3484, the doctoral program HOROS W-1253 and the DACH project I-2550).

SESSION 1 | IMMUNITY TO INFECTIONS

Poster-Nr. 002

Abstract-ID: P2 | Poster presentation: 17.11.2016

IgG opsonization of Friend virus (FV) abrogates the capacity of dendritic cells to activate specific CD8 T cells through Fcγ receptor type I (CD64)

D. Bichler[1], R. Werner[1*], M. Idrees[1], V. Oberhauser[1], B. Schiela[1], S. Verbeek[2], D. von Laer[1], U. Dittmer[3], H. Stoiber[1], Z. Bánki[1]*

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*contributed equally

Dendritic cells express Fcγ receptors (FcγR) for binding of IgG molecules immune-complexed (IC) with antigens. IC-FcγR interactions have been demonstrated to modify activation and antigen-presenting functions of DCs. Utilizing Friend virus, a mouse retrovirus model, we investigated the effect of IgG-opsonized retroviral particles on the infection of DCs and the subsequent presentation of viral antigens by DCs to specific CD8 T cells. We found that IgG-opsonization abrogates DCs infection and as a consequence significantly reduce the capacity of DCs to activate virus-specific CTL response. Effects of IgG-opsonization were mediated by the high affinity FcγR type I, CD64, expressed on DCs. Our results suggest that different opsonization patters on retroviral surface modulate antigen presenting functions of DCs, whereby in contrast to complement, IgG reduces the capacity of DCs to activate CTL responses.

The study is supported by the FWF (doctoral program HOROS W1253-B24 and the DACH project I- 2550-B30).

Poster-Nr. 003

Abstract-ID: P3 | Poster presentation: 17.11.2016

Aspergillus fumigatus responds to Natural Killer (NK) cells with upregulation of stress related genes and inhibits the immunoregulatory function of NK cells

A. Schneider[1], M. Blatzer[2], W. Posch[2], R. Schubert[3], C. Lass-Flörl[2], S. Schmidt[1], T. Lehrnbecher[1]

[1] Divisions of Pediatric Hematology and Oncology, [3] Pediatric Pulmonology, Allergology and Cystic Fibrosis, Hospital for Children and Adolescents, Johann Wolfgang Goethe-University, Frankfurt, Germany and 2 Division of Hygiene and [2]Medical Microbiology, Medical University of Innsbruck, Innsbruck, Austria

Natural Killer (NK) cells are active against *Aspergillus fumigatus*, which in turn is able to impair the host defense. Unfortunately, little is known on the mutual interaction of NK cells and *A. fumigatus*. We coincubated human NK cells with *A. fumigatus* hyphae and assessed the gene expression and protein concentration of selected molecules. We found that *A. fumigatus* up-regulates the gene expression of pro-inflammatory molecules in NK cells, but inhibited the release of these molecules resulting in intracellular accumulation and limited extracellular availability. *A. fumigatus* down-regulated mRNA levels of perforin in NK cells, but increased its intra- and extracellular protein concentration. The gene expression of stress related molecules of *A. fumigatus* such as heat shock protein hsp90 was up-regulated by human NK cells. Our data characterize for the first time the immunosuppressive effect of *A. fumigatus* on NK cells and may help to develop new therapeutic antifungal strategies.

Poster-Nr. 004

Abstract-ID: P4 | Poster presentation: 17.11.2016

Complement activation profiles in juvenile idiopathic arthritis

T. Giner[1], L. Hackl[1], J. Albrecht[1], R. Würzner[2], J. Brunner[1]

[1]Department of Pediatrics; [2]Division of Hygiene and Medical Microbiology, Medical University,Innsbruck, Innsbruck, Austria

Introduction: Juvenile idiopathic arthritis (JIA) summarizes a group of phenotypically heterogeneous chronic inflammatory disease of childhood. The innate immunity is playing a role in the pathogenesis of JIA.

Objectives: This is a controlled prospective observational study. It analyzes the three pathways of complement system (CS) and the terminal complement complex (TCC) in JIA. (Trial numberUN373).

Methods: Peripheral blood samples (PB) (n=158) of 57 JIA patients were analyzed for specific complement pathway activation (COMPL300 ELISA), complement factor H (CFH)-autoantibodies and the soluble membrane attack complex MAC (sC5b-9 ELISA) in serum (S) and EDTA-plasma (P)- The JIA subgroups were persistent Oligoarthritis (n=19), extended Oligoarthritis (n=8), rheumatoid factor positive Polyarthritis (n=4) and negative Polyarthritis (n=12), Enthesitis related arthritis (n=4); Psoriatic arthritis (n=3) and systemic JIA (n=7). As control group (n=118) healthy adults (n=100) and children (n=18) without inflammatory diseases were tested. JADAS10 Score defined acute phase of disease.

Results: JIA patients within acute phase of disease (n=53) showed lower capacity in CP (82% [38-97% IQR] vs 104% [97-115% IQR] ($p<0.001$)) and AP (34% [2-97% IQR] vs 85% [70-99% IQR] ($p<0.001$)) compared to the control group in median. Also sMAC was elevated (P 2.3 [1.27-3.43IQR] vs 1.2 [0.84-1.84] AU/ml) in patients with decreased AP in acute phase ($p<0.009$) compared to the control group. No evidence of CFH-autoantibodies was found in our study group. The sMAC levels were significantly ($p<0,009$) higher in sera (15.5 [12.03-20.91 IQR] AU/ml) and plasma (1.75 [0.9-3.46 IQR] AU/ml) compared to the control group (S 7.78 [4.9-10.32 IQR] AU/ml, P 1.22 [0.78-1.81 IQR] AU/ml) in the patients with extended and persistent OA, in PARF+ and ERA..

Conclusion: Special groups of JIA showed increased CS activation with elevated levels of MAC in PB in acute phase of disease. The additional decreased capacity in the CP and AP suppose that the complement system as an additional contributor in pathogenesis and/or course of the acute disease.

SESSION 1 | IMMUNITY TO INFECTIONS

Poster-Nr. 005

Abstract-ID: P5 | Poster presentation: 17.11.2016

Hyperimmunoglobulinemia D Syndrome is an example for an interface between metabolism and inflammation

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Mevalonate kinase (MVK) deficiency is an inborn error in metabolism which is responsible for an disorder of autoinflammation, called hyperimmunoglobulinemia D syndrome (HIDS), OMIM#260920), an autosomal recessive autoinflammatory disease, caused by mutations in the MVK gene (chromosome 12q24). The pathogenesis is responsible for an excessive Interleukin 1 production, leading to an proinfalammatory milieu. MVK is an essential enzyme in the isoprenoid pathway. Mutations in MVK gene can also lead to a severe, rare metabolic phenotype (mevalonic aciduria (MA)), which presents with fever, severe neurological impairment, growth retardation and early death.

Case report: We report on a 2 year-old Austrian boy with recurrent episodes of fever, febrile seizures, arthralgias, and splenomegaly. Rash and abdominal pain were also seen occasionally. During attacks an acute-phase response was detected. Clinical and laboratory improvement was seen between attacks. These findings led to the tentative diagnosis of HIDS. Sequencing of the MVK gene showed a homozygous c.1129G>A (p.Val377Ile, also known as V377I) mutation in the child, while the healthy non-consanguineous parents were heterozygous. The mutation is known to be associated with HIDS. Therapy with Interleukin 1 antagonists may be an option for patients with HIDS. Therefore treatment with canakinumab was initiated and a final dose of 4 mg/kg every 4 weeks resulted in the disappearance of febrile attacks and a considerable improvement of patient´s quality of life during a 6-month follow-up period. The drug has been well tolerated, and no side effects were observed.

Poster-Nr. 006

Abstract-ID: P6 | Poster presentation: 17.11.2016

Galactosaminogalactan secreted from *Aspergillus fumigatus* affects human platelet activity and stimulate complement system

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Aspergillus (A.) and mucormycetes species cause severe infections in immunocompromised patients. To understand pathomechanisms and antifungal defence in more detail we studied the role of platelets and complement, which are important innate immunity elements. Recent own studies showed that the secreted fungal polysaccharide galactosaminogalactan (GAG) might be an important player since it affects platelet activity and activates the complement system.
Supernatant (SN) of *Aspergillus* and different mucormycetes isolates were collected after 2 days fungal growth and added to human platelets. GAG secretion, platelet activation and complement deposition on platelets were studied by scanning electron microscopy, FACS, confocal laser microscopy.
Incubation of platelets with *A.fumigatus* and *A.flavus* SN resulted in deposition of secreted fungal material on the platelet surface whereas no deposition was obvious when incubating the platelets with medium and SN of mucormycetes. This deposition of fungal material correlated with expression of GAG by *A.fumigatus* and *A.flavus*. Furthermore, the two SN triggered significant platelet activation. Other GAG effects on the platelets included the deposition of complement factor C3 and the formation of the C5b-9 complex on the platelet surface. A perfect correlation between presence of GAG and platelet activation/opsonization could be underlined by the comparison with different *Aspergillus* and mucormycete species. Furthermore, GAG-induced shedding of microparticles was noticed, which represent important pro-inflammatory mediators in the human body.
Our findings underline the hypothesis that GAG might represent an important fungal immunomodulatory molecule. Putative consequences of its activity include platelet-mediated antifungal attack and support of other elements of the immune network, but also thrombus formation and excessive inflammatory reactions.

SESSION 1 | IMMUNITY TO INFECTIONS

Poster-Nr. 007

Abstract-ID: P7 | Poster presentation: 17.11.2016

Dopamine regulates iron homeostasis and innate immune responses of macrophages to Salmonella infection

S. Dichtl[1], D. Haschka[1], E. Demetz[1], O. Lutz[2], M. Nairz[1], M. Aßhoff[1], M. Seifert[1], S. Berger[1], C. Huck[3], G. Weiss[1]

[1]Department of Internal Medicine VI, Medical University of Innsbruck, Austria; [2]Austrian Drug Screening Institute, Innsbruck, Austria; [3]University of Innsbruck, Austria

Backround: Siderophores are catechol based compounds which can bind iron. Iron is an essential growth factor for mammalian cells and microbes. Based on previous observations, showing increased bacterial growth in the presence of catechols, we asked whether this may be referred to hormone mediated alterations of iron homeostasis.

Methods: We studied the effects of the catecholamine dopamine on the regulation of iron in BMDM obtained from C57BL/6 mice. The *in vivo* effects of dopamine were studied in wt mice infected with the Gram negative bacteria Salmonella typhimurium (S.tm.).

Results: Administration of dopamine to macrophages resulted in a dose dependent increase of transferrin receptor-1 and ferritin expression, which causes an increased intracellular iron content. The *in vitro* data show that the increased mobilization of iron can be used by Salmonella for their growth and proliferation. The *in vivo* administration of dopamine to mice infected with S.tm. resulted in higher bacterial burden in liver and spleen as compared mice receiving solvent. This is linked to an increased delivery of iron to bacteria in the presence of dopamine along with an impaired pro-inflammatory immune response of macrophages.

Conclusion: Our data demonstrate that dopamine may deteriorate the course of infection by promoting bacterial growth which can be a major concern from the treatment of patients with bacterial sepsis receiving catecholamines.

SESSION 1 | IMMUNITY TO INFECTIONS

Poster-Nr. 009

Abstract-ID: P9 | Poster presentation: 17.11.2016

The role of tyrosine kinase 2 (TYK2) in the immune response during endotoxemia and bacterially induced sepsis

D. Gogova[1], *A. Poelzl*[1], *R. Gawish*[1], *M. Prchal-Murphy*[2], *C. Lassnig*[1,3], *Sylvia Knapp*[4], *M. Mueller*[1,3], *B. Strobl*[1]

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Sepsis is a systemic inflammatory reaction in the course of an infection and still represents a major health care problem due to high mortality rates. TYK2 belongs to the Janus kinase family of receptor-associated tyrosine kinases and is an integral part of signaling cascades initiated by multiple cytokines. TYK2-utilizing cytokines are involved in diverse processes, such as immunity to infection, inflammation and tissue repair. Absence of TYK2 in mice is protective during lipopolysaccharide (LPS)-induced shock and it is largely unclear how TYK2 contributes to disease progression.
Using mice that lack TYK2 (*Tyk2*$^{-/-}$) and mice that express a kinase-inactive version of TYK2 (*Tyk2*^{K923E}) our aim is to define the role of TYK2 and its kinase activity during sterile inflammation and Escherichia coli-induced sepsis. We show that *Tyk2*^{K923E} mice have improved survival as compared to wild-type mice upon both lethal LPS-challenge and *E. coli* peritonitis. Interestingly, *Tyk2*^{K923E} mice are less protected from endotoxemia than *Tyk2*$^{-/-}$ mice, whereas they show a tendency toward higher resistance upon *E. coli* infection, indicating that kinase-inactive TYK2 contributes to LPS- but not E.coli-induced disease progression. Despite the well-established critical function of TYK2 in antibacterial immunity, we did not observe differences in bacterial load between wild-type, *Tyk2*$^{-/-}$ and *Tyk2*^{K923E} mice. Liver damage parameters were increased in *Tyk2*$^{-/-}$ and *Tyk2*^{K923E} compared to wild-type mice in both experimental models, whereas *Tyk2*^{K923E} mice showed increased signs for kidney damage compared to *Tyk2*$^{-/-}$ mice at late time-point after LPS challenge (when wild-type mice already succumbed to disease).
Taken together, our data suggest that kinase-inactivation of TYK2 protects against both endotoxemia and E.coli-induced sepsis, although with different efficiency. Underlying mechanisms are under investigation.

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Poster-Nr. 010

Abstract-ID: P10 | Poster presentation: 17.11.2016

Single shot booster vaccination against diphtheria does not induce sufficient long-term protection, particularly in elderly people

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Immunization is one of the most successful health intervention against infectious diseases. However, the efficacy of vaccination is reduced in old age. Our study analyzed specific immune responses following a diphtheria booster vaccination in healthy elderly (>60y; n=87) and young volunteers (25-40y; n=46). Long term protection was evaluated for 27 elderly and 17 young adults 5 years later. In addition, antigen-specific T-cells producing 9 different cytokines were quantified.
The level of protection against diphtheria was almost equal in booth age groups before the vaccination (52% for the elderly and 48% for the young donors). Antibody concentrations increased significantly 4 weeks after vaccination, but dropped substantially over 5 years leaving again 54% (elderly) and 24% (young) below protective antibody levels. Thus, compared to the elderly, young adults have a significantly better, but still insufficient maintenance of diphtheria-specific antibodies. We found correlations between diphtheria-specific antibodies and diphtheria-specific T-cells producing different cytokines. Among those were GM-CSF-producing T-cells which we detected in a higher frequency in donors with a good antibody response. To further investigate the role of GM-CSF, we set up a diphtheria-vaccination mouse model. Mice treated with GM-CSF showed a significantly better diphtheria-specific antibody response following a primary vaccination compared to mice treated with a placebo.
In conclusion, our findings demonstrate that booster vaccinations induce an insufficient long-lasting immunity against diphtheria, particularly in elderly people. GM-CSF might be a useful immune active substance to improve diphtheria vaccination. We will further study the mechanism of GM-CSF in the immune response to vaccination.

Poster-Nr. 011

Abstract-ID: P11 | Poster presentation: 17.11.2016

The viral vector vaccine VSV-GP as vaccine platform

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Introduction: Our group has recently shown that VSV pseudotyped with the glycoprotein (GP) of the lymphocytic choriomeningitis virus, VSV-GP, is a potent vaccine vector, overcoming limitations of wild type VSV. Here, we evaluated the potential of VSV-GP as a vaccine vector for infectious disease such as HIV or RSV.

Methods: We incorporated antigens from pathogens or marker genes into the genome of VSV-GP and generated infectious viruses via reverse genetics. These viruses were analyzed *in vitro* for antigen expression, location and conformation. After mouse immunization studies distribution and kinetics of infected cells and antigen-specific as well as vector-specific immune responses were analyzed.

Results: Infectious viruses containing either antigens from HIV or RSV or marker genes such as luciferase were generated. Antigens from pathogens were expressed in VSV-GP infected cells and incorporated into VSV-GP particles. The addition of an extra gene did not attenuate VSV-GP replication. After intramuscular immunization, viral replication was limited to injection side and the draining lymph nodes. No neutralizing antibodies against VSV-GP were induced even after seven boost immunizations. Therefore, homologous boost immunization was highly efficient and high titers of HIV- or RSV-specific antibodies were induced.

Discussion: Taken together, VSV-GP is non-neurotoxic, induces potent immune responses, enables boosting and thus is a promising novel vaccine vector platform.

SESSION 1 | IMMUNITY TO INFECTIONS

Poster-Nr. 012

Abstract-ID: P12 | Poster presentation: 17.11.2016

IL-17 stimulates hepcidin expression via STAT3 and NF-kB

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Background: Naïve $CD4^+$ helper T cells differentiate after TCR activation into the 3 characterizing cytokine-secreting effector cells: TH-1, TH-2, and TH-17. The TH-17 lineage is characterized by IL-17 production and is induced by IL-23, IL-6 and TGF-ß.

The role of IL-17-mediated immune functions on hepatocytes and iron homeostasis however, has not been elucidated thus far. Herein, we investigated the involvement of IL-17 in induction of hepcidin in hepatocytes *in vitro* and in the liver *in vivo*.

Materials: C57bl/6 mice were divided in 4 groups and received IL-17, IL-6 or a combination of IL-17 and a blocking antibody of IL-6 by a intraperitoneal injection. PBS was used for the control group. After different time points (1, 3, 6, 12 and 24 hours) mice were killed and the liver and serum were used for quantification of hepcidin, IL-6 and iron by PCR, Western Blotting and ELISA.

In vitro, we either used primary hepatocytes, which were isolated from C57bl/6 mice, or FL83B hepatocytes. Cells were stimulated with IL-17, IL-6 or LPS. For blocking experiments, a monoclonal antibody against IL-6, WP1066 (Jak2/STAT3 inhibitor) or CAPE (NF-kB inhibitor) were used. The cells were harvested after different time points and analyzed for the expression of hepcidin by PCR or STAT3/pSTAT3 and NF-kB by Western blotting.

Results/Conclusion: In our experiments we could clearly show that hepcidin expression in hepatocytes is in part induced by IL-17 independently of IL-6. As signaling pathways we could identify STAT3 and NF-kb. Thus, our data suggest that IL-17 might play a crucial role in iron metabolism in the liver during an acute infection.

SESSION 1 | IMMUNITY TO INFECTIONS

Poster-Nr. 013

Abstract-ID: P13 | Poster presentation: 17.11.2016

HbsAg-binding CAR-engineered T cells control virus replication in mice

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T lymphocytes are powerful components of adaptive immunity, which essentially contribute to the elimination of virus. Due to their cytotoxic capacity, T cells emerged as attractive candidates for specific immunotherapy of HBV infection. A promising approach is the genetic modification of T cells with chimeric antigen receptor (CAR). We designed a retroviral vector expressing a CAR with the viable region of antibody specificity for HbsAg, coupled with CD28 (a costimulatory receptor in T cells) and CD3-zeta (a signal-transduction component of the T-cell antigen receptor) signaling domains. $CD8^+$ T cells that expressed HBV-specific CAR recognized different HBV subtypes and were able to engraft and expand in immune-competent HBV transgenic mice. After adoptive transfer, the T cells localized to and functioned in the liver and rapidly and efficiently controlled HBV replication compared with controls, causing only transient liver damage. The large amount of circulating viral antigen did not impair or overactivate the grafted T cells. Thus, this immune cell therapy might be developed for patients with chronic hepatitis B, regardless of their HLA type.

Poster-Nr. 014

Abstract-ID: P14 | Poster presentation: 17.11.2016

Iron affects T-helper 1 cell differentiation by influencing the expression of T-cell immunoglobin and mucin-domain containing-3 (Tim-3)

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Salmonella enterica serovar typhimurium is a Gram negative, facultative intracellular bacterium which causes gastrointestinal disorders in humans and systemic, typhoid fever like infections in mice. Macrophages control S. typhimurium multiplication by inducing anti-microbial pathways including radical formation and by restricting available iron to bacteria in an Nramp1 dependent manner. Iron limitation also contributes to an effective host response by increasing effector pathways induced by interferon gamma, a cytokine originating from activated T-helper 1 cells (Th1). Studies in mouse models have shown that protection against Salmonella also requires specific Th1 $CD4^+$ T clones. We thus were interested, whether perturbations in iron homeostasis may impact on T-cell differentiation. Systemic infection of Nramp1 transgenic mice with S. typhimurium, showed that iron availability affects the differentiation of $CD4^+$ T cells to Th1 cells by influencing the expression of Tim-3. Tim-3, a negative regulator of Th1 T cells, is highly expressed in chronic infections and cancer where it contributes to the dampening of protective immunity. We found that dietary iron loading of mice prior to infection led to a time dependent increase in Tim-3 expression accompanied by a decrease in Th1 cells. Th2 $CD4^+$ T cells and regulatory T cells were not affected. *In vitro* we could show that increasing concentrations of different iron sources reduced T cell proliferation in general and induced the expression of Tim-3 by a molecular mechanism which is currently under investigation. *In vitro* differentiation of naive T cells to Th1 cells was paralleled by an iron dependent increase of Tim-3 protein and mRNA expression levels. Taken together, our data provide evidence that an increased iron availability in mice dampens the differentiation of naive $CD4^+$ T cells to protective Th1 cells by increasing the expression of the negative regulator Tim-3 resulting in an unfavorable course of the infection.

Poster-Nr. 015

Abstract-ID: P15 | Poster presentation: 17.11.2016

In vitro blockage of Candida albicans factor H binding molecule Hgt1p by antibody – an approach for in vivo immunotherapy?

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Introduction: The complement system is tightly controlled by several regulators. In particular Factor H (FH) is preferentially acquired by pathogens conveying resistance to complement attack.
Objectives: The aim of the study was to determine by an *in vitro* assay whether the FH binding molecule „high affinity glucose transporter 1" (CaHgt1p) of *Candida albicans* is a significant virulence factor. Another aim was to determine whether the binding and thus inhibition by monoclonal antibody (Hgt1p-mAb) is a possible approach for an *in vivo* immunotherapy of *C. albicans.*

Methods: An *in vitro* phagocytosis study was performed to demonstrate the ability of Hgt1p-mAb to increase the phagocytosis of *C. albicans* wild type (SN-152) by human polymorphonuclear cells (PMNs). Both wild type (SN-152) and knock-out strain (*hgt1Δ/Δ*) were treated with Hgt1p-mAb, opsonized with human serum (HS) and then stained with fluorescein isothiocyanate (FITC). Fresh human PMNs cells were co-cultured with these strains for 30´ at 30°C. Positive PMNs, with internalized C .albicans, were detected using FACS analyses.

Results: Phagocytosis experiments showed a significant ($p<0.05$) lower phagocytosis of the wild type strain in contrast to *hgt1Δ/Δ* knock-out strain, unable to bind FH. The wild type treated with Hgt1p-mAb also showed a significant ($p<0.05$), albeit small, increase in phagocytosis in comparison to untreated wild type.

At the same time our data showed a similar phagocytosis of SN-152 wild type treated with Hgt1p-mAb in comparison with *hgt1Δ/Δ* knock-out strain.

Conclusions: CaHgt1p is not only a complement inhibitor, but also a virulence factor, as corroborated by *in vitro* data. The "restored" phagocytosis of SN-152 wild type treated with Hgt1p-mAb, then comparable with *hgt1Δ/Δ* knock-out strain, represents a starting point for a possible *in vivo* immunotherapy of *C. albicans.*

SESSION 1 | IMMUNITY TO INFECTIONS

Poster-Nr. 016

Abstract-ID: P16 | Poster presentation: 17.11.2016

DCs exposed to opsonized HIV-1 are not able to restore CD8+ T cell functions during bacterial co-infection

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HIV spontaneously activates complement (C) in semen and at mucosal surfaces and is therefore opsonized with a cloud of covalently bound C3 products *in vivo*. We earlier illustrated that C opsonization of HIV-1 and HIV-2 significantly modulates dendritic cell (DC) function and antigen-presenting capacity. Upon bacterial co-infection at mucosal surfaces pathogenic bacteria and their microbial products activate DCs and thereby might alter their function and T cell-stimulatory capacity regarding HIV-1, which we investigated during this study. We found similar binding of non- (HIV) and complement-opsonized HIV (HIV-C) to immature DCs (iDCs) and LPS-matured DCs (LPS-DCs), while internalization of both, HIV and HIV-C, was significantly higher in LPS-DCs. About one third of viral particles were detected in the cytoplasm independent on the opsonization pattern in iDCs, while in LPS-DCs solely endocytosis – but not fusion - was observed. HIV-C was recently shown by our group to overcome restriction in DCs in contrast to HIV. This was not the case for LPS-DCs, which were not productively infected by HIV-C. Transfer of HIV from iDCs and LPS-DCs to $CD4^+$ T cells was similar and significantly higher than HIV-C transfer, pointing to an antiviral effect of DCs loaded with HIV-C independent on co-infection. In contrast we found a weaker capacity of HIV-C-LPS-DCs to expand and activate $CD8^+$ T cells compared to HIV-C-DCs. These results indicate an impact of co-infection on the CTL-stimulatory capacity of DCs in presence of pathogenic gram-negative bacteria during acute infection.

Poster-Nr. 017

Abstract-ID: P17 | Poster presentation: 17.11.2016

A new model of *Malassezia* skin infection to explore the fungal-host interaction in vivo

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The yeast *Malassezia* is a commensal fungus and a major constituent of the skin microflora of many mammalian organisms including humans and domestic animals. There is an increasing amount of evidence that *Malassezia* species are linked to skin-associated pathologies like *Atopic dermatitis, Seborrhoeic eczema* and *Pityriasis versicolor*. However, detailed investigations of the interactions between *Malassezia* and the host have been hampered so far by difficulties of *Malassezia* cultivation *in vitro* and more importantly the lack of *in vivo* infection models. To overcome these limitations, we established a novel infection model for epicutaneous *Malassezia* infection in mice. Topical application of *Malassezia pachydermatis* or *Malassezia furfur* on the ear of immunocompetent animals leads to a local and transient inflammation of the skin characterized by ear swelling and leukocyte infiltration. Skin inflammation depends on viable *Malassezia* yeast and exacerbates upon barrier disruption of the skin prior to infection. Moreover, experiments carried out in IL-17AF-deficient animals demonstrated that fungal control depends on the IL-23-IL-17 immune axis. In addition, *Malassezia*-mediated infection elicits an adaptive immune response characterized by IL-17A-secreting T helper cells ("Th17 cells") in the skin draining lymph node. The novel infection model will provide new insights into *Malassezia*-associated pathologies and may open up new perspectives towards the development of therapeutic approaches against infection-related skin diseases.

SESSION 1 | IMMUNITY TO INFECTIONS

Poster-Nr. 018

Abstract-ID: P18 | Poster presentation: 17.11.2016

Complement-opsonization of A. fumigatus modifies dendritic cell function

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Background: In this study, interactions of dendritic cells (DCs) with complement-opsonized and non-opsonized *Aspergillus fumigatus* strains and various mutants thereof were investigated. The opsonization pattern of the different strains and mutants, the binding and internalization by dendritic cells as well as the cytokine secretion and initial signaling pathways were investigated.

Methods: Fungi were opsonized using normal human serum as complement source. The opsonization pattern, binding of conidia to DCs and internalization were characterized by FACS analyses. Inhibition of fungal growth in presence of DCs and interactions with complement receptors were detected using confocal microscopy. Furthermore, phosphorylation of ERK1/2 and p38 were detected by immunoblot analysis.

Results: We could demonstrate in this study that melanin and β-1,3-glucan have high impact on the fungal virulence compared to the wildtype *Aspergillus* strains. With respect to dendritic cell binding and internalization complement-opsonization of conidia enhanced these processes compared to their non-opsonized counterparts independent on the fungal strain used.

Conclusion: These data revealed, that melanin and β-1,3- glucan are key effectors of masking complement deposition and binding of conidia by DCs. However opsonization of swollen conidia enhanced internalization in DCs as well as production of pro-inflammatory cytokines, thereby resulting in a favorable T_H1 immune response. These *in vitro* studies propose that the use of immune cells, like DCs or neutrophils, in combination with complement opsonins might act as potent vaccines against invasive aspergillosis.

Poster-Nr. 019

Abstract-ID: P19 | Poster presentation: 17.11.2016

The impact of biologic therapies and immunosuppressants on the risk of opportunistic fungal infections

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Background: Opportunistic infections represent a serious health threat to immunocompromised patients nowadays. One of the major causes of immunosuppression is the administration of immunosuppressive drugs and biologics that augment the risk of infections with opportunistic bacteria and fungi. In this study, we want to analyze how immunosuppressive therapies influence the disease development of invasive opportunistic mycosis by *in vitro* and *in vivo* approaches.

Methods: In order to analyze the influence of immunosuppressive therapy, we will expose cells and, in further instance, mice to the immunosuppressive agents Mycophenolate mofetil, Prednisolone and the TNFα Inhibitor Infliximab (Remicade). Our major focus lies on the investigation of the immunological response to the fungus in the murine macrophage-like cell line Raw 264.7 and in different kinds of primary immune cells, both from human and murine origin. Future experiments will include the implication of mouse models to study invasive opportunistic mycosis during immunosuppression *in vivo*.

Results: So far, we detected a decrease in the phagocytosis rate of serum-opsonized beads and the opportunistic fungal pathogen *C. albicans* during infection of Raw 264.7 cells and primary macrophages isolated from murine bone marrow during treatment with Mycophenolate Mofetil. Furthermore, phagocytosis of serum-opsonized latex beads was increased in Raw 264.7 cells during treatment with Prednisolone whilst treatment of murine primary macrophages with Prednisolone decreased the phagocytosis rate.

Conclusion: Our data suggest a strong impact on mechanisms of phagocytosis during administration of immunosuppressive medication. We detected alterations in the phagocytosis in different types of macrophages during treatment with Mycophenolate Mofetil. Additionally, we show that the commonly used glucocorticoid Prednisolone had both increasing and decreasing effects on phagocytosis, depending on the cell type that was being used.

SESSION 1 | IMMUNITY TO INFECTIONS

Poster-Nr. 020

Abstract-ID: P19 | Poster presentation: 17.11.2016

Evaluation and regulation of antiviral immune responses in human Langerhans cells and keratinocytes

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Viral infection in the skin is detected by Langerhans cells (LCs) and keratinocytes (KCs). To better understand how LCs respond functionally to viral antigens at the site of infection in the epidermis, we investigated whether viral sensing receptors that recognize double stranded RNA and downstream signaling pathways can be regulated/activated in LCs upon incubation with poly (I:C) using human skin culture models.

To efficiently disrupt the physical epidermal barrier which mainly consists of the stratum corneum, normal human skin was stripped sequentially 50 times. Punch biopsies were then placed in 24 well culture plates and PBS (control) or poly(I:C), a potent inducer of a strong inflammatory response in several cell types, were epicutaneously applied. Samples were harvested after 24 and 48 hours of incubation. Cryosections and epidermal sheets were prepared and analyzed for viral sensing receptors in skin cells using immunofluorescence. Activation of MAVS, NFκB (p65) and IRF3 was examined in LCs and KCs by confocal microscopy at a single cell level.

We found that poly(I:C) upregulated TLR3 but not PKR in KCs and failed to upregulate/induce TLR3 and PKR in LCs. In the presence of poly(I:C), MDA5 was strongly downregulated in barrier disrupted skin in LCs compared to controls. A differential expression pattern for MAVS was observed in LCs and KCs. Similarly, distinct IRF3 and p65 nuclear translocation upon poly (I:C) treatment was found in LCs and KCs.

Our data suggest that MDA5 but not TLR3 and PKR may play a key role in the innate immune response of LCs to viral infection. Understanding the signaling events of LCs to viruses might promote development of attractive therapeutic strategies.

Poster-Nr. 021

Abstract-ID: P21 | Poster presentation: 17.11.2016

Immune response and early transcriptional changes after primary and booster vaccination against Hepatitis B in young and old adults

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Many currently used vaccines are less immunogenic and effective in the elderly compared to younger adults due to age-related changes of the immune system. Most vaccines utilized in the elderly contain antigens, with which the target population had previous contact. Therefore, most studies investigating vaccine-induced immune responses in the elderly do not analyze responses to neo-antigen but rather booster responses. It can be hypothesized that age-related differences in the immune response are distinct for primary and recall responses. We therefore aimed to investigate primary and recall responses using the same antigen in young and older adults and chose Hepatitis B vaccine as a model antigen. Young (20-40 years) and elderly (>60 years) healthy volunteers received either a primary series (no prior vaccination) or a single booster shot (documented primary vaccination more than 10 years ago) of the registered vaccine Twinrix. Expression of immunity-related genes was measured before and 1 day after vaccination and antibody titers were determined at several time points with a long-term follow-up 6 months after the last vaccination. Antibody responses were lower and seemed to be delayed in the elderly compared to young adults. Non-responders after the 3-dose primary series were more frequent in the elderly group. Antibody titers after booster vaccination increased in all participants. Associations between early expression-profiles on day 1 and antibody responses were found.

SESSION 1 | IMMUNITY TO INFECTIONS

Poster-Nr. 022

Abstract-ID: P22 | Poster presentation: 17.11.2016

Superior economic efficacy of allergen molecule-based diagnosis for prescription of immunotherapy in an area with multiple pollen exposure: A real life study

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Introduction: Allergen molecule-based diagnosis has been suggested to facilitate the identification of the disease-causing allergen sources and the prescription of allergen-specific immunotherapy. Aim of the current study was to compare allergen molecules-based IgE serology with allergen extract-based skin testing for the identification of the disease-causing allergen sources in an area where patients are exposed to pollen from multiple sources (trees, grasses and weeds) at the same time and to calculate the related costs for diagnosis and treatment.

Material and Methods: Patients from Astana, Kazakhstan who suffered from pollen-induced allergy (n=95) were tested by skin prick testing with a battery of tree pollen, grass pollen and weed pollen allergen extracts and by measurement of IgE antibodies specific for marker allergen molecules (nArt v 1, nArt v 3, nAmb a 1, rPhl p 1, rPhl p 5, rBet v 1) by immunoCAP. Direct and indirect costs for diagnosis based on skin prick testing and marker allergen-based IgE serology were calculated and related to direct costs for immunotherapy resulting from SPT and serological testing.

Results: The costs for SPT-based diagnosis per patient were 68.80 Euro lower than the costs for allergen molecule-based IgE serology. However, allergen molecule-based serology was more precise in detecting the disease-causing allergen sources. Accordingly a lower number of immunotherapy treatments (n=119) was needed according to molecular diagnosis as compared to extract-based diagnosis (n= 275) which reduced the costs for diagnosis and for a three years treatment in the 95 patients from 107 891.50 Euro to 58 327.15 Euro.

Conclusions: The results from this real life study thus show that SPT is less expensive than allergen molecule-based diagnostic testing but molecular diagnosis allowed more precise prescription of immunotherapy which safes substantial costs.

Oral Presentation-Nr. 004

Abstract-ID: O4 | Oral Presentation 17.11.2016, Session 2, Innate Immunity 11.30-12.05

Human skin dendritic cell fate is differentially regulated by the monocyte identity factor KLF4 during steady state and inflammation

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Langerhans cell (LC) networks play key roles in immunity and tolerance and body surfaces. LCs are established prenatally and can be replenished from blood monocytes. Unlike skin-resident dermal/interstitial-type DCs (d/intDCs) and inflammatory dendritic epidermal cells (IDECs) appearing in dermatitis/eczema lesions, LCs lack key monocyte-affiliated markers. Inversely, LCs express various epithelial genes critical for their long-term peripheral tissue residency. DCs are functionally involved in inflammatory diseases; however, the underlying mechanisms are poorly understood. We here identified the monocyte/macrophage lineage identity transcription factor Kruppel-like factor 4 (KLF4) to be inhibited during LC differentiation from human blood monocytes. Conversely, KLF4 is maintained or induced during dDC and monocyte-derived DC/IDEC differentiation. We showed that in monocytic cells KLF4 has to be repressed to allow their differentiation into LCs. Moreover, respective KLF4 levels in DC subsets positively correlate with pro-inflammatory characteristics. We identified epithelial Notch signaling to repress KLF4 in monocytes undergoing LC commitment. Loss of KLF4 in monocytes transcriptionally de-represses RUNX3 in response to TGF-β1, thereby allowing LC differentiation marked by a low cytokine expression profile. Therefore, monocyte differentiation into Langerhans cells depends on activation of Notch signaling and the concomitant loss of KLF4.

SESSION 2 | INNATE IMMUNITY

Oral Presentation-Nr. 005

Abstract-ID: O5 | Oral Presentation 17.11.2016, Session 2, Innate Immunity 11.30-12.05

Cooperation of Langerhans cells and NK cells in the immunosurveillance of the epidermis during chemical carcinogenesis

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Immunosurveillance of tissue is an important mechanism by which the immune system prevents cancer development. Skin treatment with the carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) and the tumor promotor 12-O-tetra-decanoyl-phorbol-13-acetate (TPA) is a well established murine model for chemical carcinogenesis that results in squamous cell carcinoma (SCC) formation. There is a fair good understanding of the role of the immune system in the tumor promotion phase, but evidence for the interaction of immune cells and transformed skin cells during tumor initiation is missing. We explored the immunological processes during induction of chemical carcinogenesis and demonstrate here that two innate cell types, namely Natural Killer (NK) cells and Langerhans cells (LC), are involved in the clearance of DNA-damaged keratinocytes. In fact, the depletion of NK cells or LC resulted in an accumulation of DNA-damaged and Natural Killer Group 2D-ligands (NKG2D-L) expressing keratinocytes and the promotion of tumor growth. Mechanistically, we identified a rapid recruitment of NK cells to the epidermis after topical DMBA-treatment, a process dependent on LC and enhanced levels of TNF-α. The TNF-α induced chemokines CCL2 and CXCL10 mediated the trafficking of NK cells to the epidermis. Our findings reveal a novel mechanism how the innate immune system surveils the epidermis for cell transformation to impair tumor development during chemical carcinogenesis.

Oral Presentation-Nr. 006

Abstract-ID: O6 | Oral Presentation 17.11.2016, Session 2, Innate Immunity 11.30-12.05

Novel role for the SAA1-FPR2 axis in the initiation of type 2 immune responses

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Recent studies have demonstrated the importance of the alarmin IL-33, which is strongly induced in the airways of allergic individuals, in the initiation of aberrant type 2 responses to aero-allergens. However, the allergen-triggered pathways regulating IL-33 release in the airways remain to be established. Herein, we identify the formyl-peptide receptor 2 (FPR2) and its endogenous ligand, the acute phase protein serum amyloid A1 (SAA1) as major drivers of house dust mite (HDM)-induced IL-33 release *in vitro* and *in vivo*. Specifically, we found that HDM-induced IL-33 secretion is rapid (30-120 min), and could be completely abrogated either by FPR2 inhibition or SAA1 knockdown. *In vivo*, local inhibition of FPR2 in the lungs abrogated HDM-induced airway hyperresponsiveness, IgE synthesis, and bronchoalveolar lavage (BAL) eosinophilia, concomitant with reductions in Th2 cytokine levels, IL-13+ innate lymphoid cells (ILC2s), and BAL IL-33 levels in allergen-exposed mice. Similarly, antibody blockade of SAA1 or administration of *high density lipoprotein (HDL)*, which acts as an inhibitor of SAA1 cytokine functions, blocked HDM-induced IL-33 secretion and ILC2 recruitment. This was dependent on SAA1 recognition of the cytosolic fatty acid binding protein Der p 13 contained in HDM extract. Taken together, we report a novel mechanism of allergenicity which involves SAA1-facilitaed allergen recognition via FPR2 leading to aberrant IL-33 release and type 2 responses. This novel paradigm allows for a new view on SAA1 as a potent driver of type 2 allergic immune responses via the localized and thus restricted unmasking of its proinflammatory effects at mucosal surfaces.

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SESSION 2 | INNATE IMMUNITY

Poster-Nr. 024

Abstract-ID: P24 | Poster presentation: 17.11.2016

AllergoOncology: Macrophages critically shape the immune microenvironment in allergy and cancer in humans and in dogs

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Background: Like human, companion animals can develop allergies and cancer. In both diseases the immune system is critically involved: while it is hyper-reactive against harmless antigens in allergies, it is hypo-reactive in cancer through immune tolerance. Further, the IgG4 immunoglobulin class is typical in cured allergies, but has recently been correlated with bad prognosis in cancer. Therefore, we concentrated here on the immunoregulatory function of IgG subclasses in concert with on human and animal macrophages.

Methods: The *in vitro* polarization of macrophages into functional subtypes M1, M2a, -b or -c has been established for human and - for the first time - canine cell lines, as well as peripheral blood mononuclear cells from donors, allergic patients or cancer patients.

Results: When we investigated the immune cells surrounding the tumor mass in human colon cancer specimens, we found that macrophages were present not only at a highly density, but also in the vicinity of IgG4 expressing cells. When we investigated *in vitro* the capacity of macrophages to interact with IgG of different subclasses we found that only IgG4, but not IgG1 drives macrophages into a non-responsive tolerogenic phenotype. IgG4 also led to an expression of several cytokines and chemokines such as IL-10, IL-6, TNFa, or CCL1, and converted and reinforced the M2b-like tolerogenic macrophages.

Conclusions: Our results highlight the similarities between human and animal macrophage responses in cancer and allergy. The *in vitro* data based on cell lines and on blood cells from allergic & cancer patients will contribute to the understanding of the mechanisms and improve treatment options and prognosis in animals and humans.

SESSION 2 | INNATE IMMUNITY

Poster-Nr. 026

Abstract-ID: P26 | Poster presentation: 17.11.2016

HFE deficiency critically affects cholesterol homeostasis in mice

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Iron metabolism plays a crucial role in diseases characterized by chronic inflammation including atherosclerosis. Specifically, iron overload has been linked to an increased risk for atherosclerosis development. However, the underlying mechanisms have not been elucidated thus far.

To study this, we used dyslipidemic apoE$^{-/-}$ mice which develop spontaneous atherosclerosis and crossed them with hfe$^{-/-}$ mice, a mouse model of genetic iron overload.

Animals were fed a Western type diet enriched with iron (50 g/kg, high-iron) or poor in iron (5 mg/kg low-iron), respectively.

We observed markedly higher plasma cholesterol levels in double knockout animals compared to wild-type littermates or single knockout mice. Furthermore, feeding double knockout mice a high-iron diet led to an even more pronounced decrease in plasma cholesterol, characterized by a less atherogenic lipoprotein profile with markedly decreased low-density lipoprotein (LDL) cholesterol levels, when compared with low-iron fed littermates.

Western blot analysis of hepatic membrane compartments showed distinct higher amounts of LDL receptor in high-iron fed hfe$^{-/-}$ mice when compared to low-iron fed hfe littermates or wildtype animals. Accordingly, hfe$^{-/-}$ primary hepatocytes incubated with holo-transferrin showed an enhanced uptake of fluorescent LDL, when compared to iron-poor hfe$^{-/-}$ or wildtype cells.

The atheroprotective effect of a high-iron diet was also detected in lipid profiles of hfe single knockout mice, whereas iron did not affect the lipoprotein profile of wildtype animals.

In a subsequent long-term experiment of 20 weeks, double knockout mice on a low-iron diet displayed a significant 40% increase in atherosclerotic lesion area of the thoracic aorta.

In summary, we show that genetic alteration of iron metabolism affects cholesterol homeostasis and atherogenesis through the regulation of the hepatic LDL receptor.

Poster-Nr. 027

Abstract-ID: P27 | Poster presentation: 17.11.2016

The Ecto-ATPase CD39 inactivates Isoprenoid-derived Vγ9Vδ2 T cell phosphoantigens

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In humans, Vγ9Vδ2 T cells respond to self and pathogen-associated, diphosphate-containing isoprenoids, also known as phosphoantigens (pAgs). However, activation and homeostasis of Vγ9Vδ2 T cells remain incompletely understood. We show here that pAgs induced expression of the ecto-ATPase CD39, which however not only hydrolyzed ATP but also abrogated the γδ T cell receptor (TCR) agonistic activity of self and microbial pAgs (C_5 to C_{15}). Only mevalonate-derived geranylgeranyl diphosphate (GGPP, C_{20}) resisted CD39-mediated hydrolysis and acted as a regulator of CD39 expression and activity. GGPP enhanced macrophage differentiation in response to the tissue stress cytokine interleukin-15. In addition, GGPP-imprinted macrophage-like cells displayed increased capacity to produce IL-1ß as well as the chemokine CCL2 and preferentially activated CD161-expressing CD4$^+$ T cells in an innate-like manner. Our studies reveal a previously unrecognized immunoregulatory function of CD39 and highlight a particular role of GGPP among pAgs.

Poster-Nr. 028

Abstract-ID: P28 | Poster presentation: 17.11.2016

Different iron handling capabilities of human monocyte subsets

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Because iron significantly effects pathogen growth and impacts on host responses we questioned whether the functional diversity of human monocyte subsets may be attributed to alterations of cellular iron metabolism.

We re-analyzed existing microarray data of three published studies for differential expression of genes linked to iron metabolism. Expression of identified candidates was investigated on protein level by flow cytometry and on transcript level by fluorescence activated cell sorting and ensuing real-time PCR. Functional studies were done by adding substances altering iron metabolism and by infection assays. In the retrospective gene chip analysis we were able to identify a set of significantly regulated iron-related genes, including the sole known iron exporter ferroportin (*FPN1*). As confirmed by flow cytometry, FPN1 was up-regulated in classical monocytes, while transferrin receptor was mainly expressed on intermediate followed by classical monocytes. These results might be due to different erythrophagocytosis capabilities of the subsets and were reflected in varying bacterial loads upon infection with Salmonella.

Our results strongly suggest different iron-handling phenotypes and thus varying intracellular iron availability in the three monocyte subsets, which likely impacts on the defense of intracellular pathogens.

SESSION 2 | INNATE IMMUNITY

Poster-Nr. 029

Abstract-ID: P29 | Poster presentation: 17.11.2016

Interaction of natural and mutant fish parvalbumins and fish-derived food matrix with bronchial epithelial cells

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Background and aims: Inhalation of aerosolized fish allergens and fish matrix components is often associated with severe IgE-mediated reactions in sensitized individuals. The calcium-binding proteins parvalbumins are major fish allergens. The role of epithelial cells in allergic reactions to fish is not well understood. We explored interactions of the human bronchial epithelial cell line 16HBE14o- with natural fish parvalbumins in presence or absence of fish-derived food matrix. Furthermore, in order to explore the role of calcium binding to parvalbumins in their interaction with the cells, we included in our study a mutant carp parvalbumin in which two functional calcium-binding sites were mutated.

Methods: We used the natural parvalbumins Gad m 1 and Cyp c 1 purified from cod and carp, respectively. As a model for a mutant fish parvalbumin, a non-calcium-binding recombinant Cyp c 1 expressed in *E. coli* was used. A <3kDa fraction of fish extract was used as a fish matrix. Polarized 16HBE14o- cells were treated apically with parvalbumins with or without the respective fish-derived food matrix. Fluorescently labelled parvalbumins were detected by confocal microscopy. Concentrations of IL-6, IL-8 and CCL2 in the basolateral cell culture medium were measured by the Luminex assay.

Results: Apical exposure of the cells to parvalbumins resulted in their internalization. Concentrations of IL-6 and IL-8 were lower in the basolateral medium of cells exposed to all three parvalbumins compared with untreated cells. CCL2 release was decreased by treatment with natural but not mutant parvalbumin. Carp matrix strongly increased basolateral release of IL-6 and IL-8, in contrast to cod matrix which had no influence. This indicates a possible role of the food matrix in allergic sensitization and/or reaction via induction of a pro-inflammatory environment.

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SESSION 2 | INNATE IMMUNITY

Poster-Nr. 030

Abstract-ID: P30 | Poster presentation: 17.11.2016

Expression and regulation of NLRs in human MSCs

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Mesenchymal stem cells (MSCs) are a heterogeneous subset of stromal stem cells. MSCs can differentiate into cells of the mesodermal lineage, such as osteocytes, chondrocytes and adipocytes. Besides their tissue renewing capacity, MSCs possess also an immunomodulatory potential. They can thereby influence immune cells of the innate as well as of the adaptive immune system, mostly in an anti-inflammatory way. Pro-inflammatory stimuli, such as IFNγ, even enhance the anti-inflammatory action of MSCs. Some recent reports suggest a Toll-like receptor (TLR) mediated polarization of MSCs to either a pro- or an anti-inflammatory phenotype. However, little is known about the expression and function of NOD-like receptors (NLRs) in MSCs.

Therefore, we determined whether MSCs derived from different sources endogenously express NLRs. We found that four NLRs are expressed in unstimulated MSCs from bone marrow (BM-MSCs), white adipose tissue (WAT-MSCs) and umbilical cord (UC-MSCs). Moreover, we analysed the influence of the pro-inflammatory cytokines IFNα and IFNγ on the expression level of NLRs. To obtain a more comprehensive picture of IFNα and IFNγ effects on human MSCs we also investigated the secretion of various cytokines for three different WAT-MSC donors. While IFNα and IFNγ induced MSCs from different donors showed similar responses in the expression of NLRs and the secretion of most cytokines, we could detect diverging effects in IL-6 release.

Taken together, we show that specific NLRs are expressed in human MSCs derived from different sources. The regulation of NLR expression by IFNα and IFNγ is correlated with the expression of specific anti-inflammatory cytokines, which suggests that NLRs might be involved in the immunomodulatory function of MSCs.

Poster-Nr. 031

Abstract-ID: P31 | Poster presentation: 17.11.2016

Transcriptome and proteomic cross-analysis of *Betula Verrucosa* pollen

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Background: Birch pollen is highly allergenic, with an excess of 100 million people worldwide with a reported allergy. In particular, proteases in allergen sources have been suggested to contribute to primary sensitisation of allergens and exacerbation of allergic disorders. Until now the protease content of Betula verrucosa, a species endemic to Europe, has not been studied in detail. Hence, we aim to further identify and characterise pollen derived proteases within the Betula verrucosa species, with the future aim to further elucidate their role in sensitisation. We propose the use of transcriptome and proteomic analysis coupled with zymographic techniques to enable the identification of key proteases.

M**ethods:** Focussing on Betula verrucosa pollen extract, we aim to identify and characterise pollen derived proteases (PDPs), which may be involved in the process of allergic sensitization. Preliminary experiments using zymography show existing gelatinase and casein activity, whilst further elucidation is achieved using mass spectrometry and transcriptome analysis.

Results: By using mass spectrometry, we were able to identify non-allergenic proteases in birch pollen. We could cluster the protease into distinct families, which will provide the basis for a more detailed characterization of the effects of proteases in the early stages of allergic sensitization.

Conclusion: Up to 29 proteases from 7 different protease families have been successfully identified for Betula verrucosa , supporting reports that birch pollen has a substantial proteolytic activity which eventually leads to the degradation of tight junction proteins of epithelial cells. Whilst none of the known birch pollen allergens have been recognised as a protease, we hope to be able to investigate the role of proteolytic activity on immune-polarization and the onset of allergic sensitization.

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Poster-Nr. 032

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The anti-inflammatory effect of Glucagon like peptide-1 in experimental Glomerulonephritis

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Background: Glucagon like peptide-1 (GLP-1), acting via the GLP-1 receptor (GLP-1R), is an incretin hormone known for its ability to stimulate glucose-dependent insulin secretion and to inhibit glucagon secretion, gastric emptying and food intake. Additionally, GLP-1 has been proven to have an anti-inflammatory capacity by increasing the number and function of regulatory T cells and influencing the macrophage phenotype. We now aim to evaluate the role of GLP-1 and its receptor in nephrotoxic serum nephritis (NTS) since this murine model of rapid progressive glomerulonephritis (GN) has been proven to be dependent on T cells.

Methods: Male C57BL/6J mice aged 8 weeks were subjected to NTS and treated with liraglutide (a GLP-1R agonist) or vehicle for 14 days. Furthermore, 8-week-old GLP-1R knock-out (KO) mice were subjected to NTS for 14 days and compared with WT mice.

Results: Liraglutide treatment significantly decreased albuminuria in mice subjected to experimental glomerulonephritis for a period of 14 days. Glomerulosclerosis was also lower for the liraglutide group. Mice treated with liraglutide showed a decreased extent of renal $CD4^+$ T cell, $CD8^+$ T cell and $CD68^+$ macrophage infiltration. Concerning the expression of inflammatory related genes, a significantly decreased expression of IL-6, t-bet, INF-gamma, IL-10,TNF-alpha and FoxP3 was noted for the liraglutide group. GLP-1R gene deficiency enhanced the course of the disease, as shown by increased albuminuria levels as compared to WT controls. In addition, a significant increase of renal neutrophil infiltation was noted in the group of GLP-1R KO mice.

Conclusion: Our data support the hypothesis that GLP-1 and its receptor play a key role in the immune regulation in NTS. GLP-1R agonism by Liraglutide significantly ameliorates the course of NTS and could thereby be an attractive new therapeutic tool in the treatment of GN. Further research is ongoing to evaluate the exact role of GLP-1R in GN.

SESSION 2 | INNATE IMMUNITY

Poster-Nr. 033

Abstract-ID: P33 | Poster presentation: 17.11.2016

Novel insights into osteohematology: heterogeneity of megakaryocytes distribution at different bone compartments

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Emerging and highly developing fields of bone research are represented by osteoimmunology and osteohematology. Novel insights into the interaction between bone and the hematopoietic system substantially advanced our understanding of the contribution of myeloid and lymphatic cells to bone pathophysiology. Yet, limited knowledge is available on the exact distribution of hematopoietic or immune cell subsets within specific bone compartments. Aim of the current pilot study was to determine the skeletal heterogeneity in respect of distribution and density of megakaryocytes, macrophages, T cells and B cells using mouse models and to relate data to gender, mouse strains and bone compartments.

We studied a total number of 24 mice (male=12; female=12) from two strains (Balb/c=2; C57BL/6J=22) and isolated the spine, tibia and femur, representing the most common parts of the skeleton used for bone research. Furthermore, in tibia and femur we differentiated between bone marrow sections adjacent to cortical and trabecular bone. We stained paraffin-embedded tissue sections using immunohistochemistry and immunofluorescence with cell type-specific markers and quantified stained cells by the microscopy-based TissueFAXS platform.

First results showed significant differences in the number of megakaryocytes within the bone marrow compartments of spine vs. cortical femur, and spine vs. trabecular femur. Furthermore, bone marrow of the spine of female mice contained significantly more megakaryocytes compared to male mice. Follow-up analyses will elucidate the distribution and density of macrophages, T cells and B cells within mouse bone marrow compartments.

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Poster-Nr. 034

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The Erythropoietin-analogue ARA290 dampens innate immune cell functions thus ameliorating the course of experimental colitis

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The erythropoietin receptor (EPOR) pairs with either of two cell surface receptors to mediate the cellular responses to erythropoietin (EPO). In the erythropoietic bone marrow, EPOR subunits homodimerize to promote the maturation of erythroid progenitor cells. On other cell types including immune cells, EPOR and CD131 form heterodimers, which exerts tissue-protective effects.
Here we used the EPO analogue ARA290, which selectively activates the heterodimeric EPOR/CD131 but not the homodimeric EPOR, to study during the course of dextran sulphate sodium-induced colitis. We found that ARA290 and EPO both ameliorated the clinical course of experimental colitis but ARA290 did not affect hemoglobin levels. Treatment with ARA290 resulted in significant weight gain and improved survival of mice. Correspondingly, histopathologic analysis of colon samples revealed reduced tissue damage and inflammation in DSS-exposed mice treated with ARA290 as compared to solvent-treated DSS-mice. The infiltration of myeloid cells and the production of pro-inflammatory mediators including cytokines, chemokines and NOS-2 were significantly lower following treatment with ARA290 as compared to solvent treated littermates. In parallel, binding activity of the NF-κB subunit p65 in lamina propria macrophages was reduced in both treatment groups.
Experiments with LPS-activated primary macrophages revealed that the anti-inflammatory effects of ARA290 were dependent on JAK2 activation and were mediated via inhibition of p65. Pharmacological inhibition or genetic deletion of JAK2 in BMDMs abolished the beneficial immune modulatory effects of ARA290. ARA290 blocks the formation of pro-inflammatory cytokines and chemokines and improves the clinical course of DSS-induced colitis. The compound may thus be a promising agent for the therapy of human inflammatory bowel disease as it exerts potent anti-inflammatory effects without erythropoietic activity and without the potential for thromboembolic effects.

SESSION 2 | INNATE IMMUNITY

Poster-Nr. 035

Abstract-ID: P35 | Poster presentation: 17.11.2016

The interplay of lipids and major peanut allergens Ara h 1 and Ara h 2 and their effect on their effect on the innate immune system

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IgE-mediated food allergy is a worldwide health problem and peanuts are among the most potent allergenic foods of plant origin. Peanut proteins have been studied for many years and it is still unclear what makes them allergenic. Evidence of the adjuvant role of small molecules in allergic sensitization, such as lipids, directly bound to the allergen or present in the allergen source, is emerging. Peanut contains a significant amount of lipids.
We wanted to assess whether peanut proteins can interact with lipids, whether these act as immune adjuvant, and which type of immune response they trigger.
As dendritic cells come into contact with allergens at epithelial barriers and bridge innate and adaptive immunity, we studied how peanut lipids influence the uptake of allergens and cytokine production by monocyte-derived dendritic cells (MoDCs). Furthermore, we explored the effects of peanut allergens and lipids together on the human bronchial epithelial cell line 16HBE14o- as a model system for the sensitization via the respiratory route.
Peanut lipids decrease the dose of Ara h 1 internalized by MoDCs and the IL-4/IL-10 ratio in a non-significant manner. Furthermore, they enhance IL-1β production by MoDCs, thus favouring a pro-inflammatory environment. 16HBE14o- cells internalize Ara h 1 and Ara h 2 by different mechanisms, unaffected by the presence of lipids. This data suggest that protein-lipid interactions condition the way peanut allergens interact with innate immune system cells, which could have an effect on T cell polarization and allergic sensitization.

SESSION 2 | INNATE IMMUNITY

Poster-Nr. 036

Abstract-ID: P36 | Poster presentation: 17.11.2016

The role of STAT1 isoforms in innate immunity and T cell-mediated diseases

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Signal transducer and activator of transcription (STAT1) is a member of the JAK/STAT signalling pathway and is essential for signalling by all types of interferons (IFNs). STAT1 is crucial for the protection against bacterial and viral infections but also contributes to immune pathologies. STAT1 has two naturally occurring alternatively spliced isoforms, STAT1α and STAT1β. They differ in the C-terminal transactivation domain, which is absent in the STAT1β isoform. Accordingly, STAT1β was considered to be transcriptionally inactive if activated as homodimers in response to IFNγ and to exert dominant negative functions. Using mice that only express either STAT1α (*Stat1*$^{\alpha/\alpha}$) or STAT1β (*Stat1*$^{\beta/\beta}$) we have shown that STAT1 is transcriptionally active and capable of mediating an IFNγ-dependent immune defence against systemic *Listeria monocytogenes* infections, although with lower efficiency than STAT1α. Herein, we investigate the immunopathogenic functions of STAT1 isoforms and their role in T cell functions. *Stat1*$^{\beta/\beta}$ mice showed an intermediate survival compared to *Stat1*$^{-/-}$ and *Stat1*$^{\alpha/\alpha}$ mice upon high-dose lipopolysaccharide challenge, suggesting that STAT1β is less immune-pathogenic than STAT1α. Similar to what we have observed during acute infections, *Stat1*$^{\alpha/\alpha}$ mice were phenotypically indistinguishable from wild-type mice, confirming the notion that STAT1β does not act in a dominant negative manner in innate immunity. In contrast, we found evidence for a negative regulatory function of STAT1β in T cells: splenic CD4$^+$ T cells from *Stat1*$^{\alpha/\alpha}$ mice show an increased differentiation into type 1 helper (Th1) cells upon activation under non-polarizing conditions compared to those from wild-type mice. Ongoing experiments are directed towards assessing the differentiation of other Th subsets and the sensitivity of *Stat1*$^{\alpha/\alpha}$ mice to concavalin A-induced acute hepatitis, an experimental model for autoimmune hepatitis.

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Poster-Nr. 037

Abstract-ID: P37 | Poster presentation: 17.11.2016

Platelets as immune players in invasive *Candida* infections

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Platelets are versatile players of innate immunity. Activation in response to pathogens may lead to multifaceted antimicrobial effects, but also to thrombosis or excessive inflammation.

In *Candida (C.)* septicaemia, *C.* comes in close contact with platelets with putative subsequent processes such as mutual binding, activation and decrease of viability, which can profoundly influence the clinical outcome; therefore we studied platelet-*Candida*-interactions *in vitro* as well as in the blood of patients with Candidemia.

In vitro, adhesion of platelets to yeast cells and hyphae/pseudohyphae of *C.* was moderate, and only marginal activation of platelets could be demonstrated after co-incubation with clinical isolates of different *C.* species (*albicans, glabrata, parapsilosis, tropicalis, dubliniensis, lusitaniae, rugosa*). The presence of platelets did not affect growth or viability of the fungus.

However, in a whole-blood-model we could show strong activation of C.- adhered platelets as well as enhanced mutual binding and activation of platelets and neutrophils in the presence of *C.*

Our pilot study revealed that platelets derived from the blood of candidemia patients get significantly stimulated, with enhanced levels of the activation markers CD62P and CD63, increased numbers of circulating platelet-derived microparticles and decrease of platelet numbers (thrombocytopenia). The kinetic of the activation markers and platelet viability in the course of the disease differs between the patients, presumably due to various underlying diseases and drug regimens.

We hypothesize that stimulation of platelets by *Candida* species with subsequent activation of other elements of immunity may improve the outcome of candidemia, but might also harbour the danger of thrombosis and excessive inflammation.

Poster-Nr. 038

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Specific targeting of activated macrophages in chronic inflammatory and autoimmune diseases

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Rheumatoid arthritis (RA) is an autoimmune disease characterised by immune cell activation, chronic inflammation of synovial lining of joints and hyperplasia, which could lead to the cartilage and bone destruction and disability, if untreated. Activated macrophages are crucial players in disease pathogenicity and their numbers in inflamed synovia predict the severity of the disease. Current RA treatments often have severe side effects or are costly, therefore new therapies that specifically target activated macrophages are being developed. Folate receptor β (FRβ) was described as a marker of RA macrophages. To dissect the contribution of FRβ$^+$ macrophages to RA progression, we developed several strategies for their targeting. Firstly, we produced FRβ-specific antibodies and linked them onto liposomes using a novel non-toxic approach. We also designed bispecific antibodies, targeting FRβ and another macrophage marker, since our results revealed that FRβ was expressed by several macrophage subtypes. Finally, we used folate as a targeting moiety. All formats were specific to FRβ$^+$ cells and therefore might represent an effective way to deliver immunomodulatory drugs to the pathogenic FRβ$^+$ macrophages and RA treatment.

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SESSION 2 | INNATE IMMUNITY

Poster-Nr. 039

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Antimycotic drugs affect the innate immune functions of human platelets

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Platelets play an important role in the innate immunity harbouring both own antimicrobial functions and interacting with other immune elements. To understand the pathomechanisms in patients with invasive fungal infections, we studied the effect of antimycotic drugs on platelet activity and function. We concentrated on amphotericin B (AmB), a standard antimycotic prescribed either prophylactically to patients at risk for fungal infections or therapeutically to infected patients.

Both the desoxycholate and the liposomal formulations of amphotericin B were both capable to activate platelets as measured by increase of the activation markers CD62P and CD63. The effect was dose-dependent with active concentrations of the drugs being within the physiological range in treated patients. A time curve showed that few minutes of incubation were sufficient to achieve this effect. As a consequence, platelets revealed improved interaction with fungal hyphae and conidia of different species, a prerequisite for the antifungal functionality of platelets.

However, the shedding of microparticles from AmB-treated platelets was decreased. Since microparticles represent important pro-inflammatory mediators this process might critically influence the pathogenesis in treated patients. Our hypothesis that AmB might bind to human cholesterol and interfere with the shedding procedure was supported by confocal microscopy where morphological changes of the platelets were clearly visible. Furthermore, AmB-treated platelets were recognised by complement to be foreign followed by formation of the lytic C5b-9 complex on the platelet membrane. Taken together, the frequently prescribed drug amphotericin B strongly affects various physiological processes in the platelets, resulting in modulation of antifungal immune reaction, but also thrombosis and thrombocytopenia.

SESSION 2 | INNATE IMMUNITY

Poster-Nr. 040

Abstract-ID: P40 | Poster presentation: 17.11.2016

Intrinsic features of protein antigens contribute to immune modulation

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The skin hosts multiple dendritic cell (DC) subsets with diverse functions. A current paradigm states that immune reactions are initiated and directed through triggering of DC by pathogen-associated and endogenous danger signals. In contrast to these processes, protein antigens are traditionally regarded as passive structures and their role in shaping immune reactions is largely unnoticed. Therefore, we have investigated the involvement of skin DC subsets on immune reactions against two structurally different model antigens, *E. coli* β-galactosidase (βGal) and chicken ovalbumin (Ova), under identical conditions. In wild type mice we found robust immune responses against both antigens after epicutaneous DNA vaccination with a gene gun. In contrast, ablation of langerin+ DC almost abolished IgG1 and cytotoxic T lymphocyte (CTL) activity against βGal, whereas Ova-specific responses were strongly enhanced. We identified Langerhans cells (LC) as the subset responsible for the Ova-specific immune suppression. In contrast, reactions against βGal were not affected, indicating that this antigen required dermal langerin+ DC. The opposing findings with Ova and βGal vaccines were independent of the immune-modulating activities of the plasmid or the antigen gene products. Also the different size or dose of the two proteins was not accountable for the conflicting outcomes. However, LC-deficient mice, immunized with a βGal-Ova fusion vaccine, responded with an increased CTL immunity against βGal. We identified the H2-K^b Ova-epitope SIINFEKL responsible for the observed immune-modulating effect of OVA on βGal immunity since immunization with a SIINFEKL-minigene vaccine was sufficient to elevate CTL responses in LC-depleted mice. Based on these results, we will investigate the effects of SIINFEKL on the immunity against βGal by immunizing mice with a βGal-SIINFEKL gene vaccine.

Poster-Nr. 041

Abstract-ID: P41 | Poster presentation: 17.11.2016

The role of plasmacytoid dendritic cells in Imiquimod induced skin inflammation and melanoma clearance in mice

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

Oral Presentation-Nr. 007

Abstract-ID: O7 | Oral Presentation 17.11.2016, Session 3, Immune Cell Signaling 15.30-17.00

Bim is a key target of miR-17~92 in B cells undergoing stress responses

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Mature microRNAs (miRNAs) exert repressive control over gene expression by interacting with the 3'UTR of protein-coding RNAs (mRNAs). miRNAs affect a wide range of fundamental cellular processes from tissue homeostasis to pathology. Their small size and imperfect target recognition empowers individual miRNAs to reduce the levels of virtually hundreds of proteins, but only a fraction of potential miRNA:mRNA interactions may be operational in a given biological context. While the analysis of miRNA controlled gene-expression *in silico* and cell lines has added to our understanding of miRNA:mRNA interactions, identification of direct versus indirect and, most importantly, functionally relevant targets in the context of a complex organism remains a major challenge.

miRNAs encoded by the miR-17~92 cluster have attracted attention due to their involvement in tissue differentiation and frequent overexpression in human B cell lymphomas. Bim is a ubiquitously expressed pro-apoptotic protein whose mRNA harbours nine predicted binding sites for miR-17~92 miRNAs. Direct control of Bim by miR-17~92 has been invoked by various groups as a critical element in B cell development and lymphoid malignancies.

Addressing this issue, we generated a unique *in vivo* murine system of conditional mutagenesis, where the wild type Bim 3'UTR can be exchanged against a mutant counterpart harbouring inactivated miR-17~92 binding sites. Remarkably, steady-state B cell development was not detectably affected upon miR-17~92:Bim disruption, although miR-17~92 binding was indeed eliminated. In contrast, stress-conditions resulted in 2-fold Bim mRNA and protein upregulation.

SESSION 3 | IMMUNE CELL SIGNALING

Oral Presentation-Nr. 008

Abstract-ID: O8 | Oral Presentation 17.11.2016, Session 3, Immune Cell Signaling 15.30-17.00

Analysis of expression and function of CD39 on iTreg generated by different protocols

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Treg are a subpopulation of $CD4^+$ T-cells, associated with immunosuppression and preservation of self-tolerance. Treg can suppress proliferation and cytokine production of effector T-cells through various ways including the degradation of ATP into the suppressive molecule Adenosine through the ectoencymes CD39 and CD73. The expression of CD39 on *in vitro* induced Treg (iTreg) has not been evaluated so far. We investigated the expression and function of CD39 on iTreg in order to better understand the molecular mechanisms of immune suppression related to CD39-expression.

Naïve $CD4^+CD25^-CD39^-$ T-cells were activated with anti-CD3/CD28 coated microbeads in presence of IL-2 and TGF-β/all trans retinoic acid (atRA) or rapamycin (RAPA). After two weeks of stimulation, the phenotype of iTregs was determined by flow cytometry (CD25, CD127, CD39, CD73, FOXP3). For functional analyses iTregs were FACS-sorted into CD25highCD127low and their suppressive capacity was assessed in co-culture with CFSE-labelled $CD4^+$ T-cells, PBMCs or monocytes with or without blocking of CD39.

TGF- β/atRA was observed to strongly up-regulate the induction of CD39-expression. In contrast, CD39-expression on RAPA-iTreg was strongly down-regulated. Both RAPA-induced as well as TGF-β/atRA-induced iTreg showed a similar capacity to suppress the proliferation of $CD4^+$ T-cell and PBMC. Using pharmacological inhibitors, we found that CD39 upregulation by TGF-β/atRA was dependent on mTOR and Smad3 signaling but independent of Rock1/2 or p38 signaling. Suppression of proliferation with total $CD4^+$ T-cells and PBMCs as responder was CD39 independent but suppression of IFN-γ production in monocytes was CD39 dependent.

We show that functional and phenotypical differences exist between iTreg generated by culture in TGF-β/atRA or RAPA. These observations could help to optimize protocols for the clinical application of these cells. Further, these studies give first insights into the mechanisms of CD39 expression on iTreg.

Oral Presentation-Nr. 009

Abstract-ID: O9 | Oral Presentation 17.11.2016, Session 3, Immune Cell Signaling 15.30-17.00

The miR-15 family reinforces the transition from proliferation to differentiation in pre-B cells

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In early B lymphocyte development, pre-B cells clonally expand upon expression of a signaling-competent pre-B cell receptor (pre-BCR), but then transit into a resting state in which immunoglobulin light chain gene recombination is initiated. This bi-phasic sequence from proliferation to differentiation, which ensures proper B cell development and homeostasis, is mainly orchestrated by signals from the IL-7 receptor (IL-7R) and the pre-BCR, respectively. However, little is known about whether small non-coding RNAs contribute to fine-tuning these regulatory signaling networks. Therefore, we have screened a miRNA sponge library in a model system of early B cell development and found that cells lacking function of the miR-15 family fail to induce the transcriptional reprogramming that normally accompanies early B cell differentiation, resulting in a developmental block at the pre-B cell stage. Intriguingly, ectopic expression of validated miR-15 target genes cyclin E1 and D3 is sufficient to partially recapitulate the miR-15 family loss-of-function phenotype. Furthermore, our data indicate that the miR-15 family is actively suppressed by IL-7R and pre-BCR signaling, suggesting its integration into the regulatory circuits at the pre-B to immature B cell transition. Together, our study identifies the miR-15 family as a novel regulatory element required to promote the switch from proliferation to differentiation in pre-B cells.

SESSION 3 | IMMUNE CELL SIGNALING

Poster-Nr. 043

Abstract-ID: P43 | Poster presentation: 18.11.2016

Jak1/Jak2 inhibitor momelotinib inhibits ACVR1/ALK2, decreases hepcidin production and ameliorates Anemia of Chronic Disease (ACD) in rodents

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Objective: Results from a phase 2 study for the treatment of myelofibrosis (MF) with the Jak1/2 inhibitor momelotinib (MMB) demonstrated that MMB provided an anemia benefit, contrasting to other studies and unexpected as erythropoietin -mediated JAK2 signaling is essential for erythropoiesis. MF patients have been shown to have elevated serum hepcidin levels, paralleled by anemia and inferior overall survival. We aimed to determine whether MMB's clinical anemia benefit is also found in an ACD animal model.

Materials and Methods: Rat model of Anemia of Chronic Disease (ACD) by i.p. injection of Group A Streptococcal Peptidoglycan-Polysaccharide(PG-APS). Treatment with MMB (5mg/kg, 10mg/kg, 25mg/kg) for 3d (short term) and 21 d (long term). Analysis of effects on erythropoiesis and iron metabolism were analyzed. *In vitro* studies with HepG2 cells and cultivated BMDMs of Jak2Wt ($Jak2flox^{+/+}LysMCre^{-/-}$) and Jak2cKO ($Jak2flox^{+/+}LysMCre^{+/+}$) mice.

Results: MMB treatment in a rat model of Anemia of Chronic Disease (ACD) increased hemoglobin and red blood cell numbers to normal levels in the blood. The effect seen in ACD rats is driven by the direct inhibition of the BMP-receptor kinase ACVR1/ALK2 and the subsequent reduction of hepatocyte hepcidin production. Ruxolitinib, a JAK1/2 Inhibitor approved for the treatment of MF, had no inhibitory activity on this pathway. Further, we demonstrate the effect of MMB is not mediated by direct inhibition of Jak2-mediated ferroportin (Fpn1) degradation, as neither MMB treatment nor myeloid specific deletion of JAK2 affected Fpn1 levels, showing that Jak2 is dispensable for hepcidin dependent ferroportin degradation *in vitro* and *in vivo*.

Poster-Nr. 044

Abstract-ID: P44 | Poster presentation: 18.11.2016

DC-iphering CR-mediated HIV-1 incorporation and effects on DC function in search for novel therapeutical targets

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The immune response to viral infections involves complex interplays between the virus and the immune system and targets elimination of the pathogen with minimum damage to the host. The complement pathway is spontaneously and vigorously activated after HIV-1 entry at mucosal surfaces, recognizing structures within the viral envelope. Complement fragments covalently bind to HIV-1, which makes attachment of HIV-1 to complement receptors(CR) on dendritic cells (DCs)more likely compared to interactions of rarely found non-opsonized virus with C-type lectins.

DCs are key modulators of immunity given their pivotal role in initiating and shaping adaptive immune responses against a vast variety of pathogens and cancer. HIV-1 has evolved strategies to evade DC-mediated antiviral immunity and to make matters worse the virus exploits DCs shuttles to promote its own dissemination. We recently showed that complement-opsonization of HIV-1 (HIV-C) allowed bypassing of restriction mechanisms in DCs and this was associated with and increased quality and quantity of virus-specific immune responses due to an enhanced DC infection and co-stimulatory activity. Therefore, we are interested in unraveling in detail how differentially opsonized HIV particles enter DCs, especially HIV-C, and how that affects signaling and antigen presentation. Now we are focus in breaking down the specific functions of CR3 and CR4 in more detail with respect to HIV-1 entry, processing and signaling in DCs to identify novel therapeutic host targets.

For that purpose we are trying to generate a stable knock-out cell line for both complement receptors, CR3 and CR4.We decide to use THP-1 cell line, which is a human leukemia monocyte cell line widely used as a model to investigate the primary myeloid lineage cells. The use of primary cells is not recommended, because of the limited survival range in long protocols.

SESSION 3 | IMMUNE CELL SIGNALING

Poster-Nr. 045

Abstract-ID: P45 | Poster presentation: 18.11.2016

Creation, screening and functional potential of altered peptide ligands (APL) of the major mugwort pollen allergen Art v 1

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The activation of $CD4^+$ T lymphocyte requires T-cell antigen receptor-dependent recognition of immunogenic peptides bound to and presented by MHC class II molecules. Previous reports have shown that T cell function can be modulated by altering the sequence of immunogenic peptides. We here used K562 cells highly expressing HLA-DR1 to establish a fast, flow cytometry-based competitive binding assay for the characterization of putative APL of the immunodominant Art v 123-36 peptide of the major mugwort pollen allergen Art v 1. Different concentrations of 25 Art v 123-36-derived peptides along with the MHC loading enhancer 1-adamantaneethanol, were pre-incubated with wild-type (wt) or HLA-DR1 expressing K562 cells for two hours. Subsequently, incubation with biotinylated-HA (from haemagglutinin influenza A) reference peptide was performed and its specific binding was determined with phycoerythrin-labeled streptavidin by flow cytometry. Pre-incubation with 1-adamantaneethanol (100 µM) led to a significant 5.38±0.04 fold increase in peptide binding ($p< 0.05$). In the competitive assays two peptides with increased (IC50 competitor/wt ratio > 1.50), eight with similar (ratio 0.50 to 1.50), and 15 with decreased (ratio < 0.5) binding capabilities were identified. Functional evaluation in T cell proliferation and cytokine secretion assays identified five superagonists, nine partial agonists and three bona fide antagonists. One superagonist revealed increased binding affinity, while the partial agonists and the antagonists showed affinities similar to the wt. Furthermore, the superagonists, APL6 and APL10 revealed NF-κB activation 1.7-fold higher than the wt peptide. Surprisingly, partial agonists showed robust NF-κB activation with APL11 being exceptionally strong. Thus, investigation of different parameters of cellular activation with different kinetics and involving different sets of signaling pathways allows dissecting APL function in more detail.

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Poster-Nr. 046

Abstract-ID: P46 | Poster presentation: 18.11.2016

Macrophages are major players in a new model of chronic kidney disease

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Background: Chronic kidney disease (CKD) is characterized by long-term morbidity and mortality. We now aimed to develop a new murine model of CDK to develop future therapeutic possibilities.
Methods: DBA/2NCrl mice are susceptible to ectopic renal calcification when exposed to increased oral phosphate loads. In order to cause renal damage, these mice were fed standard chow diet (CTRL-group) or high phosphate diet (HPD-group) for four days with subsequent return to SCD for 14 (n=8) or 84 days (n=8). Serum and urine samples, as well as samples for histology and qPCR were obtained from kidney and bone.
Results: HPD mice developed CKD with calcification of the kidneys and a significant reduction in measured glomerular filtration rate (GFR) as determined by FITC-inulin clearance on day 0 and 81. Serum Fgf-23 was significantly increased in HPD-fed mice with a coherent trend towards increased serum levels of parathyroid hormone on day 18 and 88. Immunohistochemical stainings of kidneys further revealed a significant increase of infiltrating $CD4^+$ and $CD8^+$ T cells; however $CD68^+$ macrophages constituted the major infiltrating cell population.This finding was confirmed by qPCR, where mRNA levels of the macrophage markers *ccr2*, *ccr5* and *ccl2* were up regulated in HPD mice when compared to CTRL mice. With the exception of tnfa and *il6*, no other T cell marker was up regulated in mice with chronic kidney disease. Of note, evaluation of the bone and mineral status revealed that HPD mice developed a low turnover disorder.
Conclusion: We here present a new model of chronic kidney disease-mineral and bone disorder (CKD-MBD) which reflects important features of the human equivalent. This animal model is based on nephrocalcinosis, which is accompanied by inflammatory processes mainly consisting of macrophages. This animal model may serve as a useful tool for evaluating CKD-dependent end organ damage and testing new therapeutic options without the need for surgical methods.

SESSION 3 | IMMUNE CELL SIGNALING

Poster-Nr. 047

Abstract-ID: P47 | Poster presentation: 18.11.2016

Modulation of the tryptophan-kynurenine axis by airborne sensitizers

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In the recent years, special attention has been paid to volatile organic compounds (VOC) present in indoor environments. Associated adverse effects are mostly driven by chronic exposure to low concentrations and include respiratory tract irritation and sensitization leading to the development of allergy and asthma, but also neuropsychoimmunological manifestations, such as the sick building syndrome. Chemical sensitizers react non-specifically with cellular molecules and immune responses are activated after a certain threshold of insults is exceeded. However, manifestations of unspecific VOC sensitization effects may develop before obvious allergic signals are present. Thus, there is an urgent need to identify those pathways that (i) are affected at very low, sublethal VOC concentrations and (ii) have a functional connection to the development of above-mentioned adverse effects. These requirements are met by the immunoregulatory pathway of indoleamine 2,3-dioxygenase (IDO-1)-mediated tryptophan breakdown.
We investigated the effect of common indoor air pollutants such as formaldehyde and terpenes limonene and pinene on tryptophan breakdown in human peripheral mononuclear cells in the presence or absence of inflammatory stimuli. We show that IDO-1 activity was more sensitively suppressed with all airborne sensitizers in mitogen-stimulated cells compared to unstimulated cells. Moreover, the inhibition was dose-dependent and occurred already at sublethal concentrations. This effect is comparable to the activity of dinitrochlorobenzene, a skin sensitizing chemical.
We conclude that antioxidative capacity of the above mentioned compounds favors a reductive milieu, suppressing immunobiochemical pathways such as IDO-1 activity. Dysregulation of this first essential step in tryptophan metabolism has manifold consequences, as several downstream products exert bioactivities in the regulation of immunological, stress response and neurological processes.

Poster-Nr. 048

Abstract-ID: P48 | Poster presentation: 18.11.2016

mRNA expression of apoptotic factors BCL2, BCL2L11, BBC3 and DNM1L during aging and brain ischemic stroke

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Apoptosis may promote immunodepression, which is one of the characteristics of aging and also the sequela of brain ischemic stroke (BIS). Here we study mRNA expressions of the antiapoptotic factor BCL2, proapoptotic factor BBC3, apoptotic activator BCL2L11 (it inhibits BCL2) and apoptotic factor DNML1 that interact with each other on the mitochondrial level. The aim of study was to estimate the probability of their participation in immunodepression during aging and after BIS.

The study was done on Armenian population (3 groups: 37 healthy young (mean age±SE; min/max age: 37.6±1.1; 20/54), 28 healthy aged (HA) (66.7±1.5; 57/85), 39 BIS patients (71.8±1.6; 56/89)). mRNA expression in peripheral blood leukocytes (PBL) was determined by RT-PCR using PSMB2 as the reference gene. Statistical analysis was done with Graph-Pad Prism 5; $P<0.05$ considered as significant. The expression of DNM1L mRNA is higher at HA group (median: 0.154) compared with healthy young ones (0.049) and BIS patients (0.064)($p<0.001$). The mRNA expression level of BCL2L11 is lower at HA group (0.066) compared with healthy young group (0.135) and BIS patients (0.122)($p<0.01$). The expression of BCL2 mRNA is lower in both HA (0.199) and BIS patients (0.252) groups compared with young ones (0.643)($p<0.001$). The expression levels of BCL2 and BCL2L11 are lower respectively ~3 times and ~2 times in HA group compared with young ones. BCL2/BCL2L11 ratio is ~5 in young group and ~3 in HA group. There are no differences in the case of BBC3.

According our results it can be hypothesized that despite the lower level of BCL2L11 mRNA expression at HA group, apoptosis of PBL cells may be more active there contributing immunodepression because of the BCL2/BCL2L11 ratio change. The decreased BCL2 and increased BCL2L11 mRNAs expressions may contribute the increase of apoptosis of PBL cells in BIS patients, but DNM1L mRNA changes may suppose some changes in apoptotic pathway.

SESSION 3 | IMMUNE CELL SIGNALING

Poster-Nr. 049

Abstract-ID: P49 | Poster presentation: 18.11.2016

STAT1 isoform-specific functions in T cells

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Signal transducer and activator of transcription 1 (STAT1) is a transcription factor that is involved in signaling by all types of interferon (IFN), interleukin- (IL-) 21, IL-27 and IL-35 and that is crucial for the immunity to pathogens and cancer cells. Alternative splicing generates a full-length STAT1α and a C-terminally truncated STAT1β isoform. STAT1 isoforms are functionally redundant in IFNα/β- and IFNλ-dependent defence against acute infections but have distinct activities in IFNγ signaling and IFNγ-dependent innate immunity.

Within this study we investigated the functions of the two STAT1 isoforms in the IL-27 signaling cascade. We found that splenic $CD4^+$ T cells from mice that express only the STAT1α isoform *(Stat1$^{\alpha/\alpha}$)* have a higher STAT1 protein level than those from wild-type mice. IL-27 stimulation of *Stat1$^{\alpha/\alpha}$* $CD4^+$ T cells resulted in an increased activation of STAT1, whereas STAT3 activation remained unaffected. In contrast, STAT1 protein level and STAT1 activation in response to IL-27 was decreased in Stat1$^{\beta/\beta}$ compared to wild-type cells. Transcriptional profiling revealed that a subset of IL-27 responsive genes, including Th1 signature genes, showed increased expression in *Stat1$^{\alpha/\alpha}$* compared to wild-type cells, whereas transcriptional responses in *Stat1$^{\beta/\beta}$* cells shifted towards those observed in *Stat1$^{-/-}$* cells.

The absence of one STAT1 isoform did not affect the expression of the other isoform in macrophages, fibroblasts and B cells, arguing for a unique regulation of STAT1 in T cells. We show that differences in STAT1 isoform expression start during the development from double positive (DP, $CD4^+CD8^+$) to single positive (SP, $CD3^+CD4^+$ and $CD3^+CD8^+$) thymocytes. Interestingly, total STAT1 abundance and the STAT1β:STAT1α ratio increased from DP to SP thymocytes in wild-type mice, prompting the hypothesis that alternative splicing of STAT1 is used to limit STAT1-dependent responses in naïve $CD4^+$ and $CD8^+$ T cells.

Poster-Nr. 050

Abstract-ID: P50 | Poster presentation: 18.11.2016

The Fc receptor for IgM regulates T cell activation and cytotoxicity but declines with aging and T cell differentiation

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The aged immune system undergoes significant age-related changes and numerous of its physiological functions decrease, leading to the increased incidence and severity of infections as well as poor responses to vaccination. Changes occurring in the immune system of ageing humans – referred to as immunosenescence – have huge consequences on health however the exact nature of these modifications and the underlying mechanisms are still largely unknown.
Fc receptors (FcR) for IgG, IgE or IgA have been characterized extensively, but although a FcR for IgM (FcμR) has been suggested for decades the molecular identification has long been elusive. Recently, a transmembrane glycoprotein, which exhibits an exclusive IgM-binding specificity has been identified. In line with this, the major physiologic role of FcμR seems to be the internalization of IgM-coated pathogens into the cell and the shuttling of the IgM-bound cargo to the lysosome. In humans, the FcμR is only expressed on adaptive immune cells, both B and T lymphocytes and, to a lesser extent, on NK cells. In contrast to the B cells, the function of the FcμR on T cells have not been investigated. In this study, we extend the characterization of FcμR on T cells and determine its functional impact. We demonstrated that FcμR decreases with age and differentiation of T cells from naïve to TEMRA phenotype. Comparison of T cells isolated from the peripheral blood (PB) and the bone marrow (BM) of the same person revealed that the FcμR is expressed on PB T cells but nearly absent on BM T cells. This downregulation on BM T is mediated by the BM niche cytokines IL-6, IL-7 and IL-15. Furthermore, IgM binding by the FcμR accelerates TCR-mediated activation and increases the cytotoxicity.
A better understanding of the aged immune system and in particular molecules which are regulated in an age-dependent manner will have practical consequences for improvements of vaccination strategies and the prevention of immunosenescence.

SESSION 3 | IMMUNE CELL SIGNALING

Poster-Nr. 051

Abstract-ID: P51 | Poster presentation: 18.11.2016

Engagement of distinct epitopes on CD43 induces different co-stimulatory pathways in human T cells

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Co-receptors, being either co-stimulatory or co-inhibitory, play a pivotal role in T cell immunity. Several studies have indicated that CD43, one of the abundant T cell surface glycoproteins, acts not only as a potent co-receptor but also as a negative regulator for T cell activation. Here we demonstrate that co-stimulation of human peripheral blood T cells via two distinct CD43 epitopes recognized by mAbs CD43-6E5 ($T_{6E5\text{-}act}$) and CD43-10G7 ($T_{10G7\text{-}act}$) potently induced T cell proliferation. However, T cell co-stimulation via two CD43 epitopes differentially regulated activation of NFAT and NF-κB transcription factors, T cell cytokine production and effector function. $T6_{E5\text{-}act}$ produced high levels of IL-22 and IFN-γ similar to T cells activated via CD28 ($T_{CD28\text{-}act}$), whereas $T_{10G7\text{-}act}$ produced low levels of inflammatory cytokines but higher levels of regulatory cytokines TGF-β and IL-35. Compared to $T_{6E5\text{-}act}$ or to $T_{CD28\text{-}act}$, T_{10G7}-act performed poorly in response to re-stimulation and further acquired a T cell suppressive function. $T_{10G7\text{-}act}$ did not directly inhibit proliferation of responder T cells, but formed stable heterotypic clusters with dendritic cells via CD2 to constrain activation of responder T cells. Together, our data demonstrate that CD43 is a unique and polarizing regulator of T cell function.

Poster-Nr. 052

Abstract-ID: P52 | Poster presentation: 18.11.2016

T-cell derived glucocorticoids: a conversion process from an inactive precursor

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Glucocorticoids (GC) are steroid hormones which take part in a feedback mechanism in the immune system shutting down inflammatory responses. They are suggested to be key players in T cell selection in the thymus and are therefore important in shaping the peripheral T cell repertoire. GC are not only synthesized by the adrenal glands, the thymus is also able to produce GC; whether thymic epithelial cells (TEC) or thymocytes are the main source of GC is a matter of debate. Our research is centered on the study of the enzymes involved in GC synthesis in TEC and T cells, with special focus on the two main de novo GC synthesizing enzymes, CYP11A1 and CYP11B1, as well as the GC-activating enzyme 11bHSD1 which converts inactive 11-dehydrocorticosterone (11-DHC) into active corticosterone. Analysis of the expression of the GC pathway enzymes shows that CYP11A1 and 11bHSD1 are expressed at different T cell subsets in thymus and spleen, as well as in TEC, whereas we did not find any detectable expression level of the final de novo synthesis enzyme CYP11B1 neither in T cells nor in TEC. We observed differential GC effects on *in vitro* T-cell development, being $CD4^+CD8^+$ cells the most susceptible thymocyte subset to high concentrations of GC which impaired their progress in the maturation process. We analyzed T cells' capability to produce active GC *in vitro* and we found a clear conversion of the inactive precursor 11-DHC into corticosterone which resulted in T cell death specifically mediated by the glucocorticoid receptor (GR). These results suggest that GC may modulate T cell development affecting thymocytes in a different way depending on their maturation status. The absence of CYP11B1 expression suggests that if GC are needed for T cell selection and development they may not be synthesized using the de novo synthesis pathway but rather generated by the conversion of inactive 11-DHC into active corticosterone by the action of the enzyme 11bHSD1 which is expressed throughout T cell development.

SESSION 3 | IMMUNE CELL SIGNALING

Poster-Nr. 053

Abstract-ID: P52 | Poster presentation: 18.11.2016

Rapid multiplex analysis of lipid raft signaling components with single lymphocyte resolution

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Lipid rafts, a distinct class of highly dynamic cell membrane microdomains, are integral to cell homeostasis, differentiation and signaling. Raft-association of important lymphocyte receptor signaling molecules - e.g. Src family kinases – and stimulation-induced variations in raft composition was demonstrated. Furthermore, raft-association of these molecules clearly correlated with signaling function and the magnitude of response. However, biochemical quantitative analysis of lipid raft components involves laborious and time-consuming lysate fractionation protocols, relying on density gradient ultracentrifugation. These methods need a large number of input cells and completely destroy single cell information. This complicates or even precludes the examination of rare cells, developmentally heterogeneous cell populations or weakly raft-associated factors. We established a fast and reliable method that is based on the low g centrifugation of cells through a detergent gradient, requiring little starting material and effort. Lysis-resistant raft components are concentrated onto nuclear remnants, enabling multidimensional and sensitive flow cytometric quantitation of raft-associated proteins with single cell resolution. It allows easy and precise assessment of endogenously and ectopically expressed membrane components from a few cells in complex isolates as well as their dynamics due to cell differentiation, signaling and mutation. In conclusion, our approach is well suited to elucidate the role of lipid rafts in organising factors that govern signaling thresholds of crucial leukocyte receptors, including - but not limited to - the T cell antigen receptor.

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Poster-Nr. 054

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Protein kinase C theta is dispensable for suppression mediated by murine CD25⁺CD4⁺ regulatory T cell

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The activation of conventional T cells upon T cell receptor stimulation critically depends on protein kinase C theta (PKCθ). On the contrary, its role for Treg function has yet to be fully defined. Using siRNA or the potent and PKC family selective pharmacological inhibitor AEB071 we could show that murine Treg-mediated suppression *in vitro* is independent of PKCθ. Likewise, Treg cells of PKCθ-deficient mice were fully functional, showing similar suppressive activity than wild type $CD25^+CD4^+$ T cells in an *in vitro* suppression assay. Furthermore, *in vitro* differentiated wild type and PKCθ-deficient iTreg cells showed comparable Foxp3 expression as well as suppressive activity. However, we observed reduced percentage of $Foxp3^+CD25^+$ $CD4^+$ T cells in lymphatic organs of PKCθ-deficient mice. Taken together, our results suggest that while PKCθ is involved in Treg cell differentiation *in vivo*, it is dispensable for Treg-mediated suppression.

SESSION 3 | IMMUNE CELL SIGNALING

Poster-Nr. 055

Abstract-ID: P55 | Poster presentation: 18.11.2016

Evaluation of the effects of the 3-phosphoinositide-dependent kinase-1 targeting drug BX795 on allergen-specific T cells reveals promotion of interleukin-2 but complete shut-off of T helper 1 and 2 cytokine secretion

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BX795, an inhibitor of 3-phosphoinositide-dependent kinase-1 (PDK-1), was investigated for its effects on viability, allergen-specific activation, growth and factor production of major mugwort (Artemisia vulgaris) pollen allergen-specific T lymphocytes derived from double transgenic allergy mice. We assessed proliferation, T cellular activation and polarization by measuring 3H-thymidine uptake and evaluation of CD69, CD25, CD154 and CD49e expression on $CD3^+CD4^+$ T cells and T-bet, GATA-3, ROR-γt and FOXP3 expression on $CD4^+$ TCR^+ T cells. Factor production was determined by multiplexing of supernatants after T cell activation with titrated amounts of allergen. Using the IC50 dose for proliferation inhibition, no alteration in viability of BX795-treated splenocytes was observed. Noteworthy, BX795 almost completely inhibited IFN-γ secretion (72.9±10.0%, $p<0.01$) early on (24 hours) and inhibited IL-4 (88.3±6.5%, $p<0.05$), IL-13 (75.8±8.7%, $p<0.001$) and IL-10 (80.8±13.1%, $p<0.001$) secretion at 48 hours. Moreover, at 48 hours BX795 partially inhibited IL-5 (35.8±23.2%, $p<0.05$) and TNF-α (45.2±24.8%, $p<0.05$) secretion with maximal inhibition seen after 72 hours (70.6±15.5% $p<0.001$ and, 68.0±17.8% $p<0.05$, respectively). BX795 did not influence IL-17 and GM-CSF levels, however, it significantly stimulated IL-2 secretion at 48 and 72 hours (2.3±0.6-fold and 4.7±1.4- fold, $p<0.01$ and $p<0.001$, respectively). Other substances tested, displaying an equal level of proliferation inhibition, such as the ERK2 inhibitor Vx-11e drastically inhibited IL-2 secretion. The percentage of $CD4^+$ T cells co-expressing T-bet and GATA-3 was reduced non-significantly after 48h (72.5±16.5%, $p=0.0859$) In summary, BX795 specifically shuts-off Th1 and Th2 cytokines while it strongly stimulates interleukin-2 secretion. The potential clinical relevance of these effects and its application is currently being evaluated in *in vivo* experiments using double transgenic allergy mice expressing an Art v 125-36-specific and HLA-DR1 restricted TCR.

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Poster-Nr. 056

Abstract-ID: P56 | Poster presentation: 18.11.2016

Intracellular signalling through the ERK pathway upon binding of monomeric IgE and IgE-Bet v 1 complexes to CD23 on B cells

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CD23, the low affinity receptor for IgE, is found mainly on B cells. It is known to bind IgE-allergen complexes which are subsequently internalised and presented to T cells. However, CD23-mediated intracellular signalling is not yet fully understood. Increased levels of phosphorylated ERK, Fyn and AKT were reported in different human B and monocytic cell lines upon the crosslinking of CD23 with specific anti-CD23 antibodies. Nevertheless, the intracellular signalling upon binding of natural ligands of CD23, such as IgE and IgE-allergen complexes, has not been analysed yet.
We studied the intracellular signalling cascades activated through CD23 using purified monoclonal human Bet v 1-specific IgE, monomeric Bet v 1 and a recombinant Bet v 1 trimer in a human Epstein-Barr virus-transformed B cell line expressing high levels of CD23. The induction of ERK phosphorylation was studied with specific antibody probes in B cell extracts by Western blotting. The phosphorylation of ERK was induced upon the binding of IgE as well as IgE allergen complexes to CD23. The time course of induction of ERK phosphorylation differed slightly between the two conditions. In conclusion, the binding of monomeric IgE alone as well as IgE-allergen complexes to CD23 induced an intracellular signal via the ERK pathway. The established system will be used to investigate other potential signalling pathways upon engagement of CD23 with its natural ligands.

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SESSION 3 | IMMUNE CELL SIGNALING

Poster-Nr. 056a

Abstract-ID: P56a | Poster presentation: 18.11.2016

Molecular imaging of the antigen recognition dynamics in CD8$^+$ cytotoxic T-cells

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Cytolytic T-cells (CTLs) can detect with their low affinity T-cell antigen receptors (TCRs) the presence of even a single antigenic peptide-loaded MHC molecule I (pMHCI) among thousands of structurally related yet non-stimulatory pMHCs (Purbhoo et al. 2004). How they achieve this is not clear but appears to depend at least in part on the special binding conditions within the special constraints of the immunological synapse, the area of contact between a T-cell and an antigen presenting cell. Here receptors and their ligands are not only pre-oriented, but they are often enriched in specific membrane domains and also subjected to cellular forces. To relate these cell biological parameters to T-cell antigen sensitivity in a more comprehensive manner we are monitoring TCR-pMHC binding in nascent synapses with the use of molecular imaging modalities. We confront TCR transgenic CTLs with a glass-supported lipid bilayer (SLB) functionalized with pMHCI, adhesion and co-stimulatory molecules. This allows us to conduct (single molecule) measurements in noise-attenuated Total Internal Reflection (TIRF) mode, to control for ligand composition and density to quantitate their specific influence on TCR-pMHCI binding and TCR-proximal downstream signaling. We also plan to assess the role of CD8 co-receptor engagement with the use of pMHCI mutants, which are deficient in CD8 binding. In its fashion we expect to gain novel insights into cell biological and molecular processes underlying the phenomenal sensitivity of CTLs towards antigen.

Oral Presentation-Nr. 010

Abstract-ID: 10 | Oral Presentation 17.11.2016, Session 4, Adaptive Immune Regul. 17.50-18.30

The Bcl-2 prosurvival protein A1 is dispensable for T cell homeostasis upon viral infection

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The physiological role of the pro-survival BCL-2 family member A1 has been debated for a long time. Strong mRNA induction in T cells upon T cell receptor (TCR)-engagement suggested a major role of A1 in the survival of activated T cells. However, the investigation of the physiological roles of A1 was complicated by the quadruplication of the A1 gene locus in mice, making A1 gene targeting very difficult. Here, we used the recently generated $A1^{-/-}$ mouse model to examine the role of A1 in T cell immunity. We confirmed rapid and strong induction of A1 protein in response to TCR/CD3 stimulation in $CD4^+$ as well as $CD8^+$ T cells. Surprisingly, upon infection with the acute Influenza Hkx31 or the lymphocytic choriomeningitis virus (LCMV) docile strains mice lacking A1 did not show any impairment in the expansion, survival, or effector function of cytotoxic T cells. Furthermore, the ability of $A1^{-/-}$ mice to generate antigen-specific memory T cells or to provide adequate CD4-dependent help to B cells was not impaired. These results suggest functional redundancy of A1 with other pro-survival BCL-2 family members in the control of T cell-dependent immune responses.

SESSION 4 | ADAPTIVE IMMUNE REGULATION

Oral Presentation-Nr. 011

Abstract-ID: 11 | Oral Presentation 17.11.2016, Session 4, Adaptive Immune Regul. 17.50-18.30

The AMP analog AICAR modulates the Treg/Th17 axis through enhancement of fatty acid oxidation

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T cells must tightly regulate their metabolic processes to cope with varying bioenergetic demands depending on their state of differentiation. The metabolic sensor AMPK is activated in states of low energy supply and modulates cellular metabolism toward a catabolic state. Although this enzyme is known to be particularly active in regulatory T (Treg) cells, its impact on T helper (Th) cell differentiation is poorly understood. We investigated the impact of several AMPK activators on Treg differentiation and found that the direct activator AICAR (5-aminoimidazole-4-carboxamide ribonucleotide), but not the indirect activators metformin and 2-deoxyglucose, strongly enhanced Treg cell induction by specifically enhancing Treg cell expansion. Conversely, Th17 generation was impaired by the agent. Further investigation of the metabolic background of our observations revealed that AICAR enhanced both cellular mitochondrogenesis and fatty acid uptake. Consistently, increased Treg-induction was entirely reversible on inhibition of fatty acid oxidation, thus confirming the dependence of AICAR's effects on metabolic pathways alterations. Translating our findings to an *in vivo* model, we found that the substance enhanced Treg cell generation on IL-2 complex-induced immune stimulation. We provide a previously unrecognized insight into the delicate interplay between immune cell function and metabolism and delineate a potential novel strategy for metabolism-targeting immunotherapy.

Oral Presentation-Nr. 012

Abstract-ID: 12 | Oral Presentation 17.11.2016, Session 4, Adaptive Immune Regul. 17.50-18.30

Oxidative stress and age-related impairments in the maintenance of immunological memory

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Aging induces a basal level of inflammation throughout the body, a condition known as inflammaging, which contributes to immunosenescence. New strategies to counteract immunosenescence in the elderly are needed, in particular by improving the maintenance of immunological memory. It has been demonstrated that memory T cells and long-lived plasma cells home to bone marrow niches, well organised structures which promote the survival of these cells through homeostatic proliferation. $CD4^+$ and $CD8^+$ effector memory T cell survival is promoted by IL-7 and IL-15 while maintenance of long-lived plasma cells is supported by APRIL and IL-6. IL-7 is believed to be important for long-lived memory T cells while IL-15 is mostly important for short-lived CD28- senescent T cells, of which accumulation is associated with mortality in old age. The expression of effector memory cell and proinflammatory factors were investigated in bone marrow mononuclear cells (BMMCs) using qPCR and FACS, finding that, with age, IL-7 and APRIL decrease while IL-15, IL-6, TNF, IFNγ and IL1β increase. A correlation was found between proinflammatory molecules, ROS levels and the expression of IL-15 and IL-6 in BMMCs. Incubation of BMMCs with ROS scavengers N-acetylcysteine and vitamin C reduced the levels of both cytokines in this cell population. In addition, IL-15 and IL-6 were more expressed in the BM of SOD-1 knockout mice. This indicates that oxidative stress may contribute to the age-related impairments in the maintenance of immunological memory. Antioxidant treatment may be an important strategy to counteract immunosenescence by reducing the size of CD28- senescent T cell population in old age.

SESSION 4 | ADAPTIVE IMMUNE REGULATION

Oral Presentation-Nr. 013

Abstract-ID: 13 | Oral Presentation 17.11.2016, Session 4, Adaptive Immune Regul. 17.50-18.30

MAZR: Modulating regulatory T cell development and function

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$Foxp3^+$ regulatory T cells (T_{reg} cells) play a key role in maintaining immune homeostasis and in modulating immune reactions during infection and disease. Here we report that the BTB zinc finger transcription factor MAZR (also known as Patz1) severely affects $Foxp3^+$ regulatory T cell generation and differentiation. We previously identified MAZR as an important regulator of *Cd8* gene expression in DN thymocytes and the generation of MAZR knockout mice revealed an essential role for MAZR in CD4/CD8 lineage choice of DP thymocytes. Preliminary results from a comprehensive analysis of conditional MAZR-deficient mice (*Cd4*-Cre) indicate that the deletion of MAZR leads to an increase in $Foxp3^+$ T_{reg} cells *in vivo* and that MAZR-null $Foxp3^+$ T_{reg} cells displayed enhanced suppressive capacity in *ex vivo* assays. Furthermore, MAZR-null $CD4^+$ T cells differentiate with an enhanced frequency into *in vitro*-generated iTreg cells. Together, these data suggest a negative regulatory function for MAZR in T_{reg} cells. Data from our ongoing experiments will be presented.

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Poster-Nr. 057

Abstract-ID: P57 | Poster presentation: 17.11.2016

In vivo and in vitro characterization of thymoglobulin

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Thymoglobulin (ATG) is a polyclonal rabbit antibody against human thymocytes used as a T cell-depleting agent in organ transplantation. Because its polyclonal character, its effect may exceed that of pure T cell depletion. The aim of this project was to elucidate the possible role of ATG in T_{REG} induction both *in vivo* and *in vitro*.

For *in vivo* studies, humanized hCD3ε BALB/c mice were *i.v.* injected with ATG or control rabbit immunoglobulin (Ig) and then blood and lymphoid organs were harvested. To investigate the ATG effect on the prolongation of transplant survival we used a murine cervical heart allotransplant model. C57BL/6 ($H2^d$) donors graft were transplanted into hCD3ε BALB/c ($H2^b$) mice.

For *in vitro* studies, human peripheral blood mononuclear cells (PBMC) were incubated with ATG for various times at 37°C. $Foxp3^+$ T_{REG} and monocytes were phenotypically analysed by flow cytometry and functionally by *in vitro* suppression assays. Cytokines were measured by Multiplex or ELISA assays.

In vivo, ATG led to T-cell depletion in peripheral blood, spleen and lymph nodes in the hCD3ε BALB/c mice after 24h. After 7 days, T cells recovered in the circulation, however depletion in lymph nodes and thymus still persisted and were also observed 14 days post injection. Importantly, while naïve T cells were strongly depleted, T_{REG} were spared. Survival and transplant function were significantly prolonged in ATG-treated mice, in comparison to mice receiving normal Ig.

In vitro, the frequencies of $Foxp3^+$ T_{REG} increased when human PBMC were cultured with ATG as compared with rabbit Ig or without stimulation. ATG-treated cells suppressed proliferation of autologous PBMC. Monocytes stimulated with ATG down-modulated CD16 and secreted IL-10.

Our study demonstrates that ATG has additional immunomodulatory properties further to T cell depletion and this includes effects on persistence of TREG and IL-10 production by monocytes.

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SESSION 4 | ADAPTIVE IMMUNE REGULATION

Poster-Nr. 058

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Persistent alloantigen-specific immune tolerance without loss of T cell reactivity to pathogens is induced by CD28 blockade

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Donor T cells contribute to the reconstitution of protective immunity after allogeneic hematopoietic stem cell transplantation (HSCT), but have to acquire specific tolerance against recipient allo-antigens in order to avoid life-threatening graft-versus-host disease (GvHD). Systemic immunosuppressive drugs used as first line regimen potentially abrogate severe GvHD, but also impede memory responses to invading pathogens. Here we test whether blockade of CD28 co-stimulation *ex vivo* enables selective T cell tolerization to alloantigens via engagement of CD80/86-CTLA-4 signaling. Treatment of allogeneic dendritic cell/T cell co-cultures with the CD28 blocking antibody FR104 significantly abrogates allospecific immune responses as shown by decreased T cell expression of the type 1 cytokines IFN-g and IL-2. Importantly, following discontinuation of CD28 blockade, upon antigen re-exposure *in vitro*, (i) allo-tolerization persists and (ii) T cells remain pathogen-reactive, shown by clonal expansion of *Candida albicans*-specific T cells. Enhanced CTLA-4 and PD-1 immune checkpoint signaling underline persistent tolerance after antigen re-challenge of allo-tolerized T cells. Furthermore CD28-mediated allo-tolerization of T cells is sustained *in vivo*. Using an MHC-mismatched murine model, C57BL/6 T cells treated with a murine-specific FR104 mimic in the presence of BALB/c DCs ameliorate GvHD after infusion into bone marrow-transplanted BALB/c mice.

Our findings demonstrate that CD28 blockade *ex vivo* allows the generation of stably allo-tolerized T cells that prevent graft-versus-host reactions while maintaining pathogen specificity. Hence, CD28 co-stimulation blockade of donor T cells is useful as a therapeutic approach to support a patient's immune system after HSCT.

Poster-Nr. 059

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Impact of free fatty acids binding to nsLTP on their tertiary structure and allergenic activity

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Background: Plant non-specific lipid transfer proteins (nsLTPs) are relevant plant food allergens e.g. from peach (Pru p 3). They share a conserved fold with an internal cavity. Different lipid–protein complexes showed that the tunnel adapts its volume while binding a broad range of hydrophobic molecules.

Methods: The binding of lipids to Pru p 3 was monitored by adding 10 µM 1,8-ANS and measuring the decrease of 1,8-ANS fluorescence. Furthermore, molecular dynamic analysis (MD) was applied to explore the nature of interaction between nsLTP and tested ligands. W-LOGSY (Water-Ligand Observed via Gradient Spectroscopy) technique were applied to confirm results obtained *in silico*. Impact of lipid binding on the allergenicity of the protein was investigated by ELISA and BAT assay.

Results: Due to pre-incubation of Pru p 3 with lipids a concentration dependent reduction of ANS binding was observed. Pru p 3 incubated (1:1; 1:10) with lauric acid showed 19% and 66% of ANS fluorescence reduction respectively, compared with Pru p 3 without lipids. For oleic acid (1:1; 1:10) reduction was 7% and 77%, respectively. Molecular dynamic analysis suggests changes in protein structure due to binding to certain ligands. Interaction between oleic acid and Pru p 3, moved the C-terminal loop out towards the surface of the molecule. NMR based experiments confirmed binding capacity observed in MD analysis. Pre-incubation of oleic acid with Pru p 3 significantly increased IgE binding in ELISA and BAT assay as compared to stearic acid and allergen alone.

Conclusions: In this study, we observed differences in binding capacity of Pru p 3. MD simulation showed that interaction between Pru p 3 and tested ligands can lead to conformational changes that influence allergenic activity of nsLTPs. The allergen–lipid interaction may act as a potential danger signal during the allergic sensitization phase or increase allergenicity during the effector phase.

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Poster-Nr. 060

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The MAZR/RUNX3 complex is integrated into the transcriptional network controlling effector CD8$^+$ T cell differentiation

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Effector CD8$^+$ T cells play a crucial role in antiviral and antitumor immune responses. We have previously demonstrated that the transcription factor MAZR promotes cytotoxic lineage differentiation of double-positive (DP) thymocytes, in part via repressing the expression of ThPOK, a central transcription factor for helper lineage differentiation. Moreover, we recently revealed that MAZR physically interacts with the Runx transcription factors, and that they synergistically repress ThPOK expression. In this study we aim to elucidate the role of the MAZR/Runx3 complex during effector CD8$^+$ T cell differentiation. We activated MAZR- and/or Runx3-deficient CD8$^+$ T cells *in vitro* by anti-CD3/28 stimulation, and found that the synergistic activity of MAZR and Runx3 is also required for the expression of cytotoxic effector proteins such as CD8, granzyme B and T-bet. In addition, we obtained preliminary evidence showing that upon Lymphocytic Choriomeningitis Virus (LCMV) infection MAZR and Runx3 cooperatively as well as individually control the expansion and effector function of virus-specific CD8$^+$ T cells. Thus, our data indicate that the MAZR/Runx3 complex is integrated into the transcriptional network governing effector CD8$^+$ T cell differentiation. Ongoing experiments further investigate the underlying mechanisms of how the MAZR/Runx3 complex controls effector as well as memory CD8$^+$ T cell differentiation. Moreover, genome-wide analyses (i.e. RNA-seq and ChIP-seq) are currently underway, in order to gain deep insight into global transcriptional network mediated by the MAZR/Runx3 complex.

Poster-Nr. 061

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Identification and characterization of HDAC1 and HDAC2 interaction networks in Th17 cells

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The differentiation and function of $CD4^+$ T helper (Th) subsets has to be tightly regulated, since their dysregulation is linked with immune-mediated diseases. Th cell differentiation is accompanied by reversible changes in histone acetylation, mediated by the opposing activity of histone acetyltransferases (HATs) and histone deacetylases (HDACs), however many non-histone targets are emerging, indicating that HAT/HDACs act beyond the regulation of chromatin.
Results of my laboratory demonstrate an essential role for HDAC1 but not for the very closely related HDAC2 protein in regulating Th17 cells and the development of autoimmune diseases. Since HDAC1 and HDAC2 are part of larger multiprotein complexes, it is tempting to speculate that the crucial role of HDAC1 in Th17 cells is mediated by factors that interact with HDAC1 but not with HDAC2. The aim of my PhD thesis project is to test this hypothesis. I will utilize novel mouse models in combination with mass spectrometry approaches to generate a HDAC1 and HDAC2 interaction network map and to define HDAC1-specific interaction partners. I will study the role of selected HDAC1-specific interaction partners in Th17 cells to functionally link them with HDAC1 and Th17 cell function. My experimental approach will unravel molecular details of how HDAC1 regulates Th17 cells and the development of T cell-mediated (auto)immune responses.

SESSION 4 | ADAPTIVE IMMUNE REGULATION

Poster-Nr. 062

Abstract-ID: P62 | Poster presentation: 17.11.2016

Characterization of the immuno-modulary capacity of allergen-expressing virus-like nanoparticles (VNP) decorated with cytokines of choice

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Moloney murine leukemia virus(MoMLV)-derived VNP consist of viral capsid proteins and a lipid envelope but lack both the fusogenic Env protein and the integrating viral genome. Since MoMLV VNP preferentially bud form the lipid-raft-rich regions of the plasma membrane of producer cells they represent a meeting point for post-translationally lipid-modified proteins, which they tend to concentrate either on their surface or, in a shielded form, inside the viral lipid envelope and/or the viral core. Consequently, MoMLV VNP represent a safe platform for the generation of multivalent vaccines. In order to explore the potential of VNP to modulate allergen-specific immune responses, we here targeted nominal allergen to the surface or the interior of VNP and in addition decorated them with the cytokines granulocyte macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN-g), interleukin 10 (IL-10), interleukin 12 (IL-12) and transforming growth factor beta (TGF-^{2}1). Targeting of cytokines and the mugwort major allergen Art v 1 was achieved by their C-terminal fusion to the glycosyl-phosphatidyl inositol (GPI) acceptor sequence of CD16b. Expression of Art v 1 inside of VNP was accomplished by its N-terminal fusion to the viral matrix protein. Surface expression levels of cytokines and Art v 1 were confirmed by flow cytometric analyses of transfected producer cells. Immunoblotting revealed the presence of the expressed cytokines and of the two differently anchored allergens on/in VNP. VNP were produced in large-scale by applying nano-filtration and ultracentrifugation techniques. The purified and quantified particles are currently being tested in proliferation and cytokine secretion assays taking advantage of allergen-specific splenocytes of double transgenic mice specifically recognizing the immunodominant Art v 125-36 peptide in the context of HLA-DR1. The immunomodulatory capacity of VNP in primary and secondary stimulation assays will be described and potential applications of allergen-specific VNP decorated with cytokines of choice will be discussed.

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Poster-Nr. 063

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Activation of iNKTs by food derived lipids

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In contrast to conventional T lymphocytes invariant natural killer T lymphocytes (iNKTs), recognize chemically distinct CD1d-restricted antigens such as lipids. Therefore, iNKTs are a major subpopulation of T lymphocytes that recognize self- and non-self-derived lipids. Upon activation, iNKTs can either up- or downregulate immune responses by promoting the secretion of Th1 or Th2 immune regulatory cytokine patterns. So far, iNKTs have been identified as important players in different types of immune responses. To investigate the role of iNKTs in food allergy different food-derived lipid fractions from walnut and hazelnut were obtained by Folch extraction and preparative thin layer chromatography. To test whether lipid fractions contain iNKT-specific antigens, we used the murine hybridoma cell lines DN3A4-1.2 and DN3A4-1.4. In a first approach CD1d-dependent *in vitro* antigen presentation assay was performed testing total tree nut derived lipids and fractions thereof. Subsequently, a co-culture assay with the murine dendritic cell line JAWSII was established. As a readout IL-2 levels as an activation marker were measured in the supernatants. Results obtained from the CD1d-dependent *in vitro* antigen presentation assay show activation of iNKTs by total hazelnut and walnut lipid extracts. Co-culture assays are still in progress. It is expected that detailed biochemical analyses of tree nut lipids will contribute to our understanding of immunologically active substances that activate iNKTs which in turn may affect the overall allergic immune response in predisposed individuals.

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SESSION 4 | ADAPTIVE IMMUNE REGULATION

Poster-Nr. 064

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Non-allergenic virus like nanoparticles prevent sensitization in a humanized mouse model of mugwort allergy

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Allergen-specific immunotherapy requires the repeated delivery of potentially anaphylactogenic proteins. To circumvent this problem, we here explored novel non-allergenic platforms for allergen delivery based on virus-like nanonparticles (VNP). VNP are composed of virus capsid proteins and a lipid envelope but lack the infectious viral genome and are therefore regarded as a safe vaccination platform. Proteins of interest can be selectively targeted to the surface of VNP or, alternatively, to the inner leaflet, leading to their 'shielded' expression inside of VNP. In our study, VNP expressing the major mugwort pollen allergen Art v 1 either on the surface or shielded inside VNP were analyzed for their potential to activate T and B lymphocytes as well as sensitized basophils and compared to nominal allergen *in vitro* and *in vivo*. Degranulation of rat basophil leukemia cells sensitized with Art v 1-specific IgE occurred only upon exposure to VNP expressing surface-exposed but not shielded allergen. In contrast, Art v 1 protein derived from both particle types was well-presented to allergen-specific T cells, with the shielded allergen exhibiting a 3.8±2.1-fold better T cell stimulatory capacity compared to surface-exposed allergen. Upon intranasal application in Art v 1-specific 'allergy mice' VNP expressing surface-exposed allergen induced allergen-specific antibodies, including IgE, while VNP expressing shielded allergen did not. Notably, prophylactic treatment with shielded allergen significantly prevented from subsequent sensitization. Shielding of allergens inside of VNP represents a novel and versatile alternative for the *in vivo* delivery of allergens to selectively target T cells and, eventually, to prevent and cure allergies.

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SESSION 4 | ADAPTIVE IMMUNE REGULATION

Poster-Nr. 065

Abstract-ID: P65 | Poster presentation: 17.11.2016

High resolution imaging to unravel the molecular etiology of disturbed T-cell antigen recognition

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T-cell defects account for ~30% of all inherited human immune deficiencies. While recent progress in genome sequencing has helped identify gene defects in patients with compromised T-cell antigen sensitivity, the precise molecular underpinnings remain often elusive. This complicates the search for best therapy options for patients with frequent infections and/or organ-destructive autoimmunity. Here we present a cutting-edge molecular imaging platform to unravel disease-causing mechanisms in patient T-cells. We are currently devising a planar supported lipid bilayer (SLB) system, which closely mimics the physiological context of T-cell recognition and greatly facilitates single molecule and super resolution microscopy. Such high-resolution functional imaging will soon enable us to quantitate binding, signaling and redistribution defects of key receptors, effector proteins and cytoskeletal components and help us stratify human T-cell disorders according to TCR-proximity.

We will integrate imaging with state-of-the-art high-throughput genomics to identify causative mutations and with interaction proteomics to place identified genes into regulatory networks, which we will in turn verify by functional high-resolution imaging. Ultimately this hybrid approach will fill a critical gap in personalized medicine for patients suffering from inherited T-cell defects of so far unknown etiology, enabling rational therapeutic intervention and discovery of new genes involved in T-cell function.

Poster-Nr. 066

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A novel humanized mouse model to study skin immunology

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The skin comprises a complex coordinated system of epithelial tissue cells and immune cells that ensure adequate immune reactions against trauma, toxins and pathogens, while maintaining self-tolerance and preventing allergy and autoimmunity. The skin harbors a vast amount of immune cells such as T cells and innate cells that interact with each other as well as the skin tissue cells (such as keratinocytes). We hypothesize that these interactions are crucial in order to maintain homeostasis within the skin. Here we aim to analyze and understand the role of cellular interactions in human skin and manipulate them in settings of tissue damage.

Because murine and human skin differ in structure and cell composition we established a humanized mouse model to investigate human immune mechanisms in human skin *in vivo*. Importantly, we use a simplified skin tissue generated from fibroblasts and keratinocytes only. Thus, the humanized mouse model features minimal organotypic skin tissue that can be reconstituted with different immune cell types found in human skin, which will allow us to study cell-cell interactions, including T cell – APC, T cell – keratinocyte, or T cell – microbe/commensal cross-talk.

We found that we are able to successfully follow T cell infiltration of the skin. Additionally, we started to characterize local T cell – APC interactions and found that these lead to improved T cell infiltration of the skin and local T cell proliferation in the presence of a microbial antigen. In the future we will use this model to study the mechanisms of maintenance and recruitment of the huge number of skin T cells and their immunological and tissue regenerative function within the skin. The gained knowledge will set the basis for the specific manipulation of pathologic immune responses in inflammatory skin disorders in the future.

Poster-Nr. 067

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How fat influences the adaptive immune system in the bone marrow in old age

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Multipotent haematopoietic stem cells differentiate to precursor cells and further to immature T-lymphocytes in the bone marrow, which then migrate into the thymus gland. The thymus is a primary lymphatic organ and responsible for the development of T-cells to mature naïve T-cells. During puberty the thymus starts to degenerate, also called thymic involution. With age, the functional part of the thymus tissue is reduced and replaced by fat. A similar effect can be seen within the bone, there we find a reduction in bone formation and bone loss. A predominant property of age-related bone loss is the accumulation of bone marrow fat. It is already known that due to the thymic involution there is a decrease in naïve T-cells. But the effect in the bone has not yet been documented.
The aim of the study is to investigate the infiltration of immune cells in subcutaneous fat in comparison to the infiltration in bone marrow fat from lean and obese donors in the context of aging. It is known that obese people show predominantly more inflammatory cells, including macrophages that appear to be the source of fat-derived inflammatory cytokines, such as TNFα and IL-6, compared to lean individuals. With the focus on immune cells, including macrophages we try to give insight into the infiltration and localization in the adipose tissue from bone marrow in comparison to subcutaneous fat. For a better understanding we investigate the interaction of fat components on the adaptive immune system. The results of immunofluorescence staining suggest that there is a higher infiltration of macrophages in bone marrow compared to subcutaneous fat, and therefore we performed RT-PCR concerning pro- and anti-inflammatory cytokines and microarray analysis to confirm these results.

SESSION 4 | ADAPTIVE IMMUNE REGULATION

Poster-Nr. 068

Abstract-ID: P68 | Poster presentation: 17.11.2016

A phenotypical characterisation of the immune cells in the human bone marrow and the impact of aging

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Purpose: It has been shown that many antigen experienced immune cells migrate back to the bone marrow (BM), where they can remain for an extended period of time. In this study a detailed phentotypical characterisation of immune cells isolated from human BM was analysed to determine if the accumulation of highly differentiated $CD8^+$ T cells in the aging BM, limits the possibility of the accumulation of other immune cells.

Methods: Bone Marrow Mononuclear cells were isolated from human BM samples using collagenase digestion and density gradient centrifugation. Surface phenotypes were characterised using flow cytometry.

Results: Multiple correlations were seen between cell subsets found in the Aging BM. Plasma cells ($CD45^+CD19^+CD20^-$) correlated positively with Naive $CD8^+$ T cells ($CD45RO^-CCR7)^-$, and negatively with Central Memory $CD8^+$ T cells ($CCR7^+CD45RO^+$). The $CD4^+CD8^+$ double positive population, of which the functional purpose is not yet entirely known, showed a strong negative correlation with TEMRA $CD8^+$ T cells ($CCR7^-CD45RO^-$) as well as a population of Monocytes ($CD14^+CD16^-$).

Discussion: From these preliminary data, the concept of immunological BM niches comprising of limited space, suggests to affect the immune cell composition in the aging BM. The accumulation of highly differentiated $CD8^+CD28^-$ in the aging BM has previously been shown, however the impact of this subpopulation on other cell subsets has not yet been described. The accumulation of one cell type, may limit the possibility of others being maintained, and further investigation will focus on where these limitations are set and whether the antigen specificity of these long living memory cells effects their ability to migrate back to the BM.

Conclusion: The interactions between lymphocyte subsets may give us more information on the ability of the BM to harbour long living immune cells, and whether there are limiting factors determining this possibility, an important factor to consider when understanding immunological memory.

Poster-Nr. 069

Abstract-ID: P69 | Poster presentation: 17.11.2016

Stromal cells orchestrate lymph node function and development

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Lymph nodes (LNs) are strategically situated throughout the body at junctures of the blood vascular and lymphatic systems to direct immune responses against antigens draining from peripheral tissues. Fibroblastic and endothelial stromal cells of LNs have been demonstrated to modulate immune responses at multiple levels, however their origin and function for LN organogenesis have remained elusive. The current paradigm describes LN development as a programmed process that is governed through the interaction between fibroblastic lymphoid tissue organiser cells and hematopoietic lymphoid tissue inducer (LTi) cells. Using cell type-specific ablation of key molecules involved in lymphoid organogenesis, we found that initiation of LN development is dependent on LTi cell-mediated activation of lymphatic endothelial cells (LECs) and that involvement of fibroblastic stromal cells is a succeeding event. Embryonic endothelial cell activation was mediated mainly by signaling through the non-canonical NF-kB pathway and steered by sphingosine-1-phosphate receptor-dependent retention of LTi cells in the LN anlage. The finding that pharmacologically enforced LTi cell-LEC interaction promotes ectopic LN formation underscores the central lymphoid tissue organiser function of LECs. Furthermore, our data dissect the contribution of different LN stromal cells to the development and function of LNs.

Poster-Nr. 070

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Antigen dose defines T cell fate in vivo: effector versus regulatory T cells

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Immune homeostasis is governed by a fine balance of pathogenic effector T cells (Teff) and suppressive regulatory T cells (Treg). In addition of thymic Treg peripherally induced Treg (pTreg) can be generated in response to tissue antigen. However, the mechanisms controlling the generation of pTreg *in vivo* are still poorly understood. Here we **aim** to define the impact of T cell activation strength and its downstream effects on T cell differentiation and immune regulation in skin autoimmunity. In this project we want to test the **hypothesis** that low doses and chronic antigen exposure, favors pTreg generation over effector T cell differentiation. We chose to use two tetracycline-inducible systems for the expression of ovalbumin (Ova) in skin of transgenic mice. Keratin 5 promoter (K5) is expressed in the basal cell layer and involucrin promoter (INV) in the upper layers of the epidermis. In these mice we can follow adoptively transferred naïve Ova-specific T $CD4^+$ cells (DO11.10) *in vivo*. We find that K5-Ova-expression leads to the differentiation of both, Teff and pTreg cells, while INV-Ova completely blocks pTreg differentiation. Consequently, K5 results in self-resolving skin inflammation while INV leads to fatal disease. The latter was abrogated by *in vivo* pTreg generation prior to Ova-induction. We found that INV leads to higher expression levels, which we can titrate by dilution of tetracycline. This reduces proliferation, restores pTreg cell differentiation, hampers the production of effector cytokines and reduces TCR signaling. Alternatively, diminishing TCR signal strength at full dox dose with rapamycin also rescues pTreg generation. Taken together, our findings suggest that antigen load and initial TCR signal strength can be crucial in the decision of Teff versus pTreg differentiation with remarkable consequences on clinical outcome.

Poster-Nr. 071

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Is there a role for neutrophils in the adjuvanticity of alum?

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Most subcutaneous allergy vaccines contain aluminum hydroxide (alum) as adjuvant. Recently, it has been reported in mice that host-derived DNA is involved in the adjuvant effect of alum. In response to strong or particulate stimuli neutrophils have the ability to simultaneously release extracellular DNA and granular material, so-called neutrophil extracellular traps (NETs) which are able to trap and kill microbes, but also may represent danger-associated molecular patterns.
Here, we investigate alum-induced NET-formation of human neutrophils and their possible role in initiating immune responses in allergen-specific immunotherapy.
Human neutrophils were stimulated with alum, or PMA respectively ionomycin as positive controls. Strong NET-formation was induced by all stimuli as visualized by fluorescence microscopy showing co-localization of extracellular DNA and different granular proteins. In addition, alum-induced neutrophil elastase activity was found in supernatants. Inhibition experiments were performed to reveal the mechanism of NET-induction by using a plate reader assay to quantify DNA released by neutrophils over time. Ionomycin and alum induced mitochondrial reactive oxygen species (mROS), whereas PMA triggered cytoplasmatic (NADPH oxidase-dependent) ROS. Similar to ionomycin, rapid DNA-release was observed with alum together with a dependency on phagocytosis and extracellular calcium. Amine- or carbonate-modified, i.e. charged, but not uncharged latex beads (3µm), were also capable to induce NETs. First co-culture experiments of APC with NET-forming neutrophils show a decrease in antigen presenting capacity in monocytes and mdDCs, but a stimulatory effect on pDC. Together, *in vitro* alum potently induces NADPH-oxidase independent NET-formation in human neutrophils resulting in differential effects on different APC types.

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SESSION 4 | ADAPTIVE IMMUNE REGULATION

Poster-Nr. 072

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The role of allergen-specific IgG antibodies in allergen-specific T cell responses

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Background: Allergen-specific immunotherapy (AIT) is based on the administration of appropriate concentrations of allergen extracts. A beneficial response in patients has been associated with high productions of IgG4 and IgG1 antibodies, which compete with IgE for allergen binding. However, allergen-IgG complexes can also bind and cross-link Fcγ-receptors expressed on the surface of antigen-presenting cells (APC). This cross-linking may thereby increase allergen-uptake and eventually the number of HLA-peptide-complexes on the surface of these cells which may drive the resulting T cell response towards Th1.
Method and results: We will study the effects on the T cell level induced by the decrease of the IgE/IgG ratio using the major grass pollen allergen, Phl p 5. This recombinant allergen was expressed and characterized and will be incubated with human Phl p 5-specific monoclonal IgG1, IgG4 and IgE antibodies with identical paratope. In addition, sera from AIT-treated patients containing high levels of Phl p 5-specific IgG will be used. Professional APCs will be isolated from whole blood samples in order to compare surface binding, internalization and processing of IgE-, IgG-bound and unbound Phl p 5. To asses proliferative and cytokine responses, Phl p 5 specific T cell lines and T cell clones will be produced and stimulated with APCs in presence of antibody-loaded and unloaded Phl p 5. Finally, these latter aspects will also be investigated by using naïve T cells. Together, these data will show if AIT-induced IgG antibodies may not only block IgE-mediated effects but also modulate allergen-specific T cell responses during therapy.

Poster-Nr. 073

Abstract-ID: P73 | Poster presentation: 17.11.2016

Human liver- and skin-derived NK cells exhibit antigen-specific memory responses

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Until now, the adaptive immunity was considered an exclusive feature of T and B cells. NK cells are historically defined as cells of the innate immune response. However, mounting evidence suggests that NK cells can develop long lived and highly specific memory to a variety of antigens in mice and in non-human primates. The existence and consequences of antigen-specific NK cell memory in humans still needs to be proven.

We isolated human liver NK cells from individuals vaccinated against hepatitis A / B and characterized them phenotypically and functionally in cytotoxicity assays against matched and mismatched viral antigens. Furthermore, we evaluated the distribution of NK cells in epicutaneous patch test reactions, a model for delayed-type hypersensitivity reactions.

In contrast to the peripheral blood, two distinct NK cell populations were found in the liver based on their expression of CD16 and CD49a. $CD49a^{+}CD16^{-}$ NK cells (54.6% ± 4.2 of total NK cells) performed antigen-specific killing comparable to CD8 T cells. Blood-derived and $CD49a^{-}CD16^{+}$ liver NK cells did not exert antigen-specific cytotoxicity, but recognized MHC-I^{low} target cells. As $CD49a^{+}CD16^{-}$ liver NK cell exhibited a skin-homing phenotype expressing higher levels of CLA, CCR4 and CCR10, we evaluated if these NK cells are capable of migrating into the skin under steady state or inflammatory conditions. We performed immunofluorescence analysis of non-lesional skin and hapten-induced epicutaneous patch test (EPT) lesions. Although absent in healthy human skin, 57,8 ± 5.1 % of total NK cells in EPT reactions were found to belong to the $CD49a^{+}CD16^{low}$ NK cell subset arguing for the skin to be an effector site of memory NK cells.

These results suggest that memory NK cells in humans can be found in the liver and inflamed skin, which might lead to novel strategies of vaccination by harnessing this NK cell subset.

Poster-Nr. 074

Abstract-ID: P74 | Poster presentation: 17.11.2016

The soluble cytoplasmic tail of CD45 (ct-CD45) in human plasma contributes to keep T cells in a quiescent state

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The cytoplasmic tail of CD45 (ct-CD45) is proteolytically cleaved and released upon activation of human phagocytes. It acts on T cells as an inhibitory, cytokine-like factor *in vitro*. Here we show, that ct-CD45 is abundant in human peripheral blood plasma from healthy adults compared to plasma derived from umbilical cord blood and plasma from patients with rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE). Reduced ct-CD45 levels in RA and SLE patients correlated with immuno-suppressive therapy but not with disease scores. Plasma depleted of ct-CD45 enhanced T cell proliferation, while addition of exogenous ct-CD45 protein inhibited proliferation and reduced cytokine production of human T lymphocytes in response to TCR signaling. Inhibition of T cell proliferation by ct-CD45 was overcome by co-stimulation via CD28. T cell activation in the presence of ct-CD45 was associated with an upregulation of the quiescence factors Schlafen family member 12 *(SLFN12)* and Krueppel-like factor 2 *(KLF2)* as well as of the cyclin-dependent kinase (CDK) inhibitor *p27kip1*. In contrast, positive regulators of the cell cycle such as cyclin D2 and D3 as well as *CDK2* and *CDK4* were found to be downregulated in response to ct-CD45. In summary, we demonstrate that ct-CD45 is present in human plasma and sets the threshold of T cell activation.

Poster-Nr. 075

Abstract-ID: P75 | Poster presentation: 17.11.2016

Novel genetic tools to investigate the immune functions of epidermal Langerhans cells on cytotoxic T cell responses in vivo

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The skin hosts a variety of dendritic cells (DCs) which act as professional antigen presenting cells (APC) to control cutaneous immunity. Langerhans cells (LCs) are the only DC subset in healthy epidermis. However, due to the complexity of the skin DC network, their relative contribution to either immune activation or immunosuppression is still not entirely solved. To specifically address *in vivo* functions of LCs, we have generated transgenic mouse models for tamoxifen- (TAM) inducible de-novo expression of antigens in LCs, allowing for LC-restricted antigen presentation to $CD8^+$ T cells. Presentation of non-secreted ovalbumin (GFPOVA) by steady-state LCs resulted in transient activation of endogenous cytotoxic T cells (CTL) in transgenic mice. However, when these mice were challenged with Ova by gene gun (GG) immunization in the contraction phase of the primary CTL response, they did not respond with a recall of CTL memory cells, but instead, with a robust antigen-specific CTL tolerance. We found regulatory T cells (T_{regs}) enriched in the skin of tolerized mice, and depletion of T_{regs} or adoptive transfer into naive recipients revealed that T_{regs} were critically involved in CTL tolerance. By contrast, when OVA was presented by activated LCs, a recallable CTL memory response developed in transgenic mice. Thus, neoantigen presentation by epidermal LCs results in both, a robust CTL tolerance or a CTL memory and this decision-making depends on the activation state of the presenting LCs.

Poster-Nr. 076

Abstract-ID: P76 | Poster presentation: 17.11.2016

Tracking T cells in human skin: turnover and phenotype of tissue-resident memory T cells

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Most of our knowledge about the recirculation and distribution of cutaneous tissue-resident T cells (T_{rm}) is based on observations from murine studies, while the fate of T_{rm} in human skin is still poorly understood. Therefore, we followed patients who underwent myeloablative therapy, total body irradiation and subsequent allogenic hematopoietic stem cell transplantation (HSCT) in order to track the distribution, phenotype and repopulation of cutaneous T cells over time.

Fifty-one biopsies from 12 different patients taken at four time points before and after conditioning regimens/HSCT were analyzed by immunofluorescence. T cells were characterized using T cell homing molecules and markers described to be expressed by cutaneous T_{RM}. All stainings were automatically quantified using TissueQuest software.

In concordance with data of peripheral blood, the overall number of $CD4^+$ and $CD8^+$ T cells in the skin declined upon HSCT as compared to steady state (pre-conditioning). Interestingly, cutaneous $CD69^+$ and $CD103^+$ T cells remained stable throughout all time points, arguing for the presence of a pool of radiation-resistant cutaneous Trm. The majority of dermal T cells was $CCR7^-CD62L^-$, a marker profile expressed by non-recirculating T cells. Small populations of central ($CCR7^+CD62L^+$) and migratory memory T cells ($CCR7^+CD62L^-$) were present in the dermis before conditioning therapy, but disappeared from subsequent skin biopsies early after HSCT (days 0-21) and resurfaced in biopsies taken at days 95–105 after HSCT. Both $CD4^+$ and $CD8^+$ skin-resident memory T cells were primarily detected in perifollicular regions of the upper dermis.

The described results present new insights into resilience of skin-resident memory T cells and contribute to our understanding of T cells recirculating peripheral tissues. In addition, they raise the question of the importance of host-derived T cells in the initiation and propagation of graft-versus-host disease, a common complication of HSCT.

Oral Presentation-Nr. 014

Abstract-ID: 14 | Oral Presentation 18.11.2016, Session 5, Autoimmunity/Immun., 9.00-10.40

Progressive lung disease is a key feature of late onset RAG deficiency

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Objective: A widening spectrum of immunologic and clinical phenotypes are recognized among patients with hypomorphic RAG1/2. Progressive and even fatal pulmonary compromise arising from infectious and non-infectious etiologies is a significant source of morbidity and mortality in these patients.
Methods: We reviewed the immunologic and clinical phenotypes as well as the progression of end organ compromise in a cohort of RAG deficient patients surviving into adulthood.
Results: We identified nine patients with RAG deficiency surviving into late childhood and adulthood with 80% survival rate. The clinical phenotype ranged from combined immunodeficiency with autoimmunity and granulomas to selective antibody deficiency and a wide range of average Rag activity (2-62%). Non-infectious complications included both autoimmune cytopenias (20%) and other autoimmune and inflammation conditions (45%) and combination of both groups (20%). Immunologic phenotype was striking for low $CD4^+$T cell count, decrease naïve CD4 T cell, decreased B cell count and increased exhausted effector memory T cells (TEMRA). All patients had progressive lung disease: transitioning from infectious to inflammatory complications. Deaths were linked with pulmonary fibrosis secondary to hypoxia.
Conclusion: Progressive pulmonary disease is a key component of morbidity and mortality among patients with hypomorphic RAG variants in late childhood and adulthood. Severe non-infections complications are likely underestimated in the absence of monitoring lung disease. Serial lung evaluation starting with pulmonary function testing including DLCO and lung volumes should be considered in these patients and if abnormal considering yearly high resolution chest CT for detection of progressive pulmonary disease. Treatment of choice should be tailored to both infectious and inflammatory components. HSCT for worsening T cell lymphopenia should be considered before onset of rapid or progressive decline in lung function

SESSION 5 | AUTOIMMUNITY IMMUNODEF

Oral Presentation-Nr. 015

Abstract-ID: 15 | Oral Presentation 18.11.2016, Session 5, Autoimmunity/Immun., 9.00-10.40

In vivo generated peripheral regulatory T cells in skin grafting

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For genodermatoses such as the inherited skin blistering disease Epidermolysis bullosa (EB) a main therapeutic approach is *ex vivo* gene therapy, where disease-causing defective genes are replaced in tissue stem cells, skin grafts generated *ex vivo* and transplanted on to the recipient. This therapy introduces the wild-type version of the mutated protein (that is expressed by untreated skin of the recipient) and thus the recipient may mount an immune response against the neo-antigen (i.e. the wild-type protein) expressed in the graft. Therefore a critical aspect for the success of this therapy is the induction and maintenance of tolerance towards the neo-antigen.
We established mouse models in which we transplanted skin that expressed a defined neo-antigen onto otherwise syngeneic recipients. With these we determined that the immune response against an epidermal neo-antigen lead to graft rejection. Furthermore we found a $CD4^+$ T cell infiltrate in the skin graft which might be involved in acute rejection. In previous studies we found that regulatory T cells (Treg) contribute to controlling skin inflammation and we now hypothesized that antigen-specific Treg could attenuate skin graft rejection. We established a protocol to generate and expand peripheral Treg *in vivo* using a modified IL-2/anti-IL-2 complex therapy. With this approach we could increase the proportion of Treg cells in the injected skin sections to approx. 60% of the $CD4^+$ T cell population. Using our preclinical models of neo-antigen skin graft rejection we found that Treg can prolong skin graft survival in combination with small molecule inhibitors such as rapamycin. We will use our mouse models to investigate the function and maintenance of skin-resident Treg, which are both crucial for the long-term success of immuno-therapy to suppress skin-graft rejection.

Oral Presentation-Nr. 016

Abstract-ID: 16 | Oral Presentation 18.11.2016, Session 5, Autoimmunity/Immun., 9.00-10.40

Active mTORC1 signaling induces macrophage granuloma formation and sarcoidosis progression

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Aggregation of hypertrophic macrophages constitutes the basis of all granulomatous diseases such as tuberculosis or sarcoidosis and is decisive for disease pathogenesis. However, intrinsic pathways driving granuloma initiation and maintenance still remain elusive. Here we show that activation of mTORC1 in macrophages by deletion of Tsc2 was sufficient to induce hypertrophy and proliferation resulting in excessive granuloma formation *in vivo*. Remarkably, Tsc2-deficient macrophages formed mTORC1-dependent hypertrophic granulomatous structures *in vitro* and showed constitutive proliferation mediated by the neo-expression of cyclin-dependent kinase 4 (CDK4). Moreover, mTORC1 promoted metabolic reprogramming via CDK4 towards increased glycolysis, while simultaneously inhibiting NF-κB signaling and apoptosis. Inhibition of mTORC1 rapidly induced apoptosis and completely resolved granulomas in myeloid Tsc2-deficient mice. Notably, in human sarcoidosis, mTORC1 activation, macrophage proliferation, and glycolysis were identified as hallmarks that correlated with clinical disease progression. Collectively, TSC2 maintains macrophage quiescence and prevents mTORC1-dependent granulomatous disease with clinical implications for sarcoidosis.

SESSION 5 | AUTOIMMUNITY IMMUNODEF

Poster-Nr. 077

Abstract-ID: P77 | Poster presentation: 18.11.2016

Gene expression of PTPN-22 in Iranian patients with Alopecia Areata

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Objective: In present study, effect of PTPN22 gene expression was investigated in Iranian AA patients and their respective controls.

Background: Alopecia Areata (AA) is an autoimmune multifactorial disease characterized by hair loss especially from the scalp affecting approximately 5.3 million people. The gene encoding the protein tyrosine phosphatase, non-receptor type 22 (PTPN22), which is exclusively expressed in immune cells, has been considered as a risk factor associated with the pathogenesis of AA.

Methods: The study group consisted of 30 patients with AA (13 female and 17 male, mean age 26.3 ±12.5) and 15 (5 female and 10 male, mean age 30 ±5.88) healthy controls. RNA was extracted from blood samples. Thereafter, cDNA was synthesized after RNA isolation. PTPN22 expression levels were measured by Real-time PCR. Furthermore, association of this PTPN22 with some baseline clinical and demographical features was assessed.

Results: PTPN22 expression levels of patients with AA were significantly higher (4.6±3.8) than those in controls (1.14±0.56) (*p value* = 0.01). PTPN22 expression was only associated with sex of the patients with AA.

Conclusion: In present study a significant association was found between PTPN22 gene expression and susceptibility to Alopecia Areata.

Poster-Nr. 078

Abstract-ID: P78 | Poster presentation: 18.11.2016

The impact of HAX1 on lymphocyte function and development

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We identified HAX1 as an IgE-tail interacting protein. Deletion of HAX1 in mice led to a severe reduction in the number of lymphocytes and it became apparent that HAX1 plays a general crucial role for the development and also for the functionality of lymphocytes. Further, we found impaired internalization of the BCR after IgM crosslinking of splenic B cells, which apparently led to decreased BCR-mediated apoptosis. We identified KVKWI(V)F as the putative binding motif for HAX1 within the cytoplasmic domains and showed binding of HAX1 to the cytoplasmic domains of different Ig-subtypes. Because the motif KVKWI(V)F can be found in almost all Ig-subtypes, with the exception of IgA, it is likely that HAX1 is general key player in BCR mediated internalization events and BCR-mediated apoptosis. Additionally, not only B cells but also T cells are affected in numbers and distribution, and it is likely that also their functionality is affected, which has to be proven for the future However, the intrinsic effect of HAX1 on lymphocytes (on BCR-internalization and apoptosis), cannot be the sole explanation for the severe defects observed in Hax1$^{-/-}$ mice *in vivo*. We strongly suggest that a defective developmental environment within the bone marrow and the thymus also contributes to the decreased cell number of lymphocytes in Hax1$^{-/-}$ mice. However, so far the function of HAX1 in lymphocytes is totally unclear and remains to be elucidated for the future. Cellular & Molecular Immunology, 13 April 2015; doi:10.1038/cmi.2015.018.

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SESSION 5 | AUTOIMMUNITY IMMUNODEF

Poster-Nr. 079

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In vitro and in vivo immunomodulatory activity of TGF-β1-mimetic peptides selected by Phage Display technology

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Transforming growth factor-β1 (TGF-β1) belongs to a superfamily of proteins involved in many biological processes, including immune regulation. The regulatory activity of this cytokine is to modulate the differentiation state of a cell and to inhibit inflammatory cytokines, thus playing an important role in the development of immunological disorders. Hence, peptides that mimic the TGF-β1 molecule structure could be highly promising candidates for the modulation of immune responses. In the present study, TGF-β1-like peptides were selected and evaluated for their *in vitro* and *in vivo* capabilities of modulating the immune response under inflammatory conditions.

TGF-β1-mimetic peptides were selected by phage display technology. ELISA, flow cytometry, and cell reporter assays were conducted to evaluate the *in vitro* capacity of the selected peptides to recognize the TGF-β1 receptor and to modulate the immune response. *In silico* analyses were performed to predict peptides localization. *In vivo* evaluation was assessed by intravital microscopy and peritonitis assay.

The synthetic pm26TGF-β1 peptide was able to significantly down-modulate the expression of TNF-α and IL-8, up-regulate IL-10, and induce Treg cell differentiation. Furthermore, this peptide was able to decrease leucocytes rolling and neutrophils migration during an inflammatory process *in vivo*. The synthetic pm1TGF-β1 peptide was able to significantly inhibit the expression of TNF-α and IL-8 and up-regulate IL-10.

In all the performed experiments we found that the pm26TGF-β1 and pm1TGF-β1 peptides were able to modulate the immune response in an inflammatory condition. These findings imply a potential use of the selected TGF-β1-mimetic peptides as immunomodulatory compounds.

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Poster-Nr. 080

Abstract-ID: P80 | Poster presentation: 18.11.2016

E-type Prostanoid Receptor 4 (EP4) antagonist treatment ameliorates nephrotoxic serum nephritis via suppression of tubular Cxcl-1 and -5

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The lipid molecule prostaglandin E2 (PGE2) acts on four different prostaglandin E receptors designated EP1 to EP4. The purpose of the present study was to assess therapeutic effects of targeting the EP4 receptor in nephrotoxic serum nephritis (NTS).
In vivo treatment with two different doses of an EP4 receptor agonist ONO AE1-329 [280 or 1000 µg/kg bw/day], antagonist ONO AE3-208 [10 mg/kg bw/day] or vehicle was performed for 14 days of NTS. Furthermore, murine distal convoluted tubular epithelial (DCT) cells were EP4 receptor stimulated or blocked *in vitro*.
In vivo, the higher dose of the EP4 receptor agonist led to an improved NTS phenotype due to recurrent hypotensive episodes with decreased renal infiltration of immune cells. These effects were dose dependent since treatment with the low-dose agonist resulted in less episodes of hypotension and a phenotype comparable to vehicle controls. Furthermore, EP4 receptor agonist treatment significantly increased tubular proliferation *in vivo* and *in vitro*. EP4 receptor antagonist treatment significantly improved the NTS phenotype without having effects on blood pressure. It significantly decreased Cxcl-1 and -5 expression in the kidney thereby reducing interstitial neutrophil infiltration. EP4 receptor antagonist treated DCT cells also showed decreased Cxcl-1 and -5 transcription *in vitro*. In summary, treatment with high-dosages of the EP4 receptor agonist improved NTS due to recurrent ischemic conditions with decreased renal immune cell infiltration and increased tubular proliferation. In contrast, EP4 receptor antagonism improved the NTS phenotype by limiting Cxcl-1 and -5 production in tubular cells thereby reducing interstitial neutrophil infiltration.

SESSION 5 | AUTOIMMUNITY IMMUNODEF

Poster-Nr. 081

Abstract-ID: P81 | Poster presentation: 18.11.2016

Selective Immunoglobulin M deficiency in patients with hypomorphic mutations in B-cell signaling genes

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Selective Immunoglobulin M deficiency (SIgMD) or Isolated Primary Immunoglobulin M deficiency is a rare primary immunodeficiency, characterized by isolated low to absent levels of serum IgM and no IgM vaccination response. Isotype class switch, levels of other immunoglobulins and IgG vaccination response seems to be normal. Currently only a few cases of isolated IgM deficiency are reported in literature. The etiology of selective IgM deficiency remains unclear. In the present study we identified two patients with defects in the intrinsic B-cell receptor signaling pathway. One patient harbored a missense mutation in the tyrosin kinase BTK, in the other patient a biallelic mutation in BLNK was identified. Both patients presented with profound alterations within the B-cell compartment, marginal zone b cells were severely reduced. Subsequently testing of B-cell function revealed an intrinsic B-cell activation defect, with reduced of phospholipase C gamma activation and reduced calcium influx. In conclusion hypomorphic mutations in B-cell signaling genes lead to a sever reduction of MZ-B cells, impairment of B-cell function and might resulted in the clinical manifestation of selective Immunoglobulin M deficiency.

Poster-Nr. 082

Abstract-ID: P82 | Poster presentation: 18.11.2016

Characterization of anemia in a cohort of rheumatoid arthritis patients

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Purpose: As seen in a previous analysis, the prevalence of anemia in rheumatoid arthritis (RA) patients is decreasing due to increased awareness and early therapeutic intervention (from between 30 and 66% to around 12% in our cohort). (1) In a study cohort which was designed to evaluate iron metabolism in chronic inflammation, we further analyzed the characteristics and underlying pathophysiology of anemia in RA patients. Therefore, we investigated the type of anemia in RA outpatients at our tertiary center and evaluated the iron state in monocytes. We also investigated the impact of disease activity on anemia prevalence and of therapy strategies on persistence of anemia. (2-4)
Methods: We analysed RA outpatients. Laboratory parameters, disease activity (CDAI, DAS28) and drug therapy were collected. 354 patients were classified based on biochemical parameters for inflammation and iron deficiency. Anemia was defined as hemoglobin <120 mg/dl in women, and <130 mg/dl in men. Iron deficiency anemia (IDA) was defined as ferritin < 30 µg/l and C-reactive protein (CRP) <0,5 mg/dl; anemia of chronic disease (ACD) as ferritin >100 µg/l and CRP >0,5 mg/dl. Their combination (IDA/ACD) as ferritin < 100 µg/L and CRP >0,5 mg/dl); Patients with anemia not fulfilling these criteria were defined as "other". We also classified patients with abnormal iron state without anemia as iron deficient (ID) without anemia (ferrtin < 30) or ACD typical without anemia (ferritin>100 plus CRP >0,5 mg/dl). Iron parameters on monocytes were measured by polymerase chain reaction (PCR) in 88 RA patients with or without anemia. Kruskal Wallis test and Mann-Whitney test was performed to compare subgroups, Spearman-Rank-Analysis was applied to analyse correlations.
Results: 268 female (75,9%) and 85 (24,1%) male patients were analysed. (n=353). In male patients 28,4 % were anemic and 12,8% had an atypical iron status. In female patients the prevalence of anemia was 25,5% and 15,1% had an atypical iron state. As expected haemoglobin (Hb) levels were lower in female RA-patients. (p<0,001) than in male patients whereas ferritin and CRP-levels (p=0,005) were significantly higher in men reflecting the higher prevalence of ACD in the male patient group (8,6 vs. 3,9%).
Comparing the PCR-results with the serological iron parameters, in ACD and IDA/ACD there was a significant correlation between transferrin receptor saturation (p=0,01) and ferroportin mRNA expression on monocytes. (p=0,01).
There were no significant differences in treatment regimen on the prevalence or type of anemia except for corticosteroid use (p= 0,006) which was more frequent in the ACD and IDA/ ACD group. Patients using corticosteroids had in nearly 50% an abnormal iron status, patients without corticosteroids only in 25%.
Conclusion: In this study cohort with was designed to evaluate the causes of anemia and dysregulation of iron metabolism in RA-patients we could show a higher prevalence of anemia then in a previous analysis. This may be due to a selection bias; patients with anemia were more often included to the database. The characterization of anemia in RA patients showed a different distribution among pathophysiological types (IDA 27,0%, ACD 19,1%, IDA/ACD 29,2%, other 24,7%) (5) than previously described (4). The correlation between higher disease, anemia and the need of corticosteroids were shown. The association between iron parameters on monocytes, clinical and laboratory findings and treatment strategies in RA-patients has to be further investigated.

SESSION 5 | AUTOIMMUNITY IMMUNODEF

Poster-Nr. 083

Abstract-ID: P83 | Poster presentation: 18.11.2016

Elevated sodium leads to an increase in intracellular and surface expression of HSP60 on HUVECs

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Atherosclerosis is a multifactorial, immunologically driven chronic inflammatory disease. We have previously shown that T cells recognizing endogenous heat shock protein 60 (HSP60) initiate the disease, due to an autoimmune reaction1. Recent evidence in the field shows that sodium is able to induce the expression of adhesion molecules vascular adhesion molecule 1 (VCAM-1) and E-selectin on human vein endothelial cells (HUVECs), two molecules that we have previously shown to also be upregulated due to HSP60.
Here we report that increasing concentrations of sodium leads to the correlative increase in intracellular, and more importantly, surface expression of HSP60 protein on HUVECs. In addition, we show that the increase in sodium also leads to a lower cell number in cell culture and an increase in apoptosis, as shown by annexin V staining.
We therefore conclude that sodium is an additional risk factor for atherosclerosis as it leads to an upregulation of HSP60 on the surface, making it a target for auto-reactive T cells, and hence a risk factor for the "Autoimmune Concept of Atherosclerosis".

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Poster-Nr. 084

Abstract-ID: P84 | Poster presentation: 18.11.2016

Junctional epidermolysis bullosa in a newborn results from homozygosity for a novel mutation of the ITGA6 gene due to maternal uniparental disomy

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Background: Epidermolysis bullosa is a genetical disorder of varying severity that affects the skin and mucosal membranes, causing mechanical fragility and blistering. Depending on the ultrastructural level of blister formation, epidermolysis bullosa is classified into four major forms: simplex, junctional, Kindler syndrome and dystrophic.

Aims: We aimed to classify and investigate the genetic cause in a female newborn with progressive, fatal epidermolysis bullosa. In the prenatal analysis of amniotic fluid cells low-level fetal mosaic trisomy 2 was detected.

Methods: Immunofluorescence mapping, transmission electron microscopy and genetic analysis were used for diagnostic evaluation.

Results: Immunofluorescence mapping disclosed junctional split and absence of immunoreactivity for integrin α6. Sequence analysis of the ITGA6 gene on chromosome 2 revealed a homozygous mutation, leading to a premature termination codon. Interestingly, this mutation was found in a heterozygous state only in the mother, but not in the father. Segregation analysis with chromosome 2 specific STR markers exhibit exclusive maternal inheritance of chromosome 2, thus demonstrating evidence for uniparenatal disomy (UPD2).

Conclusion: Full trisomy 2 as well as high-level mosaicism would lead to spontaneous miscarriages or severe fetal malformations. Due to a very rare event of trisomy rescue a uniparental disomy can lead to the manifestation of a recessive condition in case of mutation transmission by only one parent. This case demonstrates uniparental disomy 2 as cause for a severe form of fatal junctional epidermolysis bullosa.

SESSION 5 | AUTOIMMUNITY IMMUNODEF

Poster-Nr. 085

Abstract-ID: P85 | Poster presentation: 18.11.2016

JAK1 exerts an immune inhibitory function in dendritic cells

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The JAK-STAT signaling pathway, as a mediator for cytokine-induced signal transduction, plays an important role in the control of immune responses. Therefore JAK-STAT dysregulation is often associated with various immune disorders. In this context JAK inhibitors have been approved as treatment strategies for myeloproliferative as well as various autoimmune diseases, given the role of JAKs in promoting inflammatory responses. However, we have observed a counteracting immune inhibitory function of JAK1 in dendritic cells (DCs).
Murine DCs lacking JAK1 expression exhibit enhanced T cell activation and proliferation capacity *in vitro*. Correspondingly *in vivo*, faster onset and stronger progression of experimental autoimmune encephalomyelitis (EAE) is observable in CD11cCre-JAK1fl/fl mice. These symptoms correlate to a skewed cytokine profile resulting in increased pro-inflammatory cytokine production. These observations are furthermore linked to a significantly decreased programmed death-ligand 1 (PD-L1) expression on JAK1-deficient DCs. Along these lines, total STAT1 and IRF-1 expression are reduced in DCs lacking JAK1 in both healthy and EAE-bearing mice. Furthermore PD-L1 decline goes along with an improved antigen-specific immune response indicated by an increased MHC-II+ PD-L1- and decreased MHC-II+ PD-L1+ DC population.
Our data indicate a prominent regulatory function of JAK1 in DCs in the context of autoimmune disease development. These findings should be considered when testing JAK1-specific inhibitors for the treatment of autoimmune diseases, such as rheumatoid arthritis, with regard to potential side effects.

Poster-Nr. 086

Abstract-ID: P86 | Poster presentation: 18.11.2016

Long-term outcome after combined kidney-pancreas recipients with minimized immunosuppression: a single center report

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Objective: We retrospectively analyzed long-term pancreatic and renal graft function, patient and graft survival and major complications after combined pancreatic-kidney transplantation (SPK) and Tacrolimus (Tac) or Cyclosporine A (CyA) monotherapy.

Patients and methods: Between 1979 – 2015 performed at our center, 7 out of 489 SPKs patients were converted to Tac (n=6) or CyA (n=1) monotherapy in response to hematologic side effects (n=6) or biopsy-proven BK-nephropathy (n=1). Prior to monotherapy, patients were treated with Tac plus MMF (n=5) or Tac plus Rapamycine (n = 1, study) or Tac-monotherapy (study, converted to CyA due to idiopathic thrombopenia), respectively, for a period of 62.1 (30-144) months (mean).

Results: At 133 (48-205) months all patients are alive with a stable pancreatic and renal function (mean creatinine 1.7 mg/dL +/-0.7 SD, blood glucose 97.1 mg/dL+/-12.2 SD, HbA1c 5.3%+/-0.3 SD, C-peptide 4.2 ng/L+/-2.1 SD, Tac-/CyA -level 5.6 +/- 1.8 SD / 107 ng/mL).All major complications (urosepsis, incisional hernia, portal vein thrombosis, bleeding telangiectasia of graft duodenum, idiopathic portal hypertension, mild acute rejection, idiopathic thrombopenia, n=1 each) were controllable. In one patient a biopsy proven acute vascular rejection (at month 33 within Tac-monotherapy, 155 month posttransplant, C4d negative) was treated by adding MMF (discontinued after 6 weeks due to leucopenia and diarrhea) plus prednisolone (discontinued after 5 weeks for severe skin dystrophy). No antibody mediated rejection was observed. The most recent DSA screening was negative in 2 patients and is missing for logistic reasons in 5.

Conclusion: Late after SPK, Tac-/CyA monotherapy seems to be feasible in patients suffering from side effects of non-CNI immunosuppressants. Cautious dose adjustments, careful trough level monitoring and particular attention to strict adherence to the drug treatment may be particularly relevant in this context.

SESSION 5 | AUTOIMMUNITY IMMUNODEF

Poster-Nr. 087

Abstract-ID: P87 | Poster presentation: 18.11.2016

PD-1 deficiency in CD4$^+$ T cell activation and differentiation

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Immune responses are tightly controlled to maintain immune homeostasis. This is carried out by developing a balance between responding effector T cells (Teff) and regulatory T cells (Treg). Foxp3$^+$ Treg cells can suppress Teff cells and thus maintain tolerance. They can be divided into thymic and peripheral populations. Thymic Treg cells (tTreg) develop in the thymus and have a T cell receptor (TCR) repertoire biased towards self-antigens, while peripheral Treg cells (pTreg) can be generated in response to tissue as well as foreign/neo-antigens.

The differentiation of pTreg cells is influenced by several factors, such as the dose of antigen and the expressed co-stimulatory and co-inhibitory molecules by the T cells. One of the most important regulatory molecules found on T cells is programmed cell death protein 1 (PD-1 or CD279). We **hypothesise** that this molecule plays an important role by sustaining an appropriate balance between T_{eff} and T_{reg} cells, through the regulation of TCR signaling strength and effector cytokine production.

To study the role of PD-1 in pTreg generation *in vivo* we use mouse models of T cell mediated skin autoimmunity. Expression of ovalbumin (Ova) in the skin of transgenic mice is driven by a tetracycline-inducible system and we can follow the differentiation of adoptively transferred naïve Ova-specific wild type or PD-1-deficient CD4$^+$ T cells (DO11.10) *in vivo* and analyse the clinical autoimmune disease caused by the T cell response.

We found that PD-1 regulates the severity and kinetics of clinical disease, and the T cell numbers. Interestingly, Treg percentages are unaffected by PD-1-deficiency. We are now studying role of PD-1 in regulating the suppressive function and *in vivo* stability of pTreg in the skin.

Understanding and manipulating the mechanisms, which control the choice between immune activation and tolerance, might help us to achieve a more effective therapy in cancer, allergy and autoimmune disorders.

Poster-Nr. 088

Abstract-ID: P88 | Poster presentation: 18.11.2016

IgG-isoagglutinins as examined by surface plasmon resonance analysis are decreased in patients with polysaccharide antibody deficiency

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A clinically relevant selective antibody failure can lead to significant susceptibility to infections even in patients with normal levels of serum immunoglobulins. Diagnosis of a selective defect in IgG antibody production against bacterial polysaccharides (SAD) relies upon the demonstration of defective IgG antibody production following immunization with unconjugated 23-valent pneumococcal polysaccharide vaccine (PPV) in the presence of intact IgG antibody formation against T-dependent protein antigens. A recent study (Schaballie H et al, 2016) indicates that previous immunization with pneumococcal conjugate vaccine, which has become part of the standard childhood vaccination program, might affect antibody responses to PPV, thus possibly impeding the diagnostic assessment for SAD.

Anti-ABO antibodies are thought to be natural antibodies produced early in life against environmental antigens that have carbohydrate epitopes similar to the blood group ABO antigens. In the present study we used surface plasmon resonance technology (SPR) to study the blood group anti-A/B antibody titer in an isotype-specific assay (Fischer MB et al, Front. Immunol. 2015; 6:211) in twelve SAD patients with a known defect in IgG-antibody response following PPV vaccination (age, years, median [range], 32 [4-65]). The results show that anti-A/B IgG-antibodies (resonance units, median [IQR]) were significantly ($p<0.00001$) decreased in SAD patients (20 [11 - 48]) as compared to 35 healthy controls (284 [155-534]), while conventional erythrocyte agglutination by Diamed-ID Micro Typing showed no significant differences between the two groups. These results indicate that examination of anti-A/B IgG antibodies by SPR is a useful method for the diagnosis of impaired IgG antibody production against polysaccharide antigens in patients with SAD. Furthermore, SAD includes a defect in the formation of natural IgG antibodies against carbohydrate antigens such as blood group ABO antigens.

SESSION 5 | AUTOIMMUNITY IMMUNODEF

Poster-Nr. 089

Abstract-ID: P89 | Poster presentation: 18.11.2016

Chloroquine directly suppresses activation of human CD4⁺ T-cells via modulation of AP-1 signaling

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Introduction: The anti-malarial drug chloroquine (CQ) is widely used as anti-inflammatory therapeutic for rheumatic diseases. Although its mode of action on the innate immune system is well described, there is still insufficient knowledge about direct effects on cells of the adaptive immune system. Since CD4⁺ T-cells are critically involved into the pathophysiology of rheumatic diseases, we aimed to assess the influence of CQ on these cells.

Results: CQ directly suppressed proliferation, metabolic activity and cytokine secretion of T-cells in a dose-dependent manner. In contrast, no effect on up-regulation of the T-cell activation markers CD25, CD69 and CD71 was observed. CQ inhibited activation of all T helper cell subsets, although IL-4 and IL-13 secretion by Th2 cells were less influenced compared to other Th-specific cytokines such as IL-5, IL-17 and IFN-γ. Up to 10 µM, CQ did not reduce cell viability, suggesting specific suppressive effects on T-cells. These properties of CQ and HCQ were fully reversible in re-stimulation experiments. In autophagy assays, a distinct accumulation of autophagosomes was observed, which was reversible by addition of bafilomycin A. Analyses of intracellular signaling showed that CQ and HCQ specifically inhibited activation of AP-1 by reducing phosphorylation of c-JUN, suggesting that CQ acts as an inhibitor for the kinase activity of JNK.

Conclusion: In summary, we describe selective and reversible immunomodulatory effects of CQ on human CD4⁺ T-cells. These findings provide new insights into the biological actions of autophagy and AP-1 signaling in T-cells and may help to expand the therapeutic spectrum of CQ.

Poster-Nr. 090

Abstract-ID: P90 | Poster presentation: 18.11.2016

Molecular insights into chimeric antigen receptors (CARs)

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Chimeric antigen receptor (CAR) modified T-cells proved to be highly effective in subduing refractory B-cell malignancies. Similar success was not yet observed for CARs in treating common epithelial cancers. In order to extend this therapy beyond B-cell malignancies, detailed molecular understanding of CARs in regards to their antigen sensitivity, signaling and propensity to form an immune synapse is of paramount importance. In this study we employed CARs targeting orphan-tyrosine-kinase receptor ROR1, predominantly expressed on malignant lymphatic and epithelial cells. Functionality of CAR is compared to T-cell receptor (TCR) in HLA-A0201.CMV(pp65)-specific $CD8^+$ T-cells transduced with ROR1 CAR s by means of total internal reflection fluorescence (TIRF) and fluorescence microscopy. We show that antigen threshold for activation via TCR is 1000 fold lower than CAR (i.e. 3 molecules per cell for TCR mediated activation vs 2000 for CAR mediated activation). Activation via CAR leads to an unusual immune synapse where central and peripheral supramolecular activation clusters (cSMAC) & (pSMAC) are not distinctly segregated. Surprisingly, at limiting antigen densities absence of adhesion molecule ICAM1 significantly affects the activation of CAR-T cells. These results indicate that further refinement in CAR design is required to extend their scope beyond B-cell malignancies.

SESSION 5 | AUTOIMMUNITY IMMUNODEF

Poster-Nr. 091

Abstract-ID: P91 | Poster presentation: 18.11.2016

Dissecting the immunologic properties of Amb a 1

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Background: The major ragweed pollen allergen Amb a 1 is assumed to be a pectate lyase by sequence homology. These enzymes belong to the polysaccharide lyase family 1 (PL1). Members of this family show a unique core structure termed parallel beta helix. This structure consists of a right handed helix formed by the protein backbone and stabilized by 3 parallel beta sheets stretching along the helix. For Amb a 1, it has been demonstrated that the majority of clinically important T cell epitopes is located within the beta helix, whereas IgE epitopes cluster at the N-terminal domain of the allergen. Rationale: Therefore, we sought to truncate the N- and C-terminus of Amb a 1, leaving the central beta helix intact. Using this strategy we wanted to generate a T cell-reactive hypoallergen applicable for ragweed pollen immunotherapy.

Methods: Amb a 1 TR was expressed as his-tagged fusion protein in *E. coli* and purified by affinity chromatography. In a first set of experiments, we assessed the IgE-binding properties of Amb a 1 TR by ELISA and immunoblot.

Results: We were able to purify Amb a 1 TR from the soluble fraction of *E. coli*. In Elisa and western blot experiments with sera from ragweed allergic patients the antibody-binding properties of Amb a 1 TR were reduced compared to natural Amb a 1. These first results encourage further characterization of Amb a 1 TR.

Conclusion: By dissecting the immunologic properties of Amb a 1 we aimed to find a new strategy for the design of an immune-reactive low-IgE binding variant of the major ragweed allergen. This would facilitate the development of protein-based vaccines for ragweed pollen immunotherapy.

The work was supported by the Christian Doppler Research Association, Biomay AG, and the Sparkling Science Project SPA05-193.

Poster-Nr. 092

Abstract-ID: P92 | Poster presentation: 18.11.2016

DNA in shape – aptamers against the anti-CD20 antibody Rituximab

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Conformational changes of biopharmaceuticals can impact safety, efficacy and stability of a product. An innovative tool termed aptamers, which are single-stranded DNA or RNA oligonucleotides, can help to detect subtle changes in the tertiary structure of proteins. As they present defined secondary structures with high affinity to a target they can be used as surrogate antibodies. This study aims to generate a panel of aptamers reactive to the therapeutic anti-CD20 antibody Rituximab.
The Magnetic bead-based Systematic Evolution of Ligands by Exponential enrichment (Mag-SELEX) method was used to select Rituximab-specific single-stranded DNA aptamers. Purified Rituximab was immobilized on Protein A magnetic beads and a random DNA library with 40 bases length was used. After folding, the oligonucleotides were bound to Rituximab, eluted and enriched using PCR amplification. Upon removal of the reverse strand, forward strands were applied for subsequent cycles. Selected aptamers were tested with Rituximab in an aptamer-based enzymatic assay (ELASA). The structure of the aptamers was investigated by circular dichroism spectroscopy.
In total, eight selection rounds were performed enriching DNA sequences with highest affinity to Rituximab. After the final cycle, resulting PCR products were cloned into pGEM-T vector and 54 constructs were sequenced. Seven sequences found repeatedly in different clones were selected and obtained as 5'-biotinylated oligonucleotides. Those aptamers were used for detection of Rituximab analogous to the ELISA format showing 4/7 aptamers with very high affinity while 3 presented with medium reactivity to immobilized Rituximab. Structural analysis of the different aptamers indicated a quadruplex structure.
For the first time, a panel of aptamers recognizing the monoclonal antibody Rituximab was generated. These aptamers will enable monitoring conformational coherence during production processes and detection of folding variations during storage.

SESSION 5 | AUTOIMMUNITY IMMUNODEF

Poster-Nr. 093

Abstract-ID: P93 | Poster presentation: 18.11.2016

LCMV-GP Pseudotyped Oncolytic Vesicular Stomatitis Virus for the Treatment of Ovarian Cancer

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Treatment options for advanced ovarian cancer remain limited. Metastasis commonly occurs in the peritoneal cavity. First line therapy usually fails to cure patients and eventually tumours relapse. One very promising new treatment approach is the use of oncolytic viruses (OV) that preferentially replicate in and kill tumour cells. Our group previously reported that oncolytic Vesicular Stomatitis Virus (VSV) pseudotyped with the LCMV glycoprotein (VSV-GP) is a promising, highly efficient and safe oncolytic virus. Here, we propose the use of the oncolytic VSV-GP for the treatment of ovarian cancer. Oncolytic activity was confirmed *in vitro* on a variety of ovarian cancer cell lines. However, analysis of the innate immune response of ovarian cancer cells to VSV-GP revealed IFN type I production and induction of an antiviral state of the cells as a potential mechanism leading to shortcomings in virotherapeutic treatment. *in vivo*, VSV-GP was tested in a subcutaneous ovarian cancer xenograft mouse model using the A2780 cell line. Treatment led to initial tumour remission. In an orthotopic xenograft mouse model, intraperitoneal injection of the virus led to significantly prolonged survival compared to untreated animals. In addition, combination therapy of VSV-GP with the JAK1/2-inhibitor ruxolitinib was successfully tested in both models and found to enhance the oncolytic effect. The drug inhibited the signalling pathway induced by type I IFN and could thus be used to inhibit the antiviral innate immune response and enhance intratumoral viral replication. Importantly, despite inhibiting the antiviral response, no toxicity was observed in mice receiving up to 109 pfus (plaque forming units) VSV-GP via intraperitoneal application.
In conclusion, VSV-GP was tested as a potent oncolytic virus to treat ovarian cancer. Restriction of viral replication due to the innate immune response could be overcome by the combination treatment of VSV-GP with the Jak-1/2 inhibitor ruxolitinib.

Oral Presentation-Nr. 017

Abstract-ID: 17 | Oral Presentation 18.11.2016, Session 6, Clin. Allergology, 11.00-12.00

Human dendritic cells induce divergent immune responses according to the allergenic potential of two homologous lipocalins

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Why and when the immune system initiates TH2 mediated allergic responses is still insufficiently characterized. One of the key players during the induction of allergic airway inflammation providing stimuli for TH2 cell differentiation are dendritic cells (DCs). We investigated the interaction of human monocyte derived DCs with lipocalins, a protein family comprising the majority of mammal derived respiratory allergens. We employed allergens possessing a high sequence homology with endogenous human lipocalins. Specifically we used the dog allergen Can f 1 and its human non-allergenic homologue Lipocalin-1 (Lcn-1) and the cat allergen Fel d 4 and its putative human homologue the major urinary protein (MUP).

Allergenic Can f 1 and Fel d 4 persistently induced less of the Th1 skewing maturation marker expression, tryptophan breakdown and IL-12 production in DCs in comparison to the endogenous non-allergenic Lcn-1 or MUP. As a consequence, naïve T cells stimulated by DCs treated with Can f 1 or Fel d 4 produced more of the Th2 signature cytokine IL-13 and less of the Th1 signature cytokine IFN-γ. Reactome pathway analyses of the microarray data revealed differences in the intracellular sorting and antigen presentation pathways of allergen and non-allergen treated DCs. This led us to focus our efforts on the endosomal processing pathways of the lipocalins. Various tracking experiments with markers for phagosomal/endosomal maturation or acidification and allergen specific antibodies, produced form allergic donors via the single B-cell cloning approach, will be conducted.

Our data show that human monocyte derived dendritic cells orchestrate immune responses in responding differentially to four highly homologous lipocalins according to their allergenic potential. The crosstalk of dendritic cells with lipocalins alone has the potential to direct the type of induced immune response and the endosomal processing system seems to play a crucial role during this decision process.

SESSION 6 | CLINICAL ALLERGOLOGY

Oral Presentation-Nr. 018

Abstract-ID: 18 | Oral Presentation 18.11.2016, Session 6, Clin. Allergology, 11.00-12.00

Development and characterization of a ragweed allergy vaccine based on the peptide carrier principle

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Background: More than 36 million individuals worldwide suffer from ragweed allergy and the number of patients is increasing rapidly. Beside medications combating the symptoms, only allergen specific immunotherapies (SIT) based on allergen extracts are available. Here we present for the first time the preclinical development of the ragweed allergy vaccine BM34 based on peptide-carrier principle. This concept has been previously introduced and clinically evaluated for its application in allergy therapy, lowering the side effects and therefore the number of required immunizations.

Methods: We selected peptides derived from the sequence of Amb a 1 and characterized them upon their IgE reactivity (IgE ELISA, basophil activation assays), immunogenicity *in vivo* (rabbit immunization and IgG ELISA) and potential to induce blocking IgG antibodies (inhibition ELISA with rabbit anti-peptide sera). Based on the results of these assays we designed eight Amb a 1 fusion proteins by fusing the peptides to a PreS carrier protein. Subsequently we expressed and comprehensively characterized them.

Results: Preclinical experiments demonstrated that fusion proteins lack IgE reactivity. Immunization experiments with rabbits demonstrated induction of high levels of Amb a 1 specific IgG antibodies with a great potential to block patients IgE antibodies. The most convincing vaccine candidate Amb a 1 K4 inhibited up to 94 % of patient's IgE binding. The structurally optimized cysteine variants Amb a 1 K4.2 and Amb a 1 K4.3 showed even more blocking potential.

Discussion: In summary we developed three highly promising recombinant hypoallergenic vaccine candidates consisting of recombinant PreS-fused ragweed allergen peptides for safe and efficient immunotherapy of ambrosia allergy. The promising outcome encourages us to go ahead with preclinical studies in order to pave the way towards a better and more attractive therapy for ragweed allergy.

Oral Presentation-Nr. 019

Abstract-ID: 19 | Oral Presentation 18.11.2016, Session 6, Clin. Allergology, 11.00-12.00

Modulatory capacities and possible implications of soluble Fc-epsilon-RI in the IgE-mediated immune response

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IgE-mediated allergies are potentially life threatening immunologic reactions towards otherwise harmless environmental antigens where serum IgE is the common marker for diagnosis. However, allergen-specific IgE levels not always correlate with allergic reactions. The soluble form of the high affinity receptor for IgE (sFcepsilonRI) present in serum may interfere with IgE levels. We aimed to study the presence of sFcepsilonRI among the pediatric population and its possible implications. A diverse set of individuals from different regions and clinical manifestations of allergies were analyzed for sFcepsilonRI levels by a recently developed ELISA model. We could find sFcepsilonRI ubiquitously present among all individuals irrespective of their sensitizations, clinical symptoms or region. We also observed a highly positive correlation between total and IgE-complex sFcepsilonRI measured in serum. Surprisingly, this correlation was lower in the group of peanut sensitized individuals from Ghana. Next, we studied the modulatory capacities of sFcepsilonRI by using the MelJuSo cell line stably transfected with FcepsilonRI. After cross-linking of the receptor with chimeric IgE (cIgE) and its specific ligand (NP-OVAL), we detected dose-dependent increasing levels of sFcepsilonRI in the supernatant. To measure its blocking capacity, FAB-like (Facilitated Antigen Binding) tests were performed with recombinant and purified FcepsilonRI, and the effect was compared with Omalizumab, the humanized monoclonal antibody against IgE. We could attribute a blocking capacity of sFcepsilonRI by interference of cIgE-FcepsilonRI binding comparable to Omalizumab. Therefore, sFcepsilonRI maintains the high IgE-binding affinity in serum and inhibits IgE-FcepsilonRI binding *in vitro*, placing this molecule as an important player in the complex signalling pathway of the allergic response.

Supported by the FWF Doctoral Program W 1248-B30 MCCA

Poster-Nr. 094

Abstract-ID: P94 | Poster presentation: 18.11.2016

Effect of fatty acids-binding on the IgE reactivity of different nsLTPs from fruits, nuts and seeds

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Aims: nsLTPs are important food allergens sharing a conserved 3D structure. However, different nsLTPs, even highly homologous, possess different allergenic potentials. Our major goal is to compare the immunoreactivity of different nsLTPs from fruits (Pru p 3, peach, and Mal d 3, apple); nuts (Cor a 8, hazelnut, and Jug r 3, walnut) and seeds (Hel a 3, sunflower) and to evaluate if the interaction with specific food matrix components, such as fatty acids, may affect their IgE reactivity.

Methods: All the nsLTPs were extracted from their natural sources, and most of them were also produced as recombinant proteins in *Pichia pastoris*. After purification by IEC chromatography, proteins were analyzed by SDS-PAGE, immunoblotting with anti-nsLTP antiserum, MALDI-TOF MS and N-terminal sequencing. The binding of nsLTPs to 3 different ligands (oleic, stearic and lauric acids) was analyzed by the ANS displacement assay. IgE reactivity of nsLTPs, alone or in complex with fatty acids, was tested by ELISA assay using allergic patients' sera.

Results: MS analysis and N-terminal sequencing confirmed the identity of all the purified proteins. All nsLTPs were recognized by anti-nsLTP antiserum. Different proteins showed different lipid-binding capacity, but all of them had the highest affinity for oleic acid (unsaturated) and the lowest for stearic acid (saturated). Results from IgE ELISA showed a higher IgE binding for fruit nsLTPs (Pru p 3 and Mal d 3) with respect to other nsLTPs. In general, the interaction with oleic acid seems to increase the IgE reactivity for all the proteins tested.

Conclusions: Our data suggest that nsLTPs preferentially bind the unsaturated fatty acid (i.e. oleic acid) and this interaction increases the IgE binding, thus supporting the hypothesis of a role of food matrix in modulating specific IgE responses to food allergens.

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Poster-Nr. 095

Abstract-ID: P95 | Poster presentation: 18.11.2016

The interplay between tree pollen allergens and their source

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Background: Allergenicity is defined as the capacity of a certain antigen to induce allergic sensitization. However, it is still unclear which properties discriminate between allergenic and non-allergenic proteins or strong and weak allergic sensitizers. It has been shown that pollens act as carrier for allergens, as well as bioactive allergy-promoting elements; however, little is known about the interactions between these substances and how this can influence the allergy-specific polarization of the immune system.

Aim: The aim of this study is to investigate interactions between tree pollen allergens and compounds in their source to determine the influence on the subsequent immune responses to these allergens.
Methods: Extracts of allergenic and non-/low-allergenic tree pollen were produced with several different extraction protocols and consequently differentially fractionated using various combinations of polar and non-polar solvents. Fraction contents were analyzed by SDS-PAGE and thin-layer chromatography. LPS content of fractions was determined with HEK-Blue™ cell-line assays. Preliminary immune-polarizing potential of pollen fractions was assessed with BMDC activation experiments. Results: A broad panel of polar and non-polar, protein- and non-protein-containing tree pollen extract fractions was produced. Fractions showed distinct differences in protein and lipid contents. BMDC activation was higher in tree pollen extracts than pure proteins and also differences in activation were observed between different fractions.

Conclusion and Outlook: The increased dendritic cell activating capacity of the pollen extracts combined with the already known low immunogenicity of pure allergenic proteins indicates an apparent importance of the allergen source in the course of the immune response. Further *in vitro* as well as *in vivo* experiments analysing the immunological properties of specific compounds in tree pollen extracts and their interaction with the allergens contained in them are required to create a clearer image.

This research was supported by the University of Salzburg and the Austrian Science Funds FWF project: P27589.

SESSION 6 | CLINICAL ALLERGOLOGY

Poster-Nr. 096

Abstract-ID: P96 | Poster presentation: 18.11.2016

Expression, purification and immunological evaluation of candidates for a ragweed prototype vaccine

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Among allergic patients, more than 33 million Europeans suffer from ragweed pollen allergy. Predictions demonstrate that this number of patients will increase up to 77 million by 2050 due to changing climate condition and the supporting effect of air pollution on the high allergenicity. The only allergen-specific and disease-modifying form of therapy is allergen-specific immunotherapy (SIT). Here, we present the comprehensive characterization of two fusion proteins, named Amb a 1 K4.2 and Amb a 1 K4.3 for specific immunotherapy (SIT) of ragweed allergy. They are based on a "Peptide/Carrier" concept and contain peptides of the major ragweed allergen Amb a 1 which were immunologically evaluated to ensure their ability to induce allergen-specific IgG antibodies, inhibit IgE and additionally do not show allergenicity. In order to avoid any problems in later GMP production, amino acid cysteine residues present in the original sequence of Amb a 1.0305 were deleted or exchanged with serines. After successful expression in *E. coli* and purification via Ni-NTA chromatography the fusion proteins K4.2 and K4.3 were tested in terms of hypoallergenicity, immunogenicity, stability and storage conditions. In addition, formulation experiments were performed followed by an immunization study in New Zealand rabbits. The results of IgE ELISA experiments, determination of IgG titers after immunization, IgE inhibition experiments and basophile activation tests (BAT assays) confirmed that both, K4.2 and K4.3 fusion proteins are not IgE reactive (hypoallergenic) but highly immunogenic, and that IgG induced upon immunization with the fusion proteins are able to block the binding of IgE to wild-type Amb a 1.
These promising results encourage us to move forward in the pre-clinical development of our prototype vaccine, aiming at a safe and efficacious procedure for SIT of ragweed allergy with a reduced number of annual injections.

Poster-Nr. 097

Abstract-ID: P97 | Poster presentation: 18.11.2016

Prophylactic vaccination with recombinant hypoallergenic Bet v 1-derivatives induces blocking antibody responses in non-allergic individuals

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Allergen-specific immunotherapy (AIT) is the only allergen-specific and disease-modifying treatment for allergy with long-lasting effects. Several new forms of AIT are currently evaluated for therapy of allergic patients but so far no prophylactic allergen-specific vaccines have been developed. Here we have investigated the immunological effects induced by vaccination of healthy adults with a mix of two hypoallergenic rBet v 1 fragments in order to explore the feasibility of prophylactic vaccination against birch pollen allergy. The non-allergic subjects received three subcutaneous injections at monthly intervals and one subcutaneous booster injection after one year. Blood samples were collected during the entire study period and used to compare allergen-specific IgG, IgG subclasses as well as IgM and IgA antibody levels before, after the treatment and after the booster injection. Vaccination with recombinant hypoallergenic Bet v 1 F1+F2 induced allergen-specific blocking IgG, mainly IgG_1 and IgG_4 antibodies only in the active group, which inhibited birch pollen allergic patients IgE reactivity to Bet v 1. Interestingly, the blocking activity remained high even one year after the last injection. No differences were observed regarding IgM or IgA antibodies. No relevant alterations in allergen-specific antibody responses were observed for the placebo group. Our results demonstrate that vaccination with hypoallergenic Bet v 1 fragments induces normal Bet v 1-specific IgG responses in healthy individuals. Since blocking antibodies are thought to neutralize allergens and prevent their interaction with IgE bound to mast cells and basophils, preventive treatment should induce blocking IgG antibodies without inducing IgE sensitization. Our findings thus suggest the use of hypoallergenic allergen derivatives as a feasible approach for prophylactic vaccination against allergies.

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Poster-Nr. 098

Abstract-ID: P98 | Poster presentation: 18.11.2016

Folded and highly IgE-reactive major *Parietaria* pollen allergen Par j 2 obtained by insect cell expression

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Par j 2 the major *Parietaria* allergen has a molecular weight of 11.3 kDa, belongs to the family of lipid-transfer proteins and is recognized by more than 80% of *Parietaria*-allergic patients.
Aim of this study was to express and purify a correctly folded recombinant Par j 2 molecule which mimics the structural and immunological features of the natural allergen. Recombinant Par j 2 was expressed in baculovirus-infected insect cells as well as in *Escherichia coli* and purified by affinity chromatography.

Recombinant soluble Par j 2 expressed in baculovirus-infected insect cells was characterized by SDS-PAGE under reducing and non-reducing conditions. It migrated as single band of approximately 14 kDa and the molecular mass determined by mass spectrometry MALDI MS matched the mass calculated according to the amino acid sequences of the protein.

Size exclusion chromatography showed that both, the insect cells and *E. coli* expressed recombinant proteins, occurred mainly as monomeric forms and also contained an oligomeric form of approximately 60 kDa. The analysis by circular dichroism (CD) showed that insect cell-expressed rPar j 2 assumed mainly α-helical structure whereas bacterially-expressed rPar j 2 contained mainly unordered species. When IgE reactivity of the recombinant Par j 2 proteins were compared with natural Par j 2 by ELISA using sera from *Parietaria* allergic patients from Mediterranean region and Austria, we found that insect cell-expressed Par j 2 showed higher IgE reactivity than *E. coli*-expressed Par j 2 and equally well as natural Par j 2. Our results thus show that the eukaryotic expression of Par j 2 in insect cells yielded a folded recombinant protein with superior IgE reactivity over *E. coli* expressed Par j 2. The insect cell-expressed Par j 2 can now be used to study the three-dimensional structure of the allergen and for IgE-based diagnostic testing for identifying *Parietaria* allergic patients.

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Poster-Nr. 099

Abstract-ID: P99 | Poster presentation: 18.11.2016

Lactobacilus delbrueckii subsp. *Bulgaricus* modulates the secretion of Th1/ Th2 and Treg cell-related cytokines by PBMCs from patients with atopic dermatitis

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Atopic dermatitis (AD) is an inflammatory skin disease which may be due to the imbalance between Th1-, Th2 and Treg cell-related immune responses. Evidences suggest that appropriate stimulation with probiotics may correct the skewed immune response in children with AD. The aim was to determine the effects of the yogurt culture *lactobacillus delbrueckii* subsp. *Bulgaricus* on the secretion of Th1/Th2/Treg type cytokines by peripheral blood mononuclear cells (PBMCs) from children with AD. *L. Bulgaricus* was cultivated on MRS broth. The PBMCs from 20 children with AD were separated by Ficoll-Hypaque centrifugation and co-cultured with different concentrations of UV killed bacteria in RPMI-1640 plus 10% FCS for 48/72h. The levels of IL-10, IL-4, IL-12 and IFN-γ were measured in supernatant of PBMCs by ELISA. *L. Bulgaricus* significantly up-regulated the secretion of IL-10, IL-12 and IFN-γ, whereas decreased the secretion of IL-4 by PBMCs at both incubation times 48h/72 h and both bacteria: PBMCs ratios 100:1 and 50:1, compared to control cultures ($p<0.05$). There were no significant differences between incubation times 48 h and 72 h regarding the secretion levels of IL-12, IFN-γ and IL-4. However, the secretion of IL-10 by *L. Bulgaricus*-stimulated PBMCs at incubation time 72 h and in the presence of bacteria: PBMCs ratio 100:1 was significantly higher than in incubation time 48 h and in the presence of bacteria: PBMCs ratio 50:1 ($P<0.000$ and $P<0.00$, respectively).
These data show that *L. Bulgaricus* may modulate the secretion of Th1-, Th2- and Treg-related cytokines in AD patients. Therefore, the possible potential therapeutic of *L. Bulgaricus* for treatment of AD should be consider in further investigation.

Poster-Nr. 100

Abstract-ID: P100 | Poster presentation: 18.11.2016

TBE booster vaccination is effective in allergic patients with and without SIT and contributes to reduction of the allergic Th2 bias

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Allergic diseases have drastically increased and are caused by Th2-driven immune-dysregulation. Specific immunotherapy (SIT) is the only causative treatment, leading to immuno-modulation via IL-10 and TGF-ß and to Th1-shift.
In a clinical trial we aimed to investigate whether allergy and SIT-treatment affect responsiveness to routine vaccination. Allergics +/- SIT and healthy controls received a tick-borne encephalitis (TBE) booster with subsequent evaluation of humoral and cellular immune responses. Our results show that neutralizing TBE-Ab titers were not significantly altered in both allergic groups. Prior to vaccination the presumed Th2-bias in allergics was confirmed by increased total- and TBE-specific IgG1 and elevated IL-5. TBE-booster vaccination induced a modulation of this bias, reflected by reduced total IgG1 and lack of TBE-specific IgG1 4 weeks post-booster. In SIT-patients cytokine profiles indicated general immuno-modulation in association with increased T-regs which however did not affect their ability to mount sufficient vaccine-specific Abs. Allergy and SIT led to substantial changes of T- and B-cell subsets: allergics had more late-differentiated CD4-memory T-cells and showed strong increase of plasmablasts after booster in line with highest NT-titers which were not of IgG1 subclass; in the SIT-group expanded total CD4 T-cells and increased memory-subsets of CD4 and CD8 T-cells as well as B-cells were present. TBE-booster was well tolerated by allergics because no enhanced reactogenicity and/or exacerbation of allergic symptoms were reported.
We conclude that TBE-booster vaccination was efficient both in allergic and SIT-treated patients and contributed to reduction of the Th2-bias. Allergy and SIT caused distinct changes of T- and B-cell subsets which did not impair vaccine responsiveness. Vaccination was not linked to increased reactogenicity and allergy exacerbation, indicating that routine vaccines should not be withheld from allergic patients.

Supported by investigator initiated industrial funding

Poster-Nr. 101

Abstract-ID: P101 | Poster presentation: 18.11.2016

Improving diagnosis of allergy with a novel marker for carbohydrate reactive IgE

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Background: The carbohydrate moieties of glycosylated allergens can elicit IgE reactivity in about 20% of allergic patients. Carbohydrate-specific IgE antibodies show low or no allergenic activity. Therefore, IgE cross-reactivity to carbohydrates in unrelated allergen sources can lead to false positive *in vitro* diagnostic test results.

Objective: To generate a marker for carbohydrate reactive IgE (for allergy diagnosis) by grafting N-glycosylation sites onto a non-allergenic protein backbone.

Methods: Sequences coding for one or two N-glycosylation sites were engineered into the 5' end of the horse heart myoglobin (HHM) cDNA. The artificial glycoproteins and for control purposes a non-glycosylated myoglobin variant were expressed in baculovirus-infected High-FiveTM insect cells, purified and analyzed regarding fold and aggregation by circular dichroism and gel filtration, respectively. IgE reactivity of the HHM-glycovariants was assessed by ELISA and Immunoblotting and compared to the IgE reactivity profile obtained for sera with more than 170 micro-arrayed recombinant and natural allergens including the HHM-glycovariants.

Results: HHM-glycovariants were expressed and purified from insect cells as monomeric and folded proteins. The artificial glycoproteins showed IgE reactivity with sera from patients with bee and/or wasp, as well as pollen or mold sensitization, containing carbohydrate-specific IgE. Sixty-five % of 79 serum samples from patients with IgE double positivity to bee and wasp venom extract showed IgE reactivity to the recombinant glycosylated HHM. Using the allergen chip, IgE reactivity to glycosylated natural allergens (e.g., nPhlp 4, nCyn d 1, nPla a 2, nJug r 2, nCup a 1, nCry j 1), which are not related on protein level, was detected in 62% of these sera. Pre-incubation of sera with the HHM-glycovariants inhibited IgE binding to the carbohydrate moieties of these glycoallergens.

Conclusion: The HHM-glycovariants are useful specific diagnostic markers for IgE-reactivity to carbohydrates. Using them for basophil activation studies should help to elucidate the mechanisms for low allergenic activity of IgE-reactive carbohydrates.

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SESSION 6 | CLINICAL ALLERGOLOGY

Poster-Nr. 102

Abstract-ID: P102 | Poster presentation: 18.11.2016

MBC4 is able to induce cross-reactive blocking IgG antibodies in animals

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Background: Birch pollen allergies are frequently associated with adverse reactions towards various foods, such as vegetables, nuts, fruits, and legumes. In Austria, the majority of birch pollen-allergic patients show local symptoms as itching and swelling in the oral cavity after the ingestion of apple and hazelnut. This pollen-related food allergy is caused by food allergens cross-reactive with the major birch pollen allergen Bet v 1.

Rational: To enable the concomitant treatment of birch pollen and associated food allergies towards apple and hazelnut, we designed a hybrid molecule consisting of T cell-reactive parts of birch Bet v 1, apple Mal d 1, and Cor a 1.04 from hazelnut, respectively. Moreover, the protein was mutated to alter its fold.

Methods: The molecule, termed MBC4, was produced in *E. coli* and purified to homogeneity. All parental allergens as well as MBC4 were characterized in detail physico-chemically and immunologically. Moreover, mice and rabbits were immunized to study the immunologic properties of MBC4 *in vivo*.
Results: Parental allergens revealed a Bet v 1-like folding, whereas MBC4 did not share this distinct behavior. Subsequently, the IgE-binding capacity of the hybrid protein was significantly reduced. In animal models, MBC4 induced a cross-reactive IgG response. These antibodies were able to block human IgE-binding to parental allergens as determined in mediator release assays. In addition, we observed a cross-reactive T-cell response with the hybrid protein.

Conclusion: MBC4 revealed a reduced IgE-binding capacity, retained immunogenicity, and induced blocking antibodies in animals. Therefore, it represents a promising vaccine candidate for the combined treatment of birch pollen and associated food allergies towards apple and hazelnut.

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Poster-Nr. 103

Abstract-ID: P103 | Poster presentation: 18.11.2016

Purification and characterization of an allergenic walnut vicilin-like protein

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Background: Walnuts are potent inducers of food allergic symptoms, ranging from mild up to severe reactions in sensitized individuals. So far, five allergens were identified, among those Jug r 2 belonging to the vicilin family. Based on the identification of an IgE-reactive clone from a cDNA library in 1999 (Teuber et al.), the sequence fragment of Jug r 2 was determined. However, only limited data is known about the natural form of the protein. Therefore, this study aimed to purify and characterize the physicochemical and allergenic properties of Jug r 2.

Methods: Heat treated walnuts (65°C, 90min.) were ground and defatted twice (1:5 w/v) by n-hexane. Proteins were extracted from walnut flour by 7 volumes of buffer (20mM Tris/HCl, 0.5M NaCl, 5% PVPP, 10mM DTT, pH=8) for 4 hours at 4°C. Subsequently, purification was performed starting with concanavalin A affinity followed by anion exchange and size exclusion. The purified protein was identified by MALDI-TOF mass spectrometry and LC-MS/MS. Secondary structure was assessed by CD spectroscopy and the IgE binding activity of vicilin was tested in ELISA and Western Blot using sera from 5 walnut allergic patients.

Results: Purified vicilin migrates in SDS-PAGE as a single band at around 50 kDa. CD spectroscopy confirmed a stable secondary structure of the protein. MALDI TOF mass spectrometry provided 47 kDa, which is in disagreement with the previously identified isoform with a mass of 48.4 kDa (Jug r 2 database access. n: Q9SEW4). Sequence analysis revealed two cupin domains and high sequence identity with other vicilins such as from hazelnut, Cor a 11 (72%), sesame seeds, Ses i 3 (60%) and pistachio, Pis v 3 (54%). ELISA and Western Blot confirmed IgE binding activity of the purified allergen. In contrast to the previous Jug r 2 encoding sequence, obtained by cDNA cloning the current isoform was purified directly from walnuts. Therefore, it may represent an abundant and relevant vicilin-like allergen in walnuts.

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SESSION 6 | CLINICAL ALLERGOLOGY

Poster-Nr. 104

Abstract-ID: P104 | Poster presentation: 18.11.2016

Fusion proteins of flagellin and the major birch pollen allergen Bet v 1 show enhanced immunogenicity, reduced allergenicity and intrinsic adjuvanticity

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Background: Recombinant fusion proteins of flagellin and antigens have been demonstrated to induce strong innate and adaptive immune responses. Such fusion proteins may enhance the efficacy of allergen-specific immunotherapy (AIT).
Objective: To characterize different fusion proteins of flagellin and the major birch pollen allergen Bet v 1 for suitability as allergy vaccines.

Methods: A truncated version of flagellin (NtCFlg) was genetically fused to the N- or C-terminus of Bet v 1. TLR5-binding was assessed with HEK293 cells expressing TLR5. Up-regulation of CD40, CD80, CD83, and CD86 on monocyte-derived dendritic cells (mdDC) from allergic patients was analyzed by flow cytometry. The T cell-stimulatory capacity of the fusion proteins was assessed with naïve and Bet v 1-specific T cells. IgE-binding was tested in inhibition ELISA and basophil activation tests (BAT). Mice were immunized with the fusion proteins in the absence and presence of aluminium hydroxide (alum). Murine antibody responses were monitored by ELISA and tested for blocking capacity in BAT.

Results: Both fusion proteins matured mdDC via TLR5. Compared to Bet v 1 the fusion proteins showed stronger T cell-stimulatory and reduced IgE-binding capacity and induced murine Bet v 1-specific antibodies in the absence of alum. However, only antibodies induced by immunization with NtCFlg fused to the C-terminus of Bet v 1 inhibited the binding of patients´ IgE antibodies to Bet v 1.

Conclusion: Bet v 1-flagellin fusion proteins show enhanced immunogenicity, reduced allergenicity and intrinsic adjuvanticity and thus represent promising vaccines for birch pollen AIT. However, the sequential order of allergen and adjuvant within a fusion protein determines its immunological characteristics.

Poster-Nr. 105

Abstract-ID: P105 | Poster presentation: 18.11.2016

New approach to diagnostics in children with grass pollen allergy

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The allergy diagnostics in many aspects is based on estimates of allergen specific IgE level. But allergens range is large and the cost of assays is proportional to tests number and quality of used reagents. Also capture of vein blood is a stress for the children and can't be executed in full.

The aim was search of effective diagnostic method for minimization of blood sample volume and for identification of markers for assessment of the patient sensitization without additional testing.

Materials: grass allergen IgE levels (cocksfoot g3, meadow fescue g4, timothy grass g6, meadow grass g8, brome grass g11, meadow foxtail g16, false oat-grass g204) were measured in 256 children (4 - 16 years old) with pollinosis and positive skin tests with grass allergens. The ImmunoCAP250 (Thermo Fisher Scientific) was used.

Results: high correlation was found between all investigated parameters (correlation coefficient >0,95, p<0,001). The regression analysis has been applied to assessment of interrelation of the IgE-levels to studied allergens. The cocksfoot allergen IgE level (IgE(g3)) had the largest predictive force concerning the other studied allergens IgE levels. Coefficient of determination was more 90% for all allergens except g204. The executed dispersive analysis confirmed the statistical significance of the revealed interrelation (mistake rate was 0,0005%). Grass allergens IgE levels expressed in percent from IgE(g3) were calculated: IgE(g4) averaged 101,4±17,0%, IgE(g6) - 88,7±27,3%, IgE(g8) - 119,8±24,5%, IgE(g11) - 44,5±12,8%, IgE(g16) - 78,7±19,8, IgE(g204) - 40,7±19,3%.

Conclusions: the cocksfoot allergen IgE level appears as the estimative parameter of relatives grass allergens IgE levels and allows to estimate the rate of the patient sensitization to these allergens without carrying out additional researches.

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SESSION 6 | CLINICAL ALLERGOLOGY

Poster-Nr. 106

Abstract-ID: P106 | Poster presentation: 18.11.2016

Seed storage proteins are marker allergens for patients with severe reactions to buckwheat

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In Asian countries, buckwheat (*Fagopyrum esculentum*) is a common, traditional food. Its high content of proteins but lack of gluten, makes it more and more popular as a health food also in Europe. However, for sensitized individuals, consumption can cause anaphylactic reactions. The aim of this study was to define allergen recognition patterns and to identify potential markers associated with severe symptoms.

Sera were selected by positive SPT to buckwheat and divided into two groups: (1) sensitized to buckwheat without clinical symptoms and (2) buckwheat allergy according to positive food challenges and/or convincing case history. All sera were analyzed by ImmunoCAP ISAC and IgE immunoblotting. Buckwheat proteins where extracted and separated using size exclusion followed by ion exchange chromatography. The presence of allergens in individual fractions was detected using patients' sera.

Sera from patients with systemic reactions did not recognize any of the allergens that were tested by ImmunoCAP ISAC analysis, except three sera recognizing only Fag e 2, the 2S albumin from buckwheat. Immunoblotting using these sera revealed strong IgE binding to a range of proteins from the buckwheat extract. Separation of buckwheat proteins showed the presence of seed storage proteins, such as vicilin, 2S albumin, and legumin, the latter being the most abundant, and nsLTP. The majority of sera from buckwheat allergic patients contained IgE specific to buckwheat-legumin. In addition also buckwheat-vicilin is recognized by patients' sera.

Our data demonstrate that the current buckwheat allergen repertoire available for routine CRD is not sufficient to clearly identify buckwheat allergic patients. We identified buckwheat-legumin as a marker for patients with severe reactions to buckwheat and that buckwheat vicilin could serve as additional marker.

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Poster-Nr. 107

Abstract-ID: P107 | Poster presentation: 18.11.2016

IgE cross-reactivity and T-cell cross-reactivity of allergens from defensin-like protein family

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Art v 1 from mugwort (*Artemisia vulgaris*), Amb a 4 from ragweed (*Ambrosia artemisiifolia*) and Par h 1 from feverfew (*Parthenium hysterophorus*) are pollen allergens from the from Asteraceae family. These allergens are defensin-like proteins stabilized by four disulfide bonds fused to a proline rich domain. The similarities of these three proteins on their primary structure and physicochemical features have been previously described in our group. However, the immunological features shared by these proteins from the same family are not well known. In order to shed light on this, we conducted a sensitization profile study using patients from three different geographical regions. Furthermore we performed cross-reactivity studies at the IgE and T-cell level.

Different levels of IgE reactivity to the defensin-like allergens were found in patients' sera from Austria (40), Canada (38) and Korea (27), and Art v 1 showed highest reactivity in Austrian and Korean patients. IgE cross-reactivity was demonstrated between the three allergens; however the results suggest the presence of shared and unique IgE epitopes. Furthermore, these IgE epitopes are differently affected by chemical disruption of disulfide bonds. The IgE reactivity of Amb a 4 and Par h 1 was partially retained after reduction with DTT while the IgE reactivity of Art v 1 was completely lost. On the other hand, Amb a 4 and Par h 1 did not show T-cell cross-reactivity with Art v 1 specific T-cells. Additionally, proteins showed different susceptibility to endolysosomal degradation, also suggesting differences in their immunological properties.

The defensin-like allergens Art v 1, Amb a 4 and Par h 1 share similar structural features, but distinct immunological properties. They showed partial IgE cross-reactivity with patients from different regions while T-cell cross-reactivity was not observed in our experimental settings.

SESSION 6 | CLINICAL ALLERGOLOGY

Poster-Nr. 108

Abstract-ID: P108 | Poster presentation: 18.11.2016

Anaphylaxis imaging for preclinical proof-of-principle in small animals

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The anaphylactic shock typically presents with a drop of body temperature and reduced physical activity. To reveal the pathophysiological mechanisms behind and to improve treatment options, animal models of anaphylaxis, most often in BALB/c mice, are applied. So far, monitoring for temperature and activity is done manually in these models. We aimed to refine, optimize and objectify these methods. Therefore, we developed an imaging system based on thermo- and photo-cameras for the automated, continuous measurement of the whole-body surface temperature of small animals. Simultaneously, the software calculates the horizontal and vertical movement activity of the animals purely based on image analysis. The method was evaluated in anaphylaxis mouse models of milk allergy, peanut allergy and egg allergy. These proof-of-principle experiments confirmed that the imaging technology represents a reliable, non-invasive and objective method, and can be applied for monitoring in any diseases accompanied by changes in body temperature and/or physical activity in small animals.

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Poster-Nr. 109

Abstract-ID: P109 | Poster presentation: 18.11.2016

Development of allergen-specific ELISAs for the quantification of the house dust mite allergens: Der p 5, 7, 21 and 23

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House dust mite (HDM) allergy affects more than 10% of the population in industrialized countries. Der p 1, Der p 2 and Der p 23 are known to be the major HDM allergens with highest IgE reactivity, but also Der p 5, Der p 7, Der p 21 have been identified as "mid-tier" allergens inducing responses in about 40% of subjects. Assays for measuring allergen concentrations in environmental samples, diagnostic and therapeutic allergen extracts are available only for Der p 1 and Der p 2.
The aim of this study was to develop sandwich ELISAs for the detection and quantification of Der p 5, Der p 7, Der p 21 and Der p 23 in HDM extracts and dust samples.
Allergen-specific antibodies with defined specificities were obtained by immunizing rabbits with synthetic peptides derived from different portions of the allergens and with the complete recombinant allergens. The rabbit antisera were tested for allergen reactivity towards immobilized allergens and allergens in solution and used to build sandwich ELISAs based on capturing and detecting antisera with defined specificity. Purified allergens were used for standardization and the ELISA assays were shown to be highly specific for the respective allergens. The sandwich ELISAs will be useful to measure and quantify the HDM allergens Der p 5, Der p 7, Der p 21 and Der p 23 in environmental samples, in allergen extracts used for challenge tests as well as in diagnostic and therapeutic allergen extracts.

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SESSION 6 | CLINICAL ALLERGOLOGY

Poster-Nr. 111

Abstract-ID: P111 | Poster presentation: 18.11.2016

Production and characterization of Blo t 5 variants, the major allergen of Blomia tropicalis

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Background: The mite Blomia tropicalis is an important source of allergens that is associated with allergic symptoms in the tropical and subtropical regions of the world. Among the identified IgE-binding proteins of B. tropicalis, Blo t 5 is considered its major allergen.

Objective: The aims of this study were to produce and characterize two variants of Blo t 5, and compare the allergenicity of the variants by *in vitro* assays.

Methods: The two variants were expressed in *E. coli* BL21 star, purified by ion exchange and size exclusion chromatography. The Blo t 5 variants were physico-chemically characterized by mass spectrometry (MS) analysis, circular dichroism (CD), Fourier transform infrared spectroscopy (FTIR) experiments, dynamic light scattering (DLS) and endo-/lysosomal degradation assays. The proteins were also immunologically characterized by ELISA and mediator release assay using a humanized rat basophil leukemia (huRBL) cell line.

Results: The soluble non-fusion rBlo t 5 variants presented a typical CD spectrum for proteins with α-helix content, further confirmed by FTIR measurements. Moreover, no aggregation and/or dimers were found in DLS experiments. Concerning the proteolytic stability, the short variant has shown stronger resistance towards endo-/lysosomal degradation than the long variant. No significant differences were found in IgE reactivity between the two variants using B. tropicalis allergic patients' sera. On the other hand, the long variant presented a higher capacity to induce beta-hexosaminidase release by huRBL cells.

Conclusion: We successfully expressed and characterized two variants of rBlo t 5. We showed that the absence of the N-terminus pro-sequence did not influence either the folding of the allergen or the IgE reactivity in ELISA. However, more studies will be performed to investigate the higher huRBL degranulation capacity of the long molecule version.

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Poster-Nr. 112

Abstract-ID: P112 | Poster presentation: 18.11.2016

IgE cross-reactivity between ragweed (ambrosia elatior) and grass pollen allergens

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Ragweed is one of the major sources of allergenic pollen, causing allergic reactions in sensitized individuals. Ragweed contains numerous allergens and 6 among these considered major. Previously it was known Amb a 1 was the most important Ragweed allergen: more 95% ambrosia-sensitive individuals react to it in skin tests and show high serum IgE antibody levels. More over Amb a1 has been reported to have significant sequence similarities with Phl p 4 from Timothy grass pollen. This fact is supposed as an explanation of high cross-reactivity between Ragweed (Ambrosia elatior) and Grass pollen allergens.
We tested 40 children from 4 till 12 years old with allergy symptoms caused by ambrosia pollen and measured the IgE antibodies levels to native ragweed allergen extract (w1), major ambrosia allergen Amb a1 (w230), native timothy allergen extract (g6), major and minor timothy allergen (Phl p1, Phl p 5b, Phl p 7, Phl p12, Phl p4). We used CAP Systems to determined IgE levels. All children had positive IgE levels to native ambrosia and timothy allergen extract (w1, g6). The equal positive concentrations of IgE to w1 and w230 were observed in 30% children. The IgE(w230) level was more then IgE(w1) level in 25% children. The concentration of IgE(w1) was more then IgE(w230) (both are positive) in 15%. However we observed 30% children with positive IgE(w1) levels and absence of IgE to w230. Findings reported that 30% of person sensitized to ragweed had no antibodies to Amb a1. More over patients with negative assay result had IgE to timothy grass allergens: Phl p12, Phl p1 or Phl p 5b, but not to Phl p4. So we suggest that cross-reactivity between ragweed and grass pollen occurs more often than it has been reported previously and may be explained by antibody cross-reactivity with not only Phl p4 but other allergens of timothy.

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SESSION 6 | CLINICAL ALLERGOLOGY

Poster-Nr. 113

Abstract-ID: P113 | Poster presentation: 18.11.2016

Influence of conformational and linear IgE epitopes on Ara h 2 specific IgE-binding

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Ara h 2 is the most important peanut allergen. It is stable against proteolysis and thermal denaturation. Little is known about the role of conformational versus linear IgE epitopes of this molecule. An Ara h 2 mutant, mtAra h 2 (lacking the surface exposed loops that contain most linear IgE epitopes), and the wild-type protein (wt) were expressed in the baculovirus insect cell system. The proteins were purified from the cell culture supernatants and purity of the proteins was verified by Western blotting. Aliquots of wt, mt and natural (n) Ara h 2 were reduced with dithiothreitol and alkylated with iodoacetamide. Physicochemical characteristics were determined by mass spectrometry, N-terminal sequencing and CD spectroscopy. IgE-binding was tested by ELISA using ten sera of peanut allergic patients. Mass spectrometry and N-terminal sequencing of mt, wtAra h 2 and nAra h 2 yielded masses corresponding to the predicted sizes and the correct N-termini. CD spectroscopy revealed the characteristic alpha-helical structure of the proteins. The complete reduction of all three reduced and alkylated proteins was also confirmed by CD spectroscopy. In direct ELISA, allergic patients' sera revealed a 20-50% reduced IgE-binding to the mutant compared with wt and nAra h 2. Upon reduction, wtAra h 2 revealed patient-specific decreases in IgE-binding. Depending on the Ara h 2 specific IgE levels it had either a higher IgE-binding capacity than the mt or a lower IgE-binding capacity. The reduced and alkylated mutant showed almost no IgE-binding. These results indicate that both conformational and linear IgE-binding epitopes are important for Ara h 2 specific IgE-binding. Relative contributions of linear and conformational epitopes to Ara h 2 allergenicity are variable among patients with peanut allergy.

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Poster-Nr. 114

Abstract-ID: P114 | Poster presentation: 18.11.2016

Barrier increasing factors of the respiratory epithelium

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The respiratory epithelium plays an important role as a barrier against the intrusion of environmental factors, such as pathogens and harmful molecules including allergens. Various damaging factors such as cigarette smoke extract (CSE), interferon-γ (IFN-γ) house dust mite (HDM) or rhinovirus (RV) infection decrease the epithelial barrier function, which leads to an increased penetration of allergens through the epithelium. In this project it was our aim to investigate potentially protective factors as a new approach.

For this purpose we worked with a respiratory epithelial cell line and primary human nasal epithelial cells obtained from routine surgeries. Cell types and morphological features of the cultured epithelium were investigated by flow cytometry, immunohistological assessments and ciliary beat frequency measurements. As read-out systems we used on the one hand the xCELLigence DP system to measure impedance-based cell responses and on the other hand we worked with the 2-chamber transwell system to check transepithelial resistance values of cell monolayers.

We found that various damaging factors induced different time-courses and recovery patterns of damage. The maximum barrier decreasing effects were observed after 12 hours of exposure to CSE, after 36 hours of RV infection, after 3-4 days of exposure to HDM extract and after 4-5 days of treatment with IFN-γ. Subsequently, the established models were used for investigating the capacity of various factors to protect the epithelium from damage. So far, nasal mucus proteins which had been shown to be present at higher concentrations in the mucus of non-allergic individuals compared to allergics had been tested. Additionally vitamins, amino acids, probiotic bacteria, different commercial nasal sprays and lipopolysaccharide (LPS) have been examined.

In summary we show a protective effect of betamethasone against CSE and RV, haptoglobin against damage by CSE and mainly low concentrations of LPS against HDM.

SESSION 6 | CLINICAL ALLERGOLOGY

Poster-Nr. 114a

Abstract-ID: P114a | Poster presentation: 17.11.2016

The Austrian Drug Prescription Report 2006-2014: H1-Antihistamines

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Introduction: H1-antihistamines are widely prescribed drugs to treat allergic reactions; they can be categorised in first and second generation substances (the latter causing less sedation). Second generation antihistamines can be divided into innovative substances and analogues (me-too drugs, metabolites and isomers), the latter sometimes high-priced. Since rise in public expenses on pharmaceuticals is of strong concern, prescription practice of H1-antihistamines in Austria was analysed for cost-efficiency and savings potential.

Methods: Data from all prescriptions filled in Austrian pharmacies on public expense by outpatients (2006-2014) were obtained from the Main Association of Austrian Social Security Organisations (Hauptverband der Sozialversicherungsträger). Savings potential was calculated at ATC-5 level (substance level) taking into account recommendations of authorities and the status of the medical evidence. Calculations were performed by replacing expensive brands with more cost effective brands or by replacing substances with low grades of evidence (analogues, me-too-substances etc.) by substances with higher grade evidence and choosing cost-effective brands.

Results: Public expenses on H1-antihistamines rose from €10.2m in 2006 to €11.1m in 2011; since then, the availability of generics has reduced the costs to finally €5.7m in 2014. Until 2013, the majority of second generation antihistamines prescribed were analogues. Savings potential was calculated to be €3,74m (42.4%) in 2012, €2.1m (28.5%) in 2013 and €1.16m (20.5%) in 2014.

Discussion: This report points out a relevant savings potential as well as improvement in therapy that could have been achieved by careful implementation of the authorities' and state-of the-art medical recommendations in the prescription of antihistamines in the years 2011-2014. Furthermore it demonstrates the cost reduction by the availability and use of generics.

Oral Presentation-Nr. 020

Abstract-ID: 20 | Oral Presentation 18.11.2016, Session 7, Tumor Immunology, 15.30-17.30

Combination of oncolytic virotherapy and DC-based immunotherapy for the treatment of melanoma

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VSV-GP, a novel chimeric Vesicular Stomatitis Virus (VSV) pseudotyped with the glycoprotein of the lymphocytic choriomeningitis virus represents a promising oncolytic virus (OV) that preferentially targets and kills cancer cells. Release of tumor antigens and activation of immune response by OV therapy might support dendritic cell (DC)-mediated anti-tumor immunity. Thus in our study we analyzed the efficacy and immune mechanisms of the combination of VSV-GP oncolytic virotherapy with DC-based immunotherapy. Combination of VSV-GP therapy and DC-based vaccination was investigated in the syngeneic subcutaneous B16-OVA melanoma model. SIINFEKL-loaded CpG-activated DCs (DCVacc) and VSV-GP were applied intra- and peritumorally and immune responses were analyzed in the spleen and tumor tissues. The DCVacc/VSV-GP combination therapy resulted in a significantly improved survival compared to single treatments. Surviving mice from the DCVacc/VSV-GP treated group showed a long lasting anti-tumor immunity against B16-OVA and partial anti-tumor immunity against non-OVA B16 melanoma in rechallenge experiments. Analyzing specific cytotoxic T lymphocyte (CTL) responses induced by DCVacc and VSV-GP single and combination treatments we found that both DCVacc and DCVacc/VSV-GP induced comparable levels of OVA-specific $CD8^+$ T cell responses. In addition, a strong VSV N peptide-specific CD8 T cell response was found upon VSV-GP and DCVacc/VSV-GP treatments. The improved therapeutic effect by the DCVacc/VSV-GP combination treatment correlated with increased numbers of tumor infiltrating lymphocytes (TIL) and elevated Tconv/Treg and CD8/Treg ratios seen also in non-treated collateral tumors. Furthermore, depletion of CD8 T cells but not NK cells abrogated the therapeutic effect of DCVacc/VSV-GP. Taken together, the combination of VSV-GP and DC-based immunotherapy might represent a promising therapeutic option for the treatment of melanoma.

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Oral Presentation-Nr. 021

Abstract-ID: 21 | Oral Presentation 18.11.2016, Session 7, Tumor Immunology, 15.30-17.30

MAPK-activated protein kinase MK2 exerts immune regulatory functions in the myeloid tumor microenvironment

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The MAPK signaling pathway represents a key player in inflammation. As a downstream target of p38, MAPK-activated protein kinase 2 (MK2) contributes to signal transduction regulating the expression of various targets, e.g. multiple cytokines. While MK2 was shown to promote an inflammatory macrophage phenotype, we have reported its Th1-attenuating function in dendritic cells (DCs). These differential regulatory functions prompted us to investigate MK2 in DCs and other myeloid cells in the tumor microenvironment.

Murine DCs lacking MK2 activity exhibit an enhanced potential to differentiate Th1 cells upon Toll-like receptor ligation *in vitro* and *in vivo*. In line with this, we observe reduced B16.F10 melanoma growth in CD11cCre-MK2fl/fl mice upon DC activation by LPS + whole tumor cell lysate as compared to WT littermate controls. CD11cCre-MK2fl/fl mice show an overall reduced myeloid tumor infiltration, along with enhanced DC migration to tumor-draining lymph nodes. This results in efficient priming of T cells, accumulating in tumors of CD11cCre-MK2fl/fl mice. Our findings correlate with elevated MK2 levels and increased TGF-β and IL-10 expression in WT tumor-resident myeloid-derived suppressor cells (MDSCs) and DCs as opposed to splenic DCs underlining the involvement of MK2 in immunosuppressive mechanisms in the myeloid lineage. Complex cross-regulation of MAPK signaling further highlights MK2 as a central player in immune modulation, which promotes ERK1/2 while attenuating p38 activation and transiently enhances STAT3 phosphorylation.

Our data contrast the previously described role of MK2 in pro-inflammatory mechanisms of the p38 signaling route and suggest an additional immunosuppressive feedback function in myeloid cells, which may be exploited by tumor cells to escape immune recognition. These indications are particularly interesting since MK2 represents a promising target for combination therapy, as it mediates tumor cell resistance to chemo- and radiotherapy.

Oral Presentation-Nr. 022

Abstract-ID: 22 | Oral Presentation 18.11.2016, Session 7, Tumor Immunology, 15.30-17.30

T cells from myeloma patients display features of both exhaustion and senescence

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Multiple myeloma is a still incurable plasma cell tumor which relies on the bone marrow microenvironment. Besides mutations fueling growth of the tumor cells, immune deficiencies play an essential role in disease establishment and progression. Re-arming of T cells could improve therapeutic outcomes essentially. However, the underlying defects in T-cell responses in myeloma are only partially understood so far.

We therefore assessed the functional state of T cells in multiple myeloma and observed T-cell exhaustion and senescence especially at the tumor site. Briefly, $CD8^+$ T cells expressed several molecules which are associated with T-cell exhaustion (PD-1, CTLA-4, CD160, 2B4) and T-cell senescence (CD57, lack of CD28). Despite an increased expression of Tbet, $CD8^+$ T cells failed to produce IFNg and *in vitro* T-cell activation could not restore it. Furthermore, myeloma bone marrow T-cells showed a decreased ability to proliferate and to degranulate in response to T-cell stimuli. Notably, the percentage of senescent $CD57^+CD28^-$ $CD8^+$ T cells was significantly lower in treated myeloma patients when compared to untreated patients. This suggests that terminally differentiated cells are preferentially extinguished by therapy. However, immune-checkpoint molecules were still present on T cells after treatment suggesting that interference with inhibitory molecules combined with established myeloma therapies to restore the functional activity of T-cells could be a promising way to treat myeloma patients.

SESSION 7 | TUMOR IMMUNOLOGY

Poster-Nr. 115

Abstract-ID: P115 | Poster presentation: 18.11.2016

Intradermal injection of antigen:antibody fusion proteins directed against lectin receptors targets defined dendritic cell subsets in human skin and elicits T cell responses

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Dendritic cells (DC) are essential for the induction of primary immune responses, and hence preferred targets for immunisation against cancer. Skin DC express C-type lectin receptors for recognition of pathogen-derived antigens. *In situ*, Langerhans cells (LC) express mainly Langerin/CD207, whereas DEC-205/CD205 is expressed by dermal DC and at low levels also on LC. We targeted DC *in situ* with monoclonal antibodies (mAb) against these receptors. The mAbs were injected intradermally into human skin explants. Migratory skin DC carried targeting mAb from skin explants into the culture medium over 3-4 days. Corresponding to the expression patterns of these lectin receptors in skin DC, anti-Langerin mAb was detected exclusively in epidermal LC, DEC-205 mainly in CD1a+/ CD14- dermal DC. Human anti-human and mouse anti-human DEC-205 mAb showed identical targeting patterns. Since effective vaccination requires adjuvants we co-administered the TLR-3 ligand poly I:C. This enhanced uptake of the mouse anti-human DEC-205 mAb, whereas Langerin targeting was unchanged. A model antigen (EBNA1) fused to the mAbs elicited augmented CD4 T cell responses in autologous PBMCs. Our findings demonstrate that LC can be readily targeted by Langerin mAb; in contrast, DEC-205 mAb can also be bound by dermal skin DC subsets to varying degrees. Optimal targeting conditions will be established in this model for a maximal immunisation outcome. Cancer vaccines consisting of tumor-antigen:anti-DC antibody constructs aim at extending patients' pre-existing immunity by eliciting additional tumor-specific T cells. This is expected to ultimately increase the response rates of modern immune checkpoint inhibitors.

Poster-Nr. 116

Abstract-ID: P116 | Poster presentation: 18.11.2016

Immunosurveillance in different transplantable melanoma mouse models

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Melanoma remains the most aggressive type of skin cancer due to its high metastasizing capacity accounting for poor prognosis of patients. Transplantable tumor mouse models are commonly used to test novel immunotherapeutical approaches. We were interested in the immunological features of a new melanoma mouse cell line called SM1WT1, which was derived from a spontaneously arising melanoma in $BRAF^{V600E}$ mutant transgenic mice. BRAF mutations drive tumor growth in up to 50% of human melanoma cases. For this purpose we analyzed the immunological infiltrate in SM1WT1 tumors and compared this with another transplantable melanoma mouse model, namely B16.OVA. Despite the transfer of five-times more tumor cells into flank skin of C57BL/6 mice, SM1WT1 showed a slower tumor growth when compared with B16.OVA. This was accompanied by a higher abundance of $CD45^+$ infiltrating leukocytes in SM1WT than in B16.OVA tumors. When we examined the immune cell infiltrates, we found differences in the dendritic cell (DC) composition. The most abundant DC population in both tumor models were the CD103- DCs. B16.OVA tumors contained more $CD103^+$ DC which have been described to be important for cross-presentation of tumor-derived antigens. In regard to effector cells, the tumor tissues of both melanoma models contained comparable percentages of NK cells and T cells. Interestingly, T and NK cells were more activated in the SM1WT1 tumors, as reflected by stronger expression of CD69 and granzyme B. When NK cells were depleted from tumor-bearing mice, SM1WT1 tumor growth was accelerated, whereas B16.OVA showed no significant growth differences. SM1WT1 tumors are more immunogenic than B16.OVA reflected by a larger infiltrate of immune cells. These findings provide evidence that the SM1WT1 cell line is an interesting model to investigate the potential of BRAF inhibitors alone or in combination with immunotherapy, e.g. DC therapy, in a highly immunogenic $BRAF^{V600E}$-driven melanoma tumor setting.

Poster-Nr. 117

Abstract-ID: P117 | Poster presentation: 18.11.2016

Characterization of RGS16 in the tumor-promoting immunosuppressive niche of glioblastoma multiforme

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Glioblastoma multiforme (GBM) raises challenges for immunotherapy due to its unique tumor microenvironment (TME), which impairs effective cellular anti-tumor immunity. GBM is known to secrete extracellular vesicles (EVs) harboring pro-tumorigenic signatures to enhance tumor progression, thereby, contributing to the immunosuppressive TME by interacting with GBM-associated myeloid cells (GAMs). To understand the mechanism behind the ability of GBM to modulate its TME and to transform into a highly invasive tumor, we studied infiltrating myeloid cell populations as well as EVs in this context.

We found a Regulator of G-protein Signaling family member, RGS16, being highly up-regulated and released from the murine GBM cell line GL261 via microvesicles *in vitro*. Furthermore, immunohistochemistry and -fluorescence analyses of GL261-GBM tumors showed extracellular enrichment of RGS16 within the tumor core and at the invasive margin of GBM, an area highly infiltrated by GAMs and T cells. Various immune cell populations infiltrated at high numbers, however, they appeared dysfunctional as the tumor progressed rapidly and didn't show morphological areas of T cell-mediated cytotoxicity. Based on these findings we postulate that GBM maintenance and therapeutic resistance can be associated with RGS16 functions. In this context we found RGS16 being strongly over-expressed in human GBM stem cell lines as compared to already differentiated tumor bulk *in vitro*, indicating that RGS16 may contribute to the aggressive stem cell phenotype. These observations prompted us to further investigate the contribution of RGS16 to an immunosuppressive myeloid phenotype and we found RGS16 elevated in GAMs.

Utilizing the CRISPR Type II system we are working on constructing a knockout of Rgs16 in GL261 tumor cells to further evaluate the role of Rgs16 in the GBM TME. Regarding clinical applicability, RGS16 inhibition may hinder tumor immune evasion to drive physiological immune reactions against GBM.

Poster-Nr. 118

Abstract-ID: P118 | Poster presentation: 18.11.2016

Effects of Imiquimod on hair follicle stem cells and hair cycle progression

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Topical Imiquimod (IMQ) application is widely used as a model for psoriasiform-like skin inflammation in mice. Although the effects on the epidermis are well characterized, it is unclear how IMQ affects hair follicles and cycling. Here we investigated how IMQ affects hair follicle stem cells and whether the timing of IMQ application influences the immune infiltrate. Our results show that IMQ application at mid and late telogen activated hair follicle stem cells leading to premature hair cycle entry (anagen), which was accompanied by massive infiltration of inflammatory macrophages and gamma delta T cells, whereas the number of the respective resident populations decreased. Interestingly, high resident macrophage numbers were present in Rag2 KO mice and were maintained after IMQ treatment explaining why IMQ-induced anagen was reduced. This could be rescued after macrophage depletion suggesting that resident macrophages inhibit whereas inflammatory infiltrating macrophages stimulate hair follicle stem cell activation. The expression of the anagen-inhibiting factor bone morphogenetic protein-4 was reduced by IMQ treatment as well as the activating factors Wnt showing that IMQ-induced hair follicle stem cell activation occurs by a Wnt-independent mechanism involving inflammatory cytokines such as CCL2 and tumor necrosis factor-a. Additionally also the depilation method affected the immune cell alterations after topical IMQ. On the basis of our findings, we recommend conducting experiments with IMQ in shaved skin during mid and late telogen as the biggest differences in immune cell composition are observed.

Poster-Nr. 119

Abstract-ID: P119 | Poster presentation: 18.11.2016

New concept for generation of an effective and multi-level anti-HER-2 vaccine

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Approximately 20% of breast cancers exhibit HER-2 gene overexpression. This proto-oncogene, also known as ErbB-2, encodes a 185 kDa transmembrane receptor protein and is a member of the erbB/epidermal growth factor receptor (EGFR)/class I family of receptor tyrosine kinases.
Trastuzumab which binds to the extracellular subdomain IV of the HER-2 receptor, was introduced in the late 1990's and is approved by the U.S. Food and Drug Administration (FDA) and the European Medical Agency (EMA) for passive immunotherapy in the treatment of patients with advanced HER-2 overexpressing breast cancer. A later described anti-HER-2 humanized monoclonal antibody (mAb), Pertuzumab, binds to the extracellular subdomain II of the HER-2 receptor. The two mAbs have been shown to act synergistically in inhibiting the growth of HER-2-overexpressing breast cancer cell lines *in vitro*, inhibit proliferation and induce apoptosis.
As distinct advantage over treating with mAbs, B cell multi-epitope vaccines can induce production of antibodies with anti-tumor activity as well as polyclonal humoral responses. Also, the Th-1 driven anti-tumor immune responses have been shown to be the important anti-tumor responses. Moreover, the time required for induction of such immune response is of high importance in the clinical settings. We therefore aim to develop an anti-HER-2 vaccine by combining different B-cell mimotopes with binding capacity to both Trastuzumab and Pertuzumab, a suitable Th-1 driving adjuvant as well as a delivery system which we have shown to induce quicker immune responses. Such multi-level anti-HER2 vaccine may be a more effective vaccine against all HER-2 overexpressing cancer entities.

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Poster-Nr. 120

Abstract-ID: P120 | Poster presentation: 18.11.2016

The myeloid PI3K/PTEN signaling axis in colitis and colitis associated colon cancer

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The PI3K/PTEN signaling axis regulates development and function in cells of myeloid origin. Genetic deletion of PTEN results in hyper-activation of PI3K signaling and attenuates the pro-inflammatory phenotype of innate cells. We and other have shown that myeloid PTEN-deficiency in mouse models of infection, inflammation and autoimmunity protects animals from adverse immune reactions, whereas in a model of lung fibrosis, myeloid PTEN-deficient mice showed higher disease severity. We aimed to elucidate whether the less inflammatory phenotype of PTEN-deficient innate cells would alter tumor development. To address this question, we induced colitis and colitis-associated colon cancer (CAC) in mice deficient for PTEN in myeloid ($PTEN^{fl/fl}$ LysM cre) or dendritic cells (DCs) ($PTEN^{fl/fl}$ CD11c cre). Myeloid PTEN deficiency did not affect clinical signs of colitis but resulted in an increased tumor burden in the CAC and the B16 melanoma model. Analysis of myeloid subsets by flow cytometry and mRNA microarray revealed an increase in splenic $CD8a^+$ dendritic cells that expressed the adaptive immune blocking surface molecules PD-L1 and PD-L2. This was accompanied by a decrease in splenic T-cell proliferation *ex vivo* as well as of transferred, antigen-specific $CD8^+$ T-cells *in vivo*. In contrast to PTEN deficiency in myeloid cells, DC-specific knock-out of PTEN resulted in a significantly increased mortality in models of acute and chronic colitis as well as in CAC. Furthermore, T-cell responses seem to be skewed in DC-PTEN knock-out mice, probably causing in a higher bacterial burden in the spleens of these animals.

Taken together, we show that the PI3K/PTEN signaling pathway regulates development and function of myeloid cells. Hyper-activation of PI3K signaling by deletion of PTEN reduces inflammatory responses in cells of the innate immune system and increases tumor burden by blocking adaptive immune responses.

Poster-Nr. 121

Abstract-ID: P121 | Poster presentation: 18.11.2016

Understanding the heterogeneity of B-cell subsets: from dissecting tumor complexity to prognostic modeling

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Despite accumulated knowledge positioning tumor-infiltrating B cells among powerful contributors to tumor immunity, many questions remain given complexity of multifarious B-cell subsets and unique ability to assemble into functional follicular structures. In respect of therapeutic interventions tumor infiltrating immune cells gain additional attraction due to novel promising immunotherapeutic approaches. Recently, we demonstrated that $CD20^+$ B cells organised into follicular structures at the metastatic site of patients with colorectal cancer in the liver (CRCLM) are strongly associated with a better prognosis. This is the most powerful marker allowing CRCLM patient stratification in respect of survival. For understanding their assembling mechanisms and anti-tumor effects, we aimed to perform a comparative alignment of primary and matched metastatic B-cell-attributed patient-specific immunological imprints.

To achieve this goal, we developed a computerized microscopy-based algorithm, allowing the quantitative assessment of various B-cell subsets across large-scale tissue specimens of primary and metastatic CRC. To discriminate the B-cell subsets we stained for CD20, AID, IgM, CD27, CD73 and CD138. Using CD20 as general B-cell marker we assessed the distribution and quantities of tumor-infiltrating B cells including those organised into follicular structures. The developed algorithm consolidates the complexity of tumor anatomy and the immune landscape in term of B-cell localization and distribution patterns at primary and metastatic sites and currently includes a set of 24 variables for each marker. Next, the obtained patient-specific B-cell-attributed immunological imprint, described by diverse staining-derived data sets, will be used for alignment with clinicopathological parameters and for building-up of complex prognostic/predictive survival models allowing to propose novel immune check points and/or targeting strategies.

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Poster-Nr. 122

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Role of the AP-1 protein c-Jun in Imiquimod mediated tumor clearance

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

Poster-Nr. 123

Abstract-ID: P123 | Poster presentation: 18.11.2016

EGFR signaling in tumor-associated myeloid cells promotes colorectal cancer development

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Background & Aims: Targeted EGFR inhibition serves as first line therapy in metastatic colorectal cancer (CRC) patients without RAS mutations. However, many patients with wild-type RAS do not benefit from anti-EGFR treatment and EGFR expression levels are not predictive for therapy response for poorly understood reasons.

Methods: We analyzed 116 human CRC biopsies for EGFR expression in tumor and stroma and correlated the survival to presence of $EGFR^{+}$ myeloid cells. We also generated mice lacking EGFR in intestinal epithelial cells (Vil-Cre, Vil-CreER) and myeloid cells (Lys-Cre) and induced colitis-associated cancer by AOM/DSS or bred them with $Apc^{Min/+}$ mice to induce intestinal tumors. They were compared to control ($EGFR^{f/f}$, Cre^{+}) and $EGFR^{wa2/wa2}$ mice. Intestinal tissues were analyzed for presence of colitis and tumors and intestinal barrier integrity and characterized using histology, immunohistochemistry, imaging, qRT-PCR, Western blot, and FACS.

Results: We detected EGFR expression in tumor-associated myeloid cells of human CRC, which reduced survival of metastatic patients. Furthermore, EGFR deletion in myeloid cells but not in intestinal epithelial cells impaired CRC formation in mice. EGFR downregulation in transformed intestinal epithelial cells of established tumors did not reduce tumor size demonstrating dispensability of EGFR in epithelial cells for CRC initiation and progression. By exogenous IL-6 administration or antibody-mediated IL-6 depletion we mechanistically showed that EGFR signaling in myeloid cells protects from colitis but promotes CRC via IL6-mediated STAT3 activation in tumor cells. Conclusions: This study uncovers a novel mode of action of anti-EGFR therapy in CRC and will improve stratification and individualized treatment of CRC patients.

Poster-Nr. 124

Abstract-ID: P124 | Poster presentation: 18.11.2016

Loss of skin dendritic cells and their role in a spontaneous melanoma mouse model

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Ectopic overexpression of the metabotropic glutamate receptor 1 (Grm1) in melanocytes, confers them an anti-apoptotic and hyperproliferative phenotype. This alteration, that has been detected in 40% of melanoma patient samples, leads to spontaneous melanoma development with 100% penetrance in the tg(Grm1)EPv mouse model. We have characterized the immune cell network changes that occur in the skin and the tumor draining lymph nodes in regards to skin dendritic cells (DC) as well as effector cells. Our results show a clear decrease in the skin DC, with this event already occurring very early in tumor development. Furthermore, we investigated the capacity of skin DC to prime $CD8^+$ T cells and our results show that all skin DC subsets are able to induce priming irrespective of the stage of tumor development. Understanding the processes underlying these effects will offer useful information in regards to the role of the different skin DC subsets in tumor antigen presentation and activation of $CD8^+$ T cells.

SESSION 7 | TUMOR IMMUNOLOGY

Poster-Nr. 125

Abstract-ID: P125 | Poster presentation: 18.11.2016

Molecular, structural and immunological characterization of Der p 18, a chitinase-like house dust mite allergen

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Background: The house dust mite (HDM) allergen Der p 18 belongs to the glycoside hydrolase family 18 chitinases. The relevance of Der p 18 for house dust mite allergic patients has only been partly investigated. Objective: To perform a detailed characterization of Der p 18 on a molecular, structural and immunological level.

Methods: Der p 18 was expressed in *E. coli*, purified to homogeneity, tested for chitin binding activity and its secondary structure was analyzed by circular dichroism. Der p 18-specific IgG antibodies were produced in rabbits to localize the allergen in mites using immunogold electron microscopy and to search for cross-reactive allergens in other allergen sources (i.e. mites, crustacea, mollusca and insects). IgE reactivity of rDer p 18 was tested with sera from clinically well characterized HDM-allergic patients (n=98) and its allergenic activity was analyzed in basophil activation experiments.

Results: Recombinant Der p 18 was expressed and purified as a folded, biologically active protein. It shows chitin-binding activity and partial cross-reactivity with Der f 18 from *D. farinae* but not with proteins from the other tested allergen sources. The allergen was mainly localized in the peritrophic matrix of the HDM gut and to a lower extent in fecal pellets. Der p 18 reacted with IgE from 10% of mite allergic patients from Austria and showed allergenic activity when tested for basophil activation in Der p 18-sensitized patients.

Conclusion: Der p 18 is a chitin-binding and rather genus-specific minor allergen but exhibits allergenic activity and therefore should be included in diagnostic test panels for HDM allergy.

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Poster-Nr. 126

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Overexpression of PDE4A in human T-cells acts as intrinsic checkpoint inhibitor against cAMP-induced immunosuppression

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Introduction: Malignant cells acquire physiological mechanisms of immunosuppression (also called "immune checkpoints") to protect themselves from anti-tumor immunity. Thus, manipulation of T-cells to counteract this suppression could improve adoptive therapy regimen. In this context, cyclic AMP (cAMP) is of special interest, since it acts as a common inhibitory second messenger for several suppressive substances including adenosine and prostaglandine E2 (PGE2). Consequently, we assessed, whether retroviral overexpression of the cAMP-degrading phosphodiesterase 4A (PDE4A) in human T-cells could restore their functionality under suppression by cAMP-inducing agents.

Results: PDE4A-transgenic $CD4^+$ and $CD8^+$ T-cells showed a high degree of resistance against the immunosuppressive effects of PGE2 and adenosine in proliferation and cytokine secretion assays. Importantly, no differences in the functionality under non-suppressive conditions between PDE4A- and control-vector transduced T-cells were observed. Similarly, no differences in the expression of the exhaustion markers CD244, PD-1 and TIM-3 were registered upon long-term culture. Under suppression by PGE2, up-regulation of the degranulation marker CD107a on $CD8^+$ T-cells and lysis of BW target cells were significantly increased in PDE4A-transduced T-cells compared to control-vector transduced T-cells. In co-cultures with regulatory T-cells, PDE4A-transduced T-cells showed an increased proliferation, which was especially pronounced at low Treg : Tresp ratios.

Conclusion: We show, that overexpression of PDE4A is sufficient to overcome the suppressive effects of cAMP-inducing agents in T-cells. These findings could help to improve adoptive immunotherapy protocols.

Poster-Nr. 127

Abstract-ID: P127 | Poster presentation: 18.11.2016

Laser-assisted skin immunization to target DC in mouse and human

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Skin dendritic cells (DC) are antigen presenting immune cells which induce immune responses against cutaneous infection and tumours. Due to their localization in the skin, they are able to recognize cancer cells developing in the skin and to start an immune response against tumours. The immunotherapeutic approach called epicutaneous immunization aims at loading DC subtypes directly with tumour antigens. To improve epicutaneous immunization we intend to load skin DC with antibody-antigen (Ab-Ag) conjugates against DC surface molecules, such as the lectin receptors DEC-205 and Langerin that are essential for antigen incorporation. An essential improvement of epicutaneous immunization is expected from a laser poration of the skin. An infrared laser (P.L.E.A.S.E.® Laser System, Pantec Biosolutions) creates micropores in the skin by excitation of water molecules. These micropores should allow macromolecules to diffuse into the skin, and therefore enable the transcutaneous application of molecules with high molecular weight, like antibodies. Through these pores it will be possible to deliver larger molecules such as Ab-Ag vaccines together with adjuvants for immunization. Human and murine skin samples were prepared to determine the optimal parameters for ablation of epidermis and dermis. The laser-induced thermal damage was investigated. DC targeting by antibodies against Langerin and DEC-205 was evaluated.

We were able to induce pores of definable depths and no increased apoptotic signals were found in the surrounding of the pores. However, the DC targeting efficiency after intradermal injection was found to be more effective than the new laser treatment.

Future experiments will investigate the benefit of co-applied adjuvants and the immune-stimulatory capacity of antigen-targeted DC in laser treated human and murine skin.

Poster-Nr. 128

Abstract-ID: P128 | Poster presentation: 18.11.2016

A phase I clinical trial of antigen-pulsed autologous dendritic cells in advanced colorectal cancer patients

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A phase I trial was conducted to evaluate the feasibility, safety, and efficacy of a dendritic cell(DC) based vaccination in patients with colorectal carcinoma. Autologous mature DCs derived from the peripheral blood monocytes(PBMC) were pulsed with the tumor lysate antigen. DC vaccinations were transfused i.v. every 7-10 days for three times. Twenty one patients were randomly divided into three groups receiving 1×107, 3×107, 6×107 tumor lysate pulsed DC vaccine respectively. The treatment was well tolerated with no severer than grade two adverse events associated with DC vaccination. Adverse effects associated with tumor lysate antigen-pulsed DC treatment included fever, chill, nasal congestion, running nose, fatigue, muscle pain, which were all grade I or II and alleviated after treatment correspondingly. The percentage of adverse events was 14.29%, 14.29% and 57.14% in the three stepwise increased dosage groups respectively. The number of IFN-γ-producing peripheral blood cells grew significantly, measured by ELISPOT, in 7 patients post-treatment. Conclusion: Tumor lysate antigen-pulsed DCs were good safety, well tolerated in 6 x 107 and lower dosage groups and induced specific immune responses.

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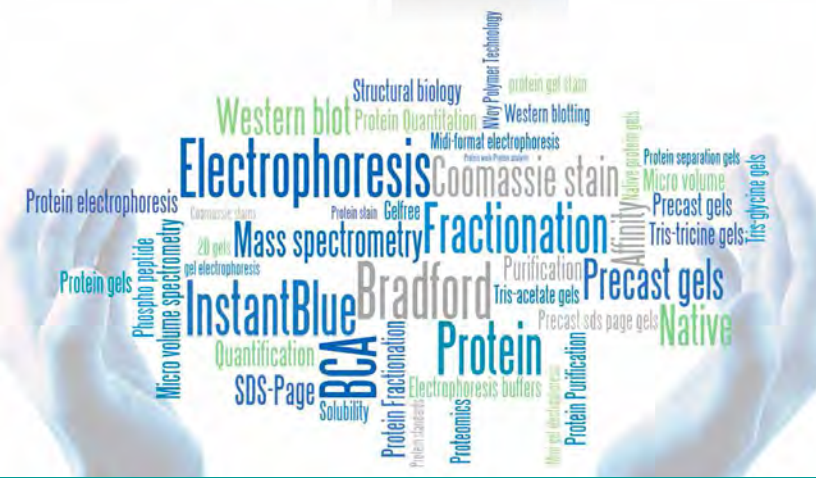

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