Myeloid Cells: Microenvironment Microorganisms & Metabolism

From Basic Science to Clinical Applications

27th Annual Conference

of the

10 – 12 October 2013

Erlangen, Germany

Program & Abstracts

FRIEDRICH-ALEXANDER UNIVERSITÄT ERLANGEN-NÜRNBERG

Universitätsklinikum Erlangen

27th Annual Meeting of the EMDS

Myeloid Cells:

Microenvironment, Microorganisms and Metabolism

October 10-12, 2013

Erlangen, Germany

www.emds2013.eu

The 27th Annual Meeting is held and organized by
the *European Macrophage and Dendritic Cell Society (EMDS)*

in cooperation with

the Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU) and
the Universitätsklinikum Erlangen.

Contents

Welcome Address

Dear colleagues and friends,

We cordially welcome you to the **27th Annual Conference of the *European Macrophage and Dendritic Cell Society* (EMDS)**, which this year is held in Erlangen, Germany. We are glad that you decided to attend this meeting, despite the fact that every year several other scientific congresses in related fields take place in autumn.

The annual EMDS meetings date back to the former macrophage symposia in the Upper Rhine area organized by scientists from Strasbourg, Basel and Freiburg (see history of the EMDS). For the first time, we have this European meeting now in Franconia in the north of the German Federal State of Bavaria. The **Friedrich-Alexander-University Erlangen-Nürnberg (FAU)**, which will celebrate its 270th anniversary next month, has a strong research focus in life science, notably in immunology. Within the *Medical Immunology Campus Erlangen*, which was founded in 2009, more than 70 independent research groups at the FAU and the University Hospital Erlangen work on a broad spectrum of basic and clinical aspects of immunology. The German Research Foundation (DFG) currently supports three Collaborative Research Centers (SFB 643 "Strategies of cellular immune intervention"; SFB 796 "Reprogramming of host cells by microbials effectors" and Transregio 130 "B cells: Immunity and autoimmunity"), three Research Training Groups and two Research Units in the field of immunology and/or infection biology. In the 2012 National Funding Atlas of the DFG Erlangen has been ranked first in the category "immunology, microbiology and virology". All this is meant to emphasize that immunology is very close to our hearts. Needless to say that many of us have had a strong and long-standing interest in macrophages and dendritic cells.

This year´s EMDS meeting focusses on the interaction of myeloid cells with the microflora and microbial pathogens, on their modulation by the microenvironment and milieu factors, and on aspects of myeloid metabolism and development. These central themes will be covered in seven symposia by 18 invited distinguished scientists. 130 abstracts were submitted, from which 23 were selected by an international review panel for short oral presentations. The more than 200 participants come from 18 countries (14 European countries as well as from the USA, Canada, Mexico, and Australia). Following the traditional format of the EMDS meetings, there is only one lecture room and the poster presentations, coffee and lunch breaks and the industrial exhibition are in one and the same area. As a novelty we will have an **extended poster session with an "Octoberfest" tent and a dinner buffet** on Thursday evening. The **banquet** and presentation of the EMDS Awards on Friday evening will take place in the **Grand Ballroom (Redoutensaal)**, which was built in 1718 to stage glittering balls.

We wish you all an enjoyable, scientifically rewarding and memorable meeting in Erlangen.

Christian Bogdan
Chairman of the 27th EMDS Meeting

Alexander Steinkasserer
Co-Chairman of the 27th EMDS Meeting

On behalf of the Local Organizing Committee and all assistants and helpers

Organizers, Committees and Assistance

The 27th Annual Meeting is held and organized by the *European Macrophage and Dendritic Cell Society (EMDS)* in cooperation with the Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU) and the Universitätsklinikum Erlangen.

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David Vöhringer, *Infection Biology*

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Conference Homepage

Christian Bodin, *Department of Dermatology*
Werbeagentur pixWork, Forchheim
Sonja Pötzsch (Meeting Coordinator)

Digital Art, Photography and Print

Sonja Pötzsch (Meeting Coordinator)
Michael Schlütter, Thierra Productions, Wilhermsdorf
Druckhaus Haspel Erlangen e.K.

Poster exhibition

Messebau Wörnlein GmbH, Nürnberg
Zeltverleih Gröschel, Roßtal

Catering and Social Event

Flinke Fee Partyservice, Erlangen
Elbl Getränkevertrieb, Neunkirchen a. Brand
Unicum GbR

Conference Office and Registrations

Liliana Bodin
Andrea Debus
Kathrin Pohl
Sonja Pötzsch (Meeting Coordinator)
Heidi Sebald
Irene Wittmann

...and many student and postdoc volunteers!!

Conference Contact

+49-173-8645010 (during the conference only)
+49-9131-8522551 (Prof. Dr. Christian Bogdan)
+49-9131-8522571 (Dr. Sonja Pötzsch)

The European Macrophage and Dendritic Cell Society is grateful to the following companies for their generous support of the 27th Annual EMDS Meeting:

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The European Macrophage and Dendritic Cell Society (EMDS)

History

The *European Macrophage and Dendritic Cell Society* (EMDS) has emerged from the activities of the former *European Macrophage Study Group* (EMSG), a loose association of scientists interested in basic and clinical aspects of monocytes, macrophages, dendritic cells and other myeloid cells in man and experimental animal models.

The EMSG was constituted in 1992 as a result of a successful series of annual conferences called THE MACROPHAGE. The annual macrophage conference originated from meetings in the Upper Rhine area organized by scientists from the universities and research centres of Strasbourg, Freiburg and Basel, but rapidly grew up to a European format.

On April 28, 1999, the *European Macrophage Society* (EMS) was founded in Regensburg. At the end of the year 2000, the members of the EMS decided to rename the society as *European Macrophage and Dendritic Cell Society* (EMDS) in order to better emphasize the two main streams of research within the Society.

The EMDS has currently ca. 500 members from 35 countries. The annual registration fee is 25 Euro. For more information please see our website: http://www.macrophage.de

Specific aims of the Society are:

- to promote outstanding, innovative studies in the field of macrophage and dendritic cell biology with respect to both basic and clinical research
- to provide a forum for the interdisciplinary exchange of basic and clinical knowledge and concepts in the field of macrophages, dendritic cells and other myeloid cells
- to accelerate the development and clinical application of new therapeutic strategies to fight infections, autoimmune diseases, inflammatory disorders, immunodeficiencies, cancer, and metabolic diseases
- to improve and support the training of young researchers who are interested in the study of macrophages, dendritic cells and other members of the myeloid lineage

Awards of the EMDS

Biannual BD Biosciences Award of the EMDS (5.000 €)
This prize is sponsored by BD Biosciences and will be awarded to a young scientist for excellency in the field of macrophage or dendritic cell biology every second year. The decision is made by the members of the EMDS Council assisted by members of the Advisory Board. Application for the prize is not possible.
Previous Awardees: Caetano Reis e Souza, London (2002); Teunis Geijtenbeek, Amsterdam (2004); Matthew Albert, Paris (2006); Falk Nimmerjahn, Erlangen (2008); Nicole Kaneider, Innsbruck (2010); Antonio Castrillo, Madrid (2012)

EMDS Conference Travel Awards (max. 800 €)
Participants (below the age of 35) of the annual EMDS Meeting are welcome to apply for a Young Investigator Travel Award. The decision is made by the organizing committee based upon the CV, the scientific quality of the submitted abstract, and the financial needs of the applicants. Applicants must be members of the EMDS.

EMDS Poster Prizes (200 €)
During the Annual EMDS Meeting, five poster prizes will be awarded to the first authors of the best presentations. The selection is made by members of the organizing committee.

Council of the EMDS (2011-2014)

President	Silvano Sozzani, Brescia, Italy
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Location of the Annual Meetings since 1992

1992 Regensburg, Germany
1993 Basel, Switzerland
1994 Lausanne, Switzerland
1995 Amsterdam, The Netherlands
1996 Verona, Italy
1997 Lübeck, Germany
1998 Paris, France
1999 Cambridge, England
2000 Krakow, Poland
2001 Vienna, Austria
2002 Basel, Switzerland
2003 Leicester, England
2004 Barcelona, Spain
2005 Amsterdam, The Netherlands
2006 Freiburg, Germany
2007 Innsbruck, Austria
2008 Brescia, Italy
2009 Regensburg, Germany
2010 Edinburgh, United Kingdom
2011 Brussels, Belgium
2012 Debrecen, Hungary
2013 Erlangen, Germany
2014 Vienna, Austria

Erlangen and its Franconian Neighbourhood at a Glimpse

Erlangen has around 103,000 inhabitants and is located in central Franconia in the north of Bavaria. The village “erlangon” on the left side of the river Regnitz was first mentioned in a charter of emperor Heinrich II. in 1002. In 1361, the village was bought by emperor Karl IV. and subsequently rapidly developed into a town with municipal rights. The newer Huguenot city (“Neustadt”) dates from 1686. The Huguenot Erlangen is one of the last remaining examples of baroque planning, laid out in a rectangular arrangement of streets and large squares. The beautiful baroque garden, the *Schlossgarten*, is located in the center of Erlangen surrounded by impressive buildings from the 18th and 19th century.

The **Friedrich-Alexander Universität Erlangen-Nürnberg (FAU)** was founded in 1743 by Karl Wilhelm Friedrich, Margrave of the Principality of Brandenburg-Ansbach. Under his son Karl Alexander, Margrave of the Franconian Principalities Brandenburg-Ansbach and Brandenburg-Bayreuth, the university rapidly gained size and reputation due to his significant support. Today, the FAU is the second largest university in Bavaria with more than 35,000 students, 5 faculties (one of which is located in Nürnberg), 23 departments and more than 600 professors. The Universitätsklinikum Erlangen, founded in 1824, comprises 24 clinics, 19 independent divisions and six institutes.

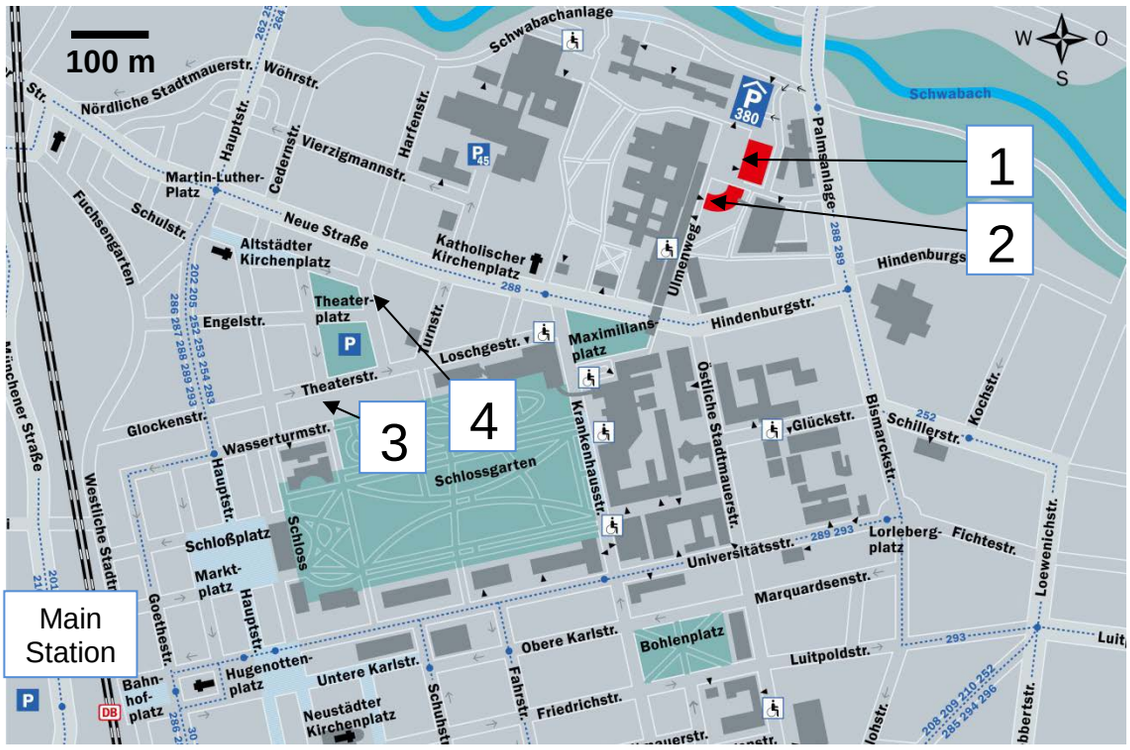
Nürnberg (Nuremberg), 25 kilometers south of Erlangen, is Bavaria’s second largest city with half a million residents. In the old part of the city, one can find innumerable historic monuments and buildings dating back to the 11th century, when Nürnberg was first mentioned in an official document. Romantic half-timbered houses, cobblestoned squares, spectacular church facades, beautiful bridges and fountains, a five kilometer long historic city wall from the 15th/16th century almost completely surrounding the old town and the majestic Imperial Castle attract more than 35 million tourists a year.

For close to 500 years, the Imperial Castle, dating back to before the year 1000, was the residence of the emperors of the Holy Roman Empire. In 1356, Nürnberg’s significance as imperial city was further augmented when Emperor Karl IV. decreed an imperial edict (so-called „Golden Bull“) that every newly elected King was required to hold his first imperial diet in Nürnberg. Subsequently, Nürnberg became the depository of the imperial regalia, the symbols of imperial majesty (crown, scepter, imperial orb, sword, and holy lance). They were kept in the “Church of Our Lady“ (Frauenkirche) until 1796. Around the year 1500, the medieval city of Nürnberg was at the height of its power and wealth. At this point in the history of Nürnberg, a continuous stream of science, paintings, and sculpture were created. For example, *Martin Behaim* designed his first Globe, *Peter Henlein* invented the first pocket watch and *Albrecht Dürer,* the city’s most celebrated son, created his world famous masterpieces.

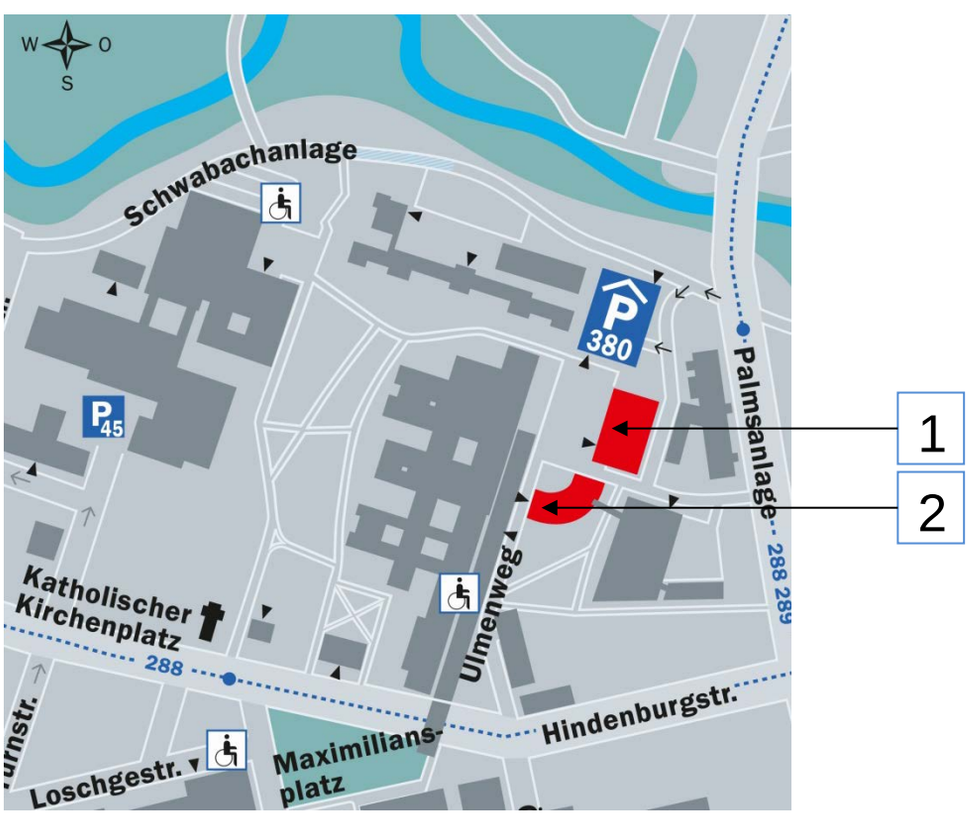
During the „Third Reich“, Adolf Hitler made Nuremberg "City of the Party Rallies", and it was here that the atrocious racial laws were adopted, and the main war criminals of the Nazi regime of terror were tried by the International Military Tribunal in the "Nuremberg Trials". Since then, the city, which was badly damaged by bombs during World War II, has been making big efforts in confronting its Nazi past. In June 2001, it became the first city to be awarded with the UNESCO human rights prize.

Bamberg, 40 kilometers north of Erlangen, was awarded "World Heritage" status by UNESCO in 1993. Unlike Nürnberg and many other cities in Germany, the town, which has around 70,000 inhabitants today, was scarcely damaged during World War II and still retains many splendid medieval architectural features and over 3,000 historical buildings. Bamberg is world famous for its cathedral (with the tomb of emperor Heinrich II. and his wife Kunigunde, the grave of Clemens II., the only grave of a pope north of the alps, and the horseman statue), its medieval town hall located in the river Regnitz, and the unique assembly of romanic, gothic, renaissance and baroque ecclestial and secular buildings on the cathedral hill.

Map of Erlangen and Conference Venue

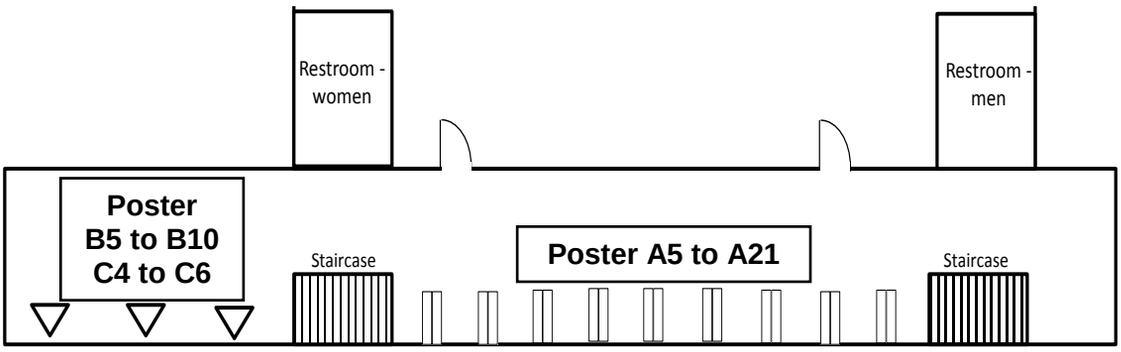


1 = Conference Venue (Neues Hörsaalgebäude, Ulmenweg 18)
2 = Council Meeting (Personalrestaurant “Palmeria”; 1st floor, Room “Erlangen”)
3 = Banquet (Redoutensaal; entrance from Theaterstraße via a small arcade)
4 = Hotel Rokokohaus (invited speakers; Theaterplatz 13, Tel. +49-9131-7830)

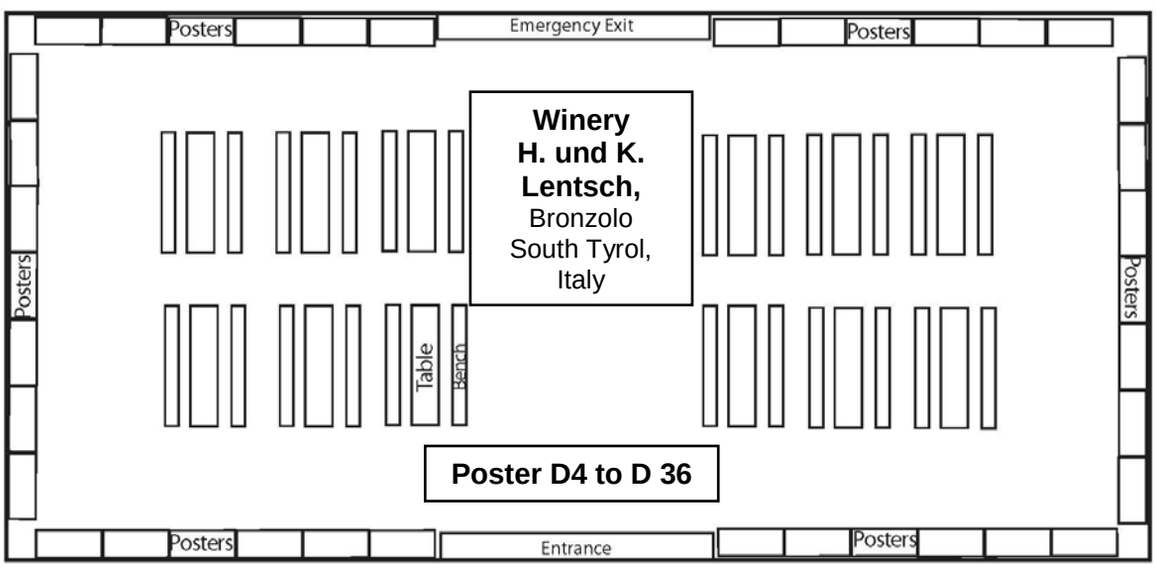


Map of the Poster Exhibition

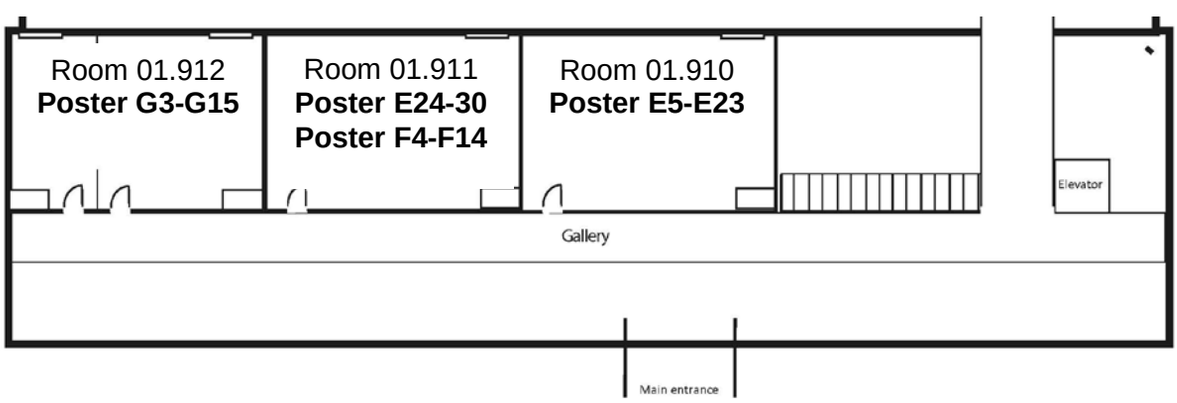
A. **Basement** (Lecture Hall building)



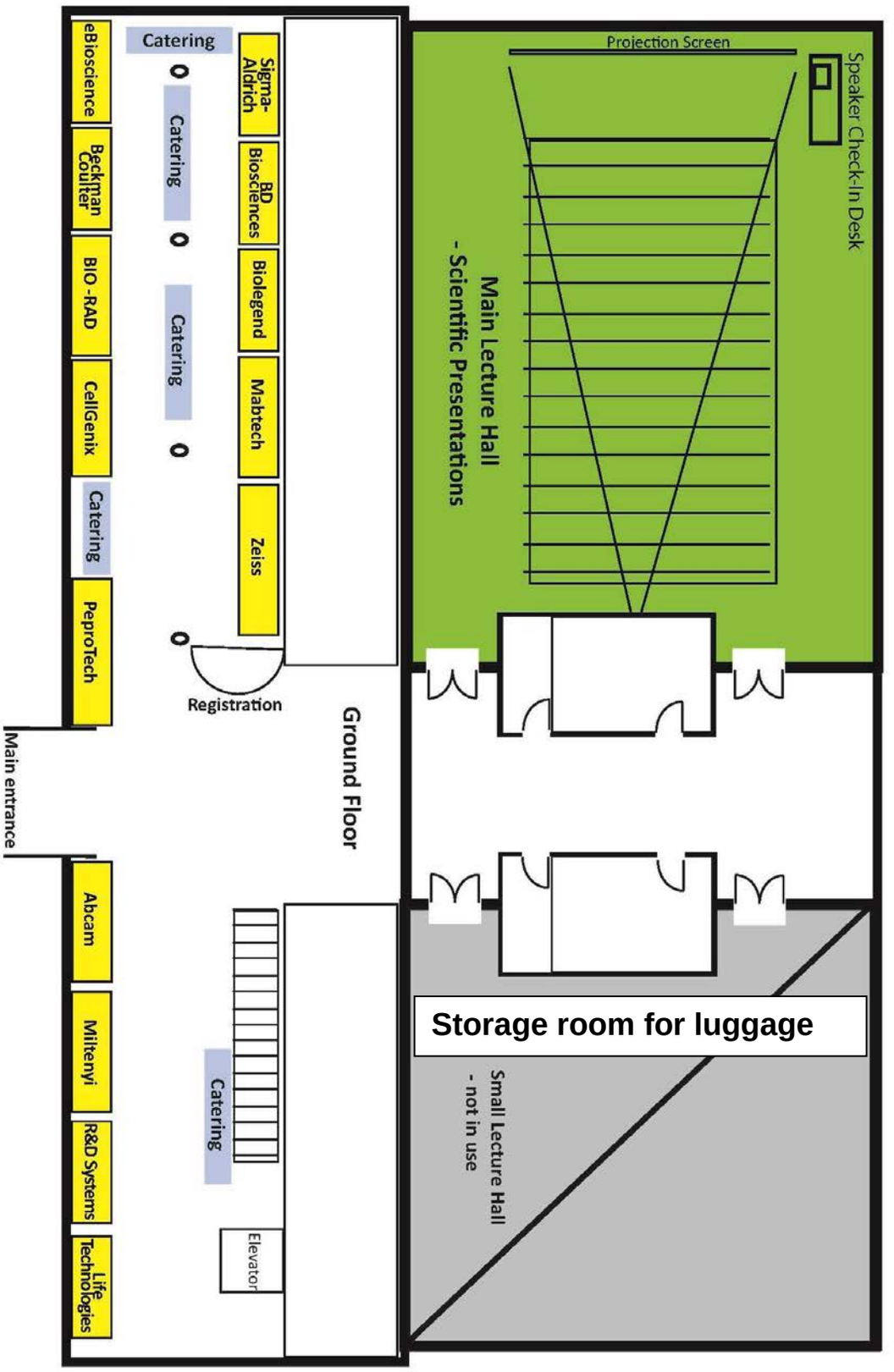
B. **Groundfloor** (Octoberfest-Tent in front the Lecture Hall building)



C. **1st Floor** (Gallery of the Lecture Hall building)



Map of the Industrial Exhibition and Lecture Hall



Instructions for Presenting Participants

Oral presentations

- **Invited symposium lectures** will be 25 min plus 5 min discussion.
- **Short oral presentations** will be 10 min plus 5 min discussion.
- **No personal computers will be accepted in the lecture room.**
- Please provide your talk as a Powerpoint file (**USB-stick**) in the **Lecture Hall the day before** (best option) or **in one of the coffee/lunch breaks prior to** your session.

Poster presentation

- All posters will be presented **throughout the meeting**.
- **Poster size:** A0 portrait (maximum width 90 cm, maximum height 150 cm)
- Please insert your **poster number** in the upper **right** angle of your poster
- During the poster sessions indicated in the program the presenting authors are kindly asked to be available at their posters for discussions

Scientific Program

SCIENTIFIC PROGRAM

Thursday, October 10, 2013

10:00 Registration opens

11:00 Meeting of the EMDS Council

12:45 Opening of the Conference
- Silvano Sozzani, President of the EMDS
- Karl-Dieter Grüske, President of the Friedrich-Alexander-Universität Erlangen-Nürnberg
- Michael Wegner, Vice Dean of the Medical Faculty
- Christian Bogdan and Alexander Steinkasserer, Chairmen of the 27th EMDS Meeting

Keynote Lecture

13:15 Carl Nathan, New York, USA
Revisiting macrophage activation

Symposium A - Myeloid Cell Development

Session Chair: Michael Rehli (Germany), Amaya Puig-Kröger (Spain)

14:00 Marc Dalod, Marseille, France
A systems biology approach to accelerate translation of knowledge on DC subsets from the mouse model to other species

14:30 Boris Reizis, New York, USA
Molecular control of plasmacytoid dendritic cell development and function

15:00 Martin Guilliams, Ghent, Belgium (A01)
Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life under influence of local GM-CSF

15:15 Chen Jianzhu, Cambridge, USA (A02)
Induction of functional human macrophages from bone marrow promonocytes by M-CSF (CSF-1) in humanized mice

15:30 Gyorgy Fejer, Plymouth, UK (A03)
Unique innate responses in a novel, self-renewing, non-transformed, GM-CSF dependent model of alveolar macrophages

15:45 Kristin Bieber, Tübingen, Germany (A04)
The innate immune systems favors emergency monopoiesis at the expense of DC differentiation to promote control of bacterial pathogens

16:00 Coffee break, industrial exhibition and posters

Symposium B - Myeloid Cells and Organ Specificity

Session Chair: Loems Ziegler-Heitbrock (Germany), Falk Nimmerjahn (Germany)

16:30 Eric Pamer, New York, USA
Inflammatory monocyte trafficking in response to pulmonary infection

17:00 Oliver Pabst, Hannover, Germany
Migration and function of intestinal myeloid cells

17:30 Alexander Misharin, Chicago, USA (B01)
Novel role for Ly6C- monocyte subsets and joint macrophages in mouse model of rheumatoid arthritis

17:45 Guy Shakhar, Rehovot, Israel (B02)
Local dissemination of antigen within networks of live DCs facilitates T cell activation in lymph nodes

18:00 Heleen Vroman, Rotterdam, The Netherlands (B03)
Dendritic cell activation status directs T helper responses in a HDM allergic asthma model

18:15 Raquel Alvarado, Ultimo, Australia (B04)
A novel molecule secreted by the parasite Fasciola hepatica modulates the response of macrophages to TLR stimulation

18:30 Industrial exhibition and Poster Session A with Franconian dinner buffet and drinks (until approx. 22:00)

SCIENTIFIC PROGRAM

Friday, October 11, 2013

Symposium C - Microflora of the Gut, Commensals

Session Chair: Silvano Sozzani (Italy), Kai Hildner (Germany)

8:30 Andreas Diefenbach, Freiburg, Germany
Commensal microbiota instructs mononuclear phagocytes

9:00 Maria Rescigno, Milan, Italy
Dendritic cells in host-microbe interactions in the gut

9:30 Arthur Mortha, New York, USA (C01)
Microbiota-driven crosstalk between myeloid cells and RORgt+ innate lymphoid cells controls T cell homeostasis in the gut

9:45 Regina Schey, Erlangen, Germany (C02)
CD101 protects from intestinal inflammation

10:00 Manfred Nairz, Innsbruck, Austria (C03)
The erythropoietin-analogue ARA290 ameliorates the course of experimental colitis

10:15 Coffee break, industrial exhibition and posters

Symposium D - Microbial Pathogens

Session Chair: Thomas Decker (Austria), Roland Lang (Germany)

10:45 Julie Blander, New York, USA
Rabs and toll-like receptors: Firing up cross-presentation in dendritic cells

11:15 Gordon Brown, Aberdeen, UK
C-type lectins in immunity and homeostasis

11:45 Pavel Kovarik, Vienna, Austria (D01)
Streptococcus pyogenes RNA activates Toll-like receptor signaling in mouse but not human cells and confers resistance to infection in mice

12:00 David Sancho, Madrid, Spain (D02)
Recognition of Leishmania parasites by the macrophage-inducible C-type lectin Mincle subverts adaptive immunity and promotes susceptibility to infection

12:15 Peter Crauwels, Langen, Germany (D03)
LC3 associated phagocytosis in human primary macrophages can reduce Leishmania specific T cell proliferation

12:30 Lunch break, industrial exhibition and Poster Session B

Symposium E - Microenvironment and Tumor - Part 1

Session Chair: Jo van Ginderachter (Belgium), Dimitrios Mougiakakos (Germany)

14:00 Michael Shurin, Pittsburgh, USA
Interdifferentiation and control of myeloid regulatory cells in the tumor immunoenvironment

14:30 Gerold Schuler, Erlangen, Germany
Immune intervention by adoptive transfer of dendritic cells

15:00 Geert Raes, Brussels, Belgium (E01)
Myeloid cells as targets for nanobody-mediated imaging of inflammation and cancer

15:15 Raffaella Bonecchi, Milan, Italy (E02)
Downregulation of the chemokine decoy receptor D6 unleashes Kaposi's sarcoma growth

15:30 Coffee break, industrial exhibition and posters

SCIENTIFIC PROGRAM

Friday, October 11, 2013

Symposium E - Microenvironment and Tumor - Part 2

Session Chair: Maciek Siedlar (Poland), Udo Gaipl (Germany)

16:00 Carl Figdor, Nijmegen, The Netherlands
Blood and tumor microenvironment in melanoma after dendritic cell vaccination

16:30 Karolina Palucka, New York, USA
Reprogramming the immune environment in cancer via dendritic cells

17:00 Sandra van Vliet, Amsterdam, The Netherlands (E03)
Tumor-associated glycans trigger the C-type lectin MGL on dendritic cells and dampen anti-tumor immunity

17:15 Lisa Vogelpoel, Amsterdam, The Netherlands (E04)
Human M2 macrophages promote inflammation in rheumatoid arthritis via synergy between Fc gamma receptors and TLRs

17:30 EMDS Business Meeting - EMDS Members only

19:30 Social Evening and Presentation of the EMDS Awards at the Redoutensaal Erlangen (until approx. 1:00)

Saturday, October 12, 2013

Symposium F - Tissue Injury and Repair

Session Chair: Angel Corbi (Spain), Martin Herrmann (Germany)

8:30 Michel Gilliet, Lausanne, Switzerland
Innate sensing of nucleic acids by DC in immunity and autoimmunity

9:00 Rikard Holmdahl, Stockholm, Sweden
Redox control of autoimmunity and inflammation

9:30 Ajay Chawla, San Francisco, USA
Type 2 immunity at crossroads of metabolism and regeneration

10:00 Efstathios Stamatiades, London, UK (F01)
Nr4a1-dependent monocytes monitor endothelial cells and orchestrate their necrosis by neutrophils in response to a TLR7-mediated danger signal

10:15 Johanna Schott, Heidelberg, Germany (F02)
Translational control of specific mRNAs is important for cellular survival and the anti-inflammatory feedback during macrophage activation

10:30 Matthias Gebhardt, Berlin, Germany (F03)
High salt (NaCl) affects H3K4 trimethylation and alternative macrophage activation

10:45 Coffee break, industrial exhibition and posters

Symposium G - Hypoxia, Micronutrients, Metabolism

Session Chair: Luigi Varesio (Italy), Diana Dudziak (Germany)

11:15 Bernhard Brüne, Frankfurt, Germany (G01)
The tumor microenvironment under the influence of HIF-1α in myeloid cells

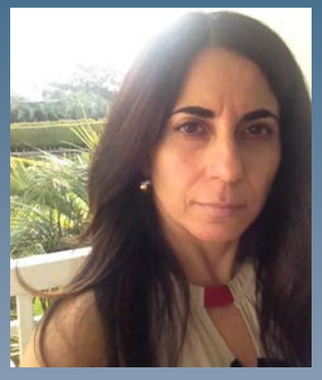
11:30 Jonathan Jantsch, Erlangen, Germany (G02)
Hypoxia impairs the NO-dependent leishmanicidal activity of macrophages and prevails in the skin lesions of Leishmania major-infected mice

11:45 Randall Johnson, Cambridge, England
HIF-1 vs HIF-2 balance: a key factor in regulating the micro-environment

12:15 Ursula Grohmann, Perugia, Italy
IDO: an eclectic metabolic enzyme serving immune regulation

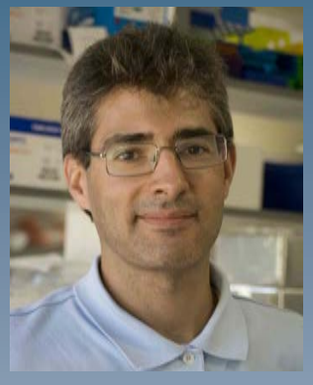
12:45 Farewell and end of conference

Invited Speakers of the 27th Annual Meeting of the EMDS
Erlangen, Germany, October 10 - 12 2013


Julie Blander


Gordon Brown


Ajay Chawla


Marc Dalod

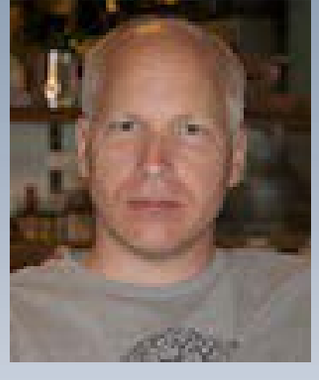

Andreas Diefenbach

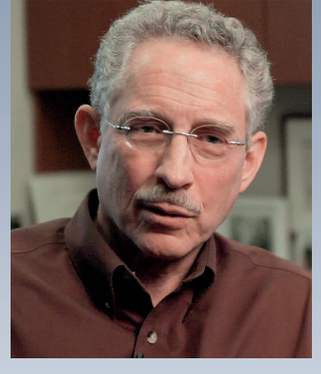

Carl Figdor


Michel Gilliet


Ursula Grohmann


Rikard Holmdahl


Randall Johnson


Carl Nathan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Karolina Palucka


Eric Pamer


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Abstracts

of the

Short Oral Communications

A01

Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life under influence of local GM-CSF.

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Tissue-resident macrophages can develop from circulating adult monocytes, or from primitive yolk-sac-derived macrophages. The precise ontogeny of alveolar macrophages (AMFs) is unknown. By performing BrdU labeling, parabiosis and radiation chimera experiments in adult mice, we found that circulating monocytes contributed minimally to the steady state AMF pool. Mature AMFs were undetectable before birth and only fully colonized the alveolar space by 3 days after birth. Before birth, F4/80^{hi}CD11b^{lo} primitive macrophages and Ly6C^{hi}CD11b^{hi} fetal monocytes sequentially colonized the developing lung around E12 and E15, respectively. The first signs of AMF differentiation appeared around the saccular stage of lung development (E18). Adoptive transfer identified fetal monocytes but not primitive macrophages as the main precursors of AMFs. Fetal monocytes transferred to the lung of neonatal mice acquired an AMF phenotype via defined intermediate developmental stages over the course of one week, and persisted in the lungs for at least three months. Early AMF commitment from fetal monocytes was absent in GM-CSF-deficient mice, while short-term perinatal intrapulmonary cytokine therapy rescued AMF development for weeks. We have sorted cells in the defined intermediate developmental stages and are currently performing a thorough micro-array analysis to unravel the molecular cues that regulate AMF development. This is the first demonstration that tissue-resident macrophages can also develop from fetal monocytes that adopt a stable tissue phenotype shortly after birth in response to instructive cytokines and then self-maintain throughout life.

A02

Induction of Functional Human Macrophages from Bone Marrow Promonocytes by M-CSF (CSF-1) in Humanized Mice

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Engraftment of human CD34^{+} hematopoietic stem/progenitor cells into immunodeficient mice leads to robust reconstitution of human T and B cells but not monocytes and macrophages. To identify the cause underlying the poor monocyte and macrophage reconstitution, we analyzed human myeloid cell development in humanized mice and found that it was blocked at the promonocyte stage in the bone marrow. Expression of human M-CSF or GM-CSF by hydrodynamic injection of cytokine-encoding plasmid completely abolished the accumulation of promonocytes in the bone marrow. M-CSF promoted the development of mature monocytes and tissue resident macrophages whereas GM-CSF did not. Moreover, correlating with an increased human macrophages at the sites of infection, M-CSF treated humanized mice exhibited an enhanced protection against influenza virus and *Mycobacterium* infection. Our study identifies the precise stage at which human monocyte/macrophage development is blocked in humanized mice and reveals overlapping and distinct functions of M-CSF and GM-CSF in human monocyte and macrophage development. The improved reconstitution and functionality of monocytes/macrophages in the humanized mice following M-CSF expression provide a superior *in vivo* system to investigate the role of macrophages in physiological and pathological processes.

A03

Unique Innate Responses in a Novel, Self-renewing, Non-transformed, GM-CSF-Dependent Model of Alveolar Macrophages

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Macrophages represent the first line of defence against invading pathogens and comprise a group of cells diverse in their origin, development, lifespan and function. A significant part of our knowledge on pathogen elicited macrophage responses comes from currently available *ex-vivo* models using M-CSF differentiated bone-marrow derived macrophages (BMMs) with a limited lifespan. We developed a novel method yielding non-transformed, GM-CSF dependent, differentiated, but continuously growing macrophages (MPI cells) from mouse foetal liver. Global gene expression profiles and surface markers indicated that MPI cells and BMMs are distinct cell types and suggested the similarity of MPI cells to lung alveolar macrophages (AMs). A GM-CSF/STAT5 dependent novel innate reactivity pattern indicated a close functional relationship between these two cell-types. MPI cells and AMs, unlike other known macrophages, sense rough-form LPS requiring LBP/CD14. They also have dramatically increased sensitivity to mycobacterium, its component cord factor and adenovirus. Upon stimulation, MPI cells and AMs show a strongly pro-inflammatory cytokine response without anti-inflammatory IL-10 production, including the effective and unconventional production of IL-1α. In conventional systems, TLR ligands induce the production of pro-ILα and an independent signal triggers the cleavage and secretion of mature ILα. In MPI cells however, ultrapure LPS alone stimulates the secretion of pro-ILα, a process not observed previously in macrophages. We demonstrate the importance of this new pathway by showing that LPS induced lung damage requires ILα production in mice. MPI cells therefore revealed novel innate immune pathways and represent a new tool to study macrophage functions, especially AM specific mechanisms.

A04

The innate immune system favors emergency monopoiesis at the expense of DC differentiation to promote control of bacterial pathogens

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Dendritic cells (DCs) are critical in host defense against infection, bridging the innate and adaptive immune systems. How pathogens influence DC commitment remains still elusive. Here we employed a previously described mouse model of systemic bacterial infection to analyze *in vivo* the impact of different bacteria on DC development. We found that exposure to bacterial infection reduced the numbers of bone marrow hematopoietic progenitors of the monocyte and DC lineages in a TLR4-dependent manner, irrespectively of the individual pathogen. This reduction occurred concomitant to increased numbers of monocyte progenitors in the bone marrow and monocytes in the spleen during infection, whereas the number of newly generated DCs is reduced. Mechanistically bacterial infection led to increased MafB expression in monocyte dendritic cell progenitors (MDPs), whereas the expression of PU.1 was unaltered, indicating a biased differentiation of myeloid progenitors into monocytes. Our study support the notion, that systemic bacterial infection leads to a general attrition of myeloid progenitors in the bone marrow and cDCs in the periphery, which can be compensated by emergency monopoiesis not only to sustain but even increase the numbers of innate immune monocytes to promote pathogen control.

B01

Novel role for Ly6C- monocyte subsets and joint macrophages in mouse model of rheumatoid arthritis

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Monocytes and macrophages play a key role in the pathogenesis of rheumatoid arthritis. However, role of the individual subsets of monocytes and macrophages in the initiation, perpetuation and/or resolution of arthritis is unknown. To uncover their role we utilized multiple strategies to deplete selective subsets of monocytes and macrophages in the K/BxN serum transfer arthritis mouse model. We found that contrary to the current dogma monocytes, and not neutrophils, were necessary for initiation of arthritis. Moreover, we found that only non-classical Ly6C^{-} monocytes were required for induction of arthritis, while classical Ly6C^{+} monocytes were dispensable. Further, we identified that naïve mouse joint contains heterogeneous population of macrophages, which differ in their origin, turnover and function, namely tissue-resident macrophages and bone marrow-derived macrophages. Selective depletion of the tissue-resident macrophages accelerated development of arthritis, while depletion of bone marrow-derived macrophages had no effect on arthritis. While blood monocytes were necessary for initiation and development of arthritis, they were not necessary for its resolution. In contrast, tissue macrophages were crucial for the resolution, since their depletion delayed resolution of arthritis and was associated with increased histological joint damage. These data suggest that unlike other models (myocardial infarction, infectious diseases) resolution of arthritis does not require second wave of monocyte recruitment into the joint, but rather dependent on a molecular rheostat within macrophages, which controls the switch of their phenotype from “proinflammatory/classically activated” to a “wound healing/regulatory”. Phagocytosis of apoptotic neutrophils (efferocytosis) is potentially one of the mechanisms controlling this switch.

B02

Local dissemination of antigen within networks of live DCs facilitates T cell activation in lymph nodes

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To initiate primary immune responses, naïve T cells must locate DCs that present their cognate Ag in the T zones of lymphoid organs, where we have previously visualized immigrant DCs as they join networks of sessile resident DCs. We investigated whether such immigrant DCs transfer Ag to resident DCs. Such Ag distribution could serve as a “retention signal” for T cells to continue migrating on the local DC networks until they locate the immigrant DCs. Using *in vitro* imaging of DC networks we showed that rare Ag-loaded DCs transfer Ag to bystander DCs. CD8 T cells arrested and interacted with these bystander DCs while fluxing calcium. The presence of presentation-competent bystander DCs boosted T cell activation and proliferation. *In vivo*, the spatial aspects of the interaction between CD8 T cells and Ag presenting cells were visualized by two-photon microscopy of LNs. When H2Kb$^{-/-}$ presentation-deficient immigrant DCs were used, T cells clustered on nearby bystander DCs, and were efficiently activated, suggesting Ag-transfer. Several mechanisms seem to act in parallel to transfer Ag between DCs: in vitro Ag transfer occurs only between live functioning DCs. *In vivo*, efficient T cell stimulation required the migration of viable Ag-loaded DCs to the draining LNs and proximity between immigrant and resident DCs. DC-derived exosomes participate in Ag transfer but play a non-exclusive role. Thus, immigrant DCs facilitate T cell activation by actively transferring, partly through exosomes, Ag to resident DCs.

B03

Dendritic cell activation status directs T helper responses in a HDM allergic asthma model

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Allergic asthma is a chronic Th2 mediated inflammatory disease of the airways in response to inhaled antigens such as house dust mite (HDM). Dendritic cells (DCs) are essential in both the sensitization as the challenge phase of allergic asthma. Activation of DCs is regulated by A20, a negative regulator of NF-kappaB. We hypothesize that mice lacking A20 in DCs will show an increased Th2 mediated immune response in an HDM-driven model for allergic asthma. $A20^{fl/fl}$*Cd11c*Cre^{+} mice (DC-A20 mice) were sensitized with 1 µg HDM or PBS and challenged with 10 µg HDM. Additionally, C57BL/6 mice were intratracheally sensitized with bone marrow-derived immature and HDM-matured A20-DCs and challenged with HDM. Exposure of DC-$A20^{WT}$ mice and DC-$A20^{HZ}$ mice to HDM induced massive eosinophilic infiltration in the airways, increase in mucus-producing goblet cells and expression of IL-5 and IL-13 by $CD4^{+}$ T cells. Surprisingly, DC-$A20^{KO}$ mice were resistant to induction of Th2-driven allergic inflammation, but showed an increased amount of infiltrating neutrophils and IL-17A expression in CD4+ T cells. Complementary to this, no increased mucus production was found. C57BL/6 mice sensitized with HDM-matured $A20^{WT}$ DCs and challenged with HDM showed a typical Th2-mediated immune response, while this was absent in mice sensitized with $A20^{KO}$ DCs. In two allergic asthma models we showed that lack of A20 in DCs results in a shift from a Th2 towards a Th17 immune response in contrast with our hypothesis. This indicates that DC activation is imperative to direct T helper responses in allergic inflammation.

B04

A novel molecule secreted by the parasite *Fasciola hepatica* modulates the response of macrophages to TLR stimulation

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In response to tissue damage, elicited through injury or infection, mammals secrete host defence peptides (HDPs) to perform a range of immune modulatory functions, the net result of which is a balance between pro- and anti-inflammatory immune responses. In particular, the mammalian cathelicidin HDPs have a crucial role in regulating TLR-dependent innate inflammatory responses. This means that they function to maintain homeostasis in response to natural shedding of microflora-TLR agonists as well as controlling the systemic inflammatory response to infection or tissue damage. The immune response elicited by helminth (worm) parasites is akin to the innate immune response to tissue injury and wound healing. We have identified a novel family of molecules secreted by medically-important helminth pathogens (termed helminth defence molecules; HDMs) that exhibit striking structural and biochemical similarities to the mammalian cathelicidins. In addition, like the mammalian HDP, LL37, the HDM from the helminth parasite *Fasciola hepatica* (FhHDM-1) regulates the innate immune response to TLR stimulation. Treatment of macrophages with FhHDM-1 inhibits the secretion of pro-inflammatory cytokines in response to stimulation with bacterial lipopolysaccharide. Furthermore, FhHDM-1 also reduces the NLRP-3 dependent activation of caspase-1, thus preventing secretion of IL-1β from macrophages. We propose that the function of FhHDM-1 is similar to the predicted role for the mammalian HDPs; inhibition of an excessive inflammatory response. This ensures the longterm survival of the worm and simultaneously protects the host from exacerbated tissue damage as a result of its migration.

C01

Microbiota-driven crosstalk between myeloid cells and RORγt$^+$ innate lymphoid cells controls T cell homeostasis in the gut.

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The intestinal microbiota and tissue resident myeloid cells promote regulatory immune responses that maintain mucosal homeostasis in the host. However, the cellular cues that translate microbial signals into regulatory host immunity remain unclear. Here we show that deficient GM-CSF production strongly altered myeloid cell effector functions and lead to reduced regulatory T cells (Tregs), increased IFNγ-producing T cells and increased susceptibility to colitis. Strikingly, we observed that RORγt$^+$ innate lymphocyte cells (ILCs) are the primary source of GM-CSF in the gut and that ILC-driven GM-CSF production was dependent on the ability of macrophages to sense microbial signals. Importantly, we discovered that IL-1β produced by intestinal macrophages in response to commensal signals controlled ILC production of GM-CSF, which in turn promoted myeloid cell-mediated control of T cell homeostasis in the intestine. Our findings reveal that commensal microbes promote a crosstalk between innate myeloid and lymphoid cells that leads to immune homeostasis in the intestine.

C02

CD101 protects from intestinal inflammation

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Complex interactions between environmental factors, genetic factors and dysregulated immune responses contribute to the pathogenesis of inflammatory bowel disease (IBD). However, molecules and signaling pathways controlling the interactions of the intestinal microflora with the immune system of the patient and their role in the maintenance of mucosal homeostasis have been rarely identified.

CD101 is a negative costimulatory molecule that is primarily expressed in the gut on subsets of dendritic cells, macrophages and granulocytes as well as on regulatory and memory T cells. We have recently characterized CD101 as critical factor for the control of intestinal inflammation in a chronic T cell transfer colitis model. CD101 was absent from naïve CD45RB^{high} CD25-negative T cells in wild-type mice before the adoptive transfer. CD101-deficient recipient mice exhibited significantly more severe intestinal pathology than CD101-expressing littermate recipients correlating with an acquisition of CD101-expression on donor T cells and enhanced IL-10-production by myeloid recipient cells. Co-transferred regulatory T cells that expressed CD101 suppressed intestinal inflammation more efficiently than their CD101-negative counterparts. In contrast, a lack of CD101 expression on myeloid recipient or donor T cells inhibited the generation of regulatory T cells, triggered T cell proliferation and promoted the differentiation of naïve T cells into effector Th17 lymphocytes. Conversely, human IBD patients exhibited lower numbers of CD101-expressing T cells in the periphery and in inflamed intestinal tissues than healthy control individuals and patients with other gastrointestinal diseases. Based on these findings we propose that CD101 is a novel clinical marker for tissue-specific inflammation in IBD.

C03

The Erythropoietin-Analogue ARA290 Ameliorates the Course of Experimental Colitis

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Erythropoietin (EPO) is a cytokine whose main function is to stimulate the production of red blood cells after binding of EPO to its homodimeric receptor (EPOR) on erythroid progenitors. Based on the fact that immune cells express and alternative, heterodimeric receptor composed of EPOR and CD131, we have recently reported that EPO ameliorates the course of experimental colitis due to its ability to reduce the binding activity of the transcription factor NF-κB in macrophages. The potential benefits of high-dose EPO therapy in humans are outweighed by the high risk of thromboembolic complications, though.
We used the EPO analogue ARA290, a nonapeptide known to selectively activate the heterodimeric EPOR, and tested its *in vivo* efficacy in the dextran-sulfate sodium (DSS) model of experimental colitis. Moreover, we are using cell line and primary macrophages to investigate the production of cytokines and signaling pathways involved *in vitro*.
We could demonstrate that ARA290 ameliorates the clinical course of DSS-colitis as efficient as does EPO without affecting haemoglobin levels. DSS-exposed mice treated with solvent showed substantial weight loss and reduced survival. However, treatment with ARA290 or EPO resulted in significantly reduced weight loss and improved survival. Correspondingly, histopathologic analysis of colon samples revealed significantly reduced tissue damage and inflammation in DSS-exposed mice treated with ARA290 or EPO as compared to solvent-treated DSS-mice. When analyzing supernatants of colonic organ cultures for cytokine levels, we found that TNF, IL-1ß, IL-6, IL-12p70, IL-23, IFN-γ and lL-17A concentrations were significantly lower following treatment with ARA290 or EPO.
ARA290 is efficient in improving the clinical course of DSS-induced colitis. It inhibits the production of pro-inflammatory cytokines, which are key mediators in the pathogenesis of the disease, but does not stimulate erythropoiesis. Thus, ARA290 may be a promising agent for the therapy of humans affected by inflammatory bowel disease as it exerts potent anti-inflammatory effects without unintended thromboembolic side effects.

D01

Streptococcus pyogenes RNA Activates Toll-like Receptor Signaling in Mouse but not Human Cells and Confers Resistance to Infection in Mice

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Streptococcus pyogenes is a major Gram-positive pathogen in humans but for unclear reasons it is not pathogenic in mice. In our current work we show that mice commence a successful immune defense against *S. pyogenes* through engagement of Toll-like receptor (Tlr) 2 and via endosomal TLR-mediated recognition of streptococcal rRNA. These Tlr pathways are non-redundant since only their combined activation results in a full-blown inflammation and host protection. In mice, bacterial rRNA is known to trigger specifically Tlr13 which is lacking in humans. Remarkably, we find that human innate immune cells fail to recognize *S. pyogenes* through bacterial RNA and that the sole TLR signaling engaged in these cells is the TLR2 pathway. Phylogenetic analysis reveals that Tlr13 is missing in primates but occurs sporadically in other mammals including mice and rats, and it can be found in other vertebrates and kingdoms including insects and plants. Our data suggest that the evolutionary trimmed pathogen recognition in humans compared to mice contributes to human susceptibility to *S. pyogenes* infections.

D02

Recognition of Leishmania parasites by the macrophage-inducible C-type lectin Mincle subverts adaptive immunity and promotes susceptibility to infection

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Leishmania utilizes a variety of strategies to escape from an effective immune response. We describe that targeting of the C-type lectin receptor Mincle (Clec4e) by *L. major* results in the inhibition of the adaptive immunity against the parasite. We find that *L. major* parasites encode for a cytosolic ligand for Mincle that is exposed upon infection in vivo. The recognition of this ligand by Mincle promotes the infiltration of neutrophils and monocytes to the infected dermal site, correlating with an enhanced chemokine production early after infection. Mincle-deficient mice are more resistant to *L. major* intradermal infection in the ear, exhibiting a reduction in the dermal pathology and the parasite burden. Accordingly, Mincle deficiency enhances the adaptive immunity against the parasite, boosting the early priming of $CD4^+$ T cells specific for *L. major*- derived antigen. This improved priming correlates with an increased activation and migration of the dermal dendritic cells to the draining lymph nodes. These findings suggest that *L. major* exploits their recognition by Mincle to modulate and subvert the development of acquired resistance.

D03

LC3 associated phagocytosis in human primary macrophages can reduce *Leishmania* specific T cell proliferation

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Antigen specific human T-cells contribute in controlling *Leishmania* (*Lm*) infection. The human macrophages (hMDM) may act both as host cell for *Lm* and as antigen presenting cell, in which phagosomal and autophagosomal maturation are involved in MHC loading. In this study we investigated the response of naïve T-lymphocytes to *Lm* infected hMDM.
Upon coculturing autologous human PBMCs with *Lm* infected hMDM, we observed a proliferating population consisting out of $CD3^+$ cells. The $CD3^+$ cells were $CD4^+CD45RO^+$ and proliferated in a MHCII dependent manner. This demonstrates that unexposed individuals have *Lm* specific memory T-cells. Moreover, we could show that this T-cell proliferation led to a reduced parasitic infection rate in hMDM. In the presence of apoptotic promastigotes during infection of hMDM we found a significant lower T-cell proliferation. Focusing on the intracellular fate of apoptotic promastigotes inside hMDM we found them to enter a different compartment as compared to viable promastigotes. Where viable promastigotes enter a maturing phagolysosome, apoptotic promastigotes entered a single membrane compartment positive for LC3, typical for LC3 associated phagocytosis (LAP). Upon chemically activating autophagy in hMDM following *Lm* infection, T-cell proliferation was significantly reduced, confirming the role of the autophagy machinery as a negative regulator of T-cell proliferation during *Lm* infection.
In conclusion, we found *Leishmania* to induce a *Lm* specific memory T-cell response in unexposed individuals. Apoptotic promastigotes recruit LC3 to the phagosome, leading to a reduced T-cell proliferation, suggesting LAP as a novel pathogenic silencing mechanism in human primary macrophages.

E01

Myeloid Cells as Targets for Nanobody-mediated Imaging of Inflammation and Cancer

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Non-invasive whole-body molecular in vivo imaging holds promise for refined monitoring of inflammation and cancer, not only providing information about the amount of inflammation, but also on the location and type of inflammation and on cells and/or receptors involved. Myeloid cells such as macrophages and dendritic cells play a crucial role in a range of pathological and physiological processes. Due to the high degree of versatility of these cells upon activation and/or differentiation, myeloid cells and the activation states they acquire in response to various triggers represent potential in vivo sensors for the status of the immune system during inflammation and cancer.
As single-domain antigen-binding fragments derived from heavy-chain antibodies occurring naturally in camelids, Nanobodies offer the high binding affinity and specificity of antibodies, combined with the favourable pharmacokinetics of small molecules. As such, high contrast information (i.e. high signal to noise ratio's) can be obtained already 1-3 hours post tracer inoculation. We provide evidence that Nanobodies raised against selected myeloid cell markers are excellent probes for molecular imaging of (i) pro-angiogenic tumor-associated macrophages residing in hypoxic tumor areas, or (ii) inflammatory foci in rheumatoid arthritis. Thus, we obtain proofs-of-principle for tracking myeloid cells during inflammation and cancer using Nanobodies, offering perspectives for in-depth preclinical testing and mode-of-action analysis of new therapeutic compounds as well as clinical applications.

E02

Downregulation of the chemokine decoy receptor D6 unleashes Kaposi's sarcoma growth

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D6 is an atypical chemokine receptor acting as a decoy and scavenger for inflammatory CC chemokines. Consistent with its restricted expression on lymphatic endothelial cells, D6 expression was observed on Kaposi's sarcoma (KS) spindle cells, which have lymphatic origin. When analyzed in a longitudinal cohort of KS patients, D6 expression levels inversely correlated with KS aggressiveness as rapidly progressing lesions has significant higher levels as compared to slowly progressive lesions. To investigate the impact of D6 expression on KS biology, a KS-derived cell line was reconstituted for D6 expression and injected in nude mice. D6-expressing tumors presented decreased angiogenesis and growth rate, and reduced infiltration of neutrophils and macrophages. Macrophage adoptive transfer increased the growth rate of D6-competent neoplasms, and inhibition of monocyte recruitment by CCR2 blockade reduced macrophage infiltration and growth of D6-incompetent tumors. Both in the preclinical model and in human KS lesions low levels of D6 are associated not only with an increased macrophage infiltrate, but also with their skewing toward an M2-like proangiogenic phenotype. These results indicate that during KS progression downregulation of D6 expression unleashes chemokine-mediated macrophage recruitment and acquisition of an M2-like phenotype. Targeting CCR2 should be considered as a therapeutic option for KS patients.

E03

Tumor-associated glycans trigger the C-type lectin MGL on dendritic cells and dampen anti-tumor immunity

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The concordant interplay between diverse classes of pattern recognition receptors, including the C-type lectin family, shapes innate immune responses and primes the immune system for adaptive immunity. Dendritic cells (DCs), the sentinels of the immune system, express several C-type lectins allowing them to respond to specific glycosylation patterns on pathogens and tumor cells. We have previously demonstrated that the C-type lectin MGL can distinguish between healthy tissue and colorectal cancer through its specific recognition of the glycan structure Tn antigen (αGalNAc-Ser/Thr) on tumor-derived MUC1. Our new data show that high MGL-ligand expression in stage III colorectal cancer patients is an independent prognostic marker associated with worse disease free survival and a higher disease-related death rate. Furthermore, the presence of MGL ligands was correlated with activating mutations in the KRAS-BRAF pathway, providing a link between oncogenic transformation and the immune system. Furthermore, engagement of MGL on DCs by tumor-associated Tn antigens triggered MGL-dependent signaling, characterized by the activation of ERK-p90RSK-CREB axis and enhanced secretion of the anti-inflammatory cytokine IL-10. Moreover, MGL-conditioned DCs adopt an inhibitory programming, leading to induction of T cell anergy and a failure to generate strong T cell responses. Together, our results imply a direct effect of the aberrant tumor glycosylation on DCs. Therefore, we hypothesize that glycans, expressed by tumor cells act as an immune evasion strategy to negatively influence anti-tumor immunity through the induction of tolerogenic DCs.

E04

Human M2 macrophages promote inflammation in rheumatoid arthritis via synergy between Fc gamma receptors and TLRs

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In rheumatoid arthritis (RA), macrophages are considered to be the main producers of disease-associated pro-inflammatory cytokines TNFα, IL-1β, and IL-6. In RA synovia, both M1 and M2 macrophages can be identified. However, the role of M2 macrophages, which are known for induction of anti-inflammatory responses in numerous other disorders, is still unclear in the context of RA. To address this, we stimulated human *in vitro* generated M2 macrophages with RA-associated stimuli such as complexed IgG (c-IgG), TLR ligands and pro-inflammatory cytokines. Strikingly, while exposure to individual RA-associated stimuli induced anti-inflammatory responses, the specific combination of c-IgG and TLR ligands synergized for selective induction of TNFα, IL-1β, and IL-6. This pro-inflammatory response did not differ between RA patients and healthy donors, suggesting that the responsible mechanism does not depend on cell-intrinsic or inflammation-associated differences. Additionally, c-IgG-TLR ligand co-stimulation of M2 macrophages specifically promoted Th17 responses. Furthermore, we showed that induction of pro-inflammatory cytokines upon this co-stimulation was dependent on Fc gamma receptors. Notably, the anti-inflammatory cytokine profile of M2 macrophages could be completely restored using Syk inhibitor R406, thereby providing a possible mechanistic explanation for the therapeutic efficacy of this RA drug. Thus, our data show that upon exposure to c-IgG, the anti-inflammatory response of human M2 macrophages to TLR stimulation is converted into a strong, RA-associated, pro-inflammatory response. This finding may be a starting point for identification of new therapeutic agents that can attenuate inflammation in RA by restoring the anti-inflammatory function of M2 macrophages.

F01

Nr4a1-dependent monocytes monitor endothelial cells and orchestrate their necrosis by neutrophils in response to a TLR7-mediated danger signal

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The functions of *Nr4a1*-dependent Ly6C^{low} monocytes have remained enigmatic. We show herein that they are highly enriched within capillary vessels and scavenge microparticles from their luminal side in a steady state. Induction of inflammation by a TLR7-dependent nucleic acid signal in the kidney cortex triggers G□-dependent intravascular retention of Ly6C^{low} monocytes, which recruit neutrophils in a TLR7-dependent manner to mediate focal necrotic death of endothelial cells, while the monocytes remove cellular debris. Prevention of Ly6C^{low} monocyte development, crawling, or retention in *Nr4a1*$^{-/-}$, *Itgal*$^{-/-}$, and *Tlr7*$^{host-/-BM+/+}$ and *Cx3cr1*$^{-/-}$ mice, respectively, abolished neutrophil recruitment and endothelial killing. Prevention of neutrophil recruitment by monocytes in *Tlr7*$^{host+/+BM-/-}$ bone-marrow chimeras or by antibody-mediated neutrophil depletion also abolished endothelial cell necrosis. *Nr4a1*-dependent Ly6C^{low} monocytes are therefore intravascular housekeepers that orchestrate the necrosis by neutrophils of endothelial cells that signal a local threat sensed via TLR7 followed by the in situ phagocytosis of cellular debris.

F02

Translational control of specific mRNAs is important for cellular survival and the anti-inflammatory feedback during macrophage activation

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We analyzed the regulation of mRNA translation during the course of macrophage activation. To identify individual mRNAs whose translation is specifically regulated in RAW264.7 cells after 1 h of LPS treatment, we fractionated mRNAs by sucrose gradient centrifugation and quantified them by microarray analysis. Transcript levels were monitored globally with RNASeq for the first 2 h of the response. A comparison between mRNA levels and translation revealed that polysomes buffer changes in transcript levels. Therefore, increased transcript levels translate efficiently into increased protein levels only when translation is favored. This is especially relevant during the early phase of the macrophage response, when mRNA levels change dramatically. While mRNA levels of cytokines as well as mediators of negative feedback loops are strongly induced, negative feedback genes are activated more strongly at the level of translation than cytokines. The strongest activation was observed for four inhibitors of the NFkB signaling pathway. At the level of mRNA abundance and translation, Ier3 showed the highest correlation with Tnf. They share several promoter and 3'UTR elements, which indicates that co-regulation of these two genes was evolutionarily selected for. The analysis of BMDM from Ier3 knockout mice showed that Ier3 protects macrophages from LPS-induced cell death, which was previously described to result from auto-/paracrine secretion of Tnf. Taken together, our analysis reveals that translational control during macrophage activation is important for cellular survival as well as feedback inhibition of NFkB signaling and thereby promotes the resolution of inflammation.

F03

High salt (NaCl) affects H3K4 trimethylation and alternative macrophage activation

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High dietary salt (NaCl) is a major risk factor for cardiovascular disease (CVD). We have shown that macrophages play a key role in the regulation of interstitial tonicity and that NaCl-induced hypertonicity amplifies Th17 polarization. Therefore, we hypothesize that NaCl-induced hypertonicity also affects macrophage polarization. Here we have investigated the effect of NaCl-induced hypertonicity on the IL4/IL13-induced alternative activation of M2 macrophages (AAM). Bone marrow cells from C57BL/6 mice were differentiated in the presence of M-CSF for 7 days to generate M0 macrophages. To induce AAM, M0 macrophages were then incubated with IL4/IL13 for 24h in the presence of 0-40mM NaCl. Quantitative RT-PCR was used to analyze AAM signature genes (e.g. *Mrc1, Arg1, Fizz1, Ym-1, PD-L2*). NaCl specifically blunted the upregulation of these genes and *Klf4* and *Irf4*, two transcription factors essential for M2 polarization, whereas osmotic stress by mannitol or urea was non-effective. In previous experiments Sgk1 played a pivotal role in Th17 polarization. In contrast, Sgk1 was not involved in the NaCl effect on M2 macrophages. It has been shown that histone modifications are essential for AAM. In RAW264.3 macrophages, IL4/IL13 induced an increase in H3K4 trimethylation in a number of genes including Mrc1. H3K4 trimethylation and M2 gene signature were blunted by NaCl. We also found that incubation with increased [NaCl] altered STAT6 phosphorylation upon IL4/IL13 stimulation. Therefore, we propose that NaCl-induced hypertonicity alters chromatin modifications and signaling, which subsequently affect gene expression important for M2 polarization and function.

G01

The tumor microenvironment under the influence of HIF-1α in myeloid cells

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Cancer immunoediting implies both, eliminating tumors but also an educational program affecting immune competent cells in the tumor microenvironment resulting in the escape from immune attack, allowing progression and metastasis. Macrophages highlight the dichotomous role of immune cells that both antagonize and enhance tumor development and progression. In the tumor microenvironment cell death by apoptosis with the concomitant formation of sphingosine-1-phosphate (S1P), TGFß and additional factors polarizes macrophages to acquire a tumor supportive phenotype. Upregulation of the cyclooxygenase-2/microsomal prostaglandin E-synthase pathway, activation of CREB with the concomitant expression of arginase II, stimulation of PPARγ, and regulation/activation of hypoxia inducible factors (HIF) are decisive factors for promoting a tumor supportive macrophage phenotype. However, targeted deletion of HIF-1α in macrophages reduces tumor growth in the transgenic *polyoma middle T oncogene* (PyMT) breast cancer mouse model only marginally, whereas in a methylcholanthrene (MCA)-induced fibrosarcoma model tumor growth is nearly eliminated. The loss of HIF-1α impaired MCA-metabolism and reduced DNA damage (γ-H2AX-assay) due to impaired cytochrome P450 (CYP 1A1) expression, pointing to a role of HIF-1 in tumor initiation and carcinogen formation in response to polycyclic aromatic hydrocarbons. Further evidence will be provided showing that HIF-1 limits differentiation of precursors into plasmacytoid dendritic cells (pDCs). Hypoxia suppressed Flt3-L-induced differentiation of bone marrow cells to pDCs in wild-type but not HIF-1α(fl/fl) LysM-Cre bone marrow cells by up-regulating inhibitor of DNA binding 2 (ID2). Likely, this restricts the numbers of these potentially autoreactive cells and lowers their anti-tumor response, which is required to reject tumors in mice.

G02

Hypoxia impairs the NO-dependent leishmanicidal activity of macrophages and prevails in the skin lesions of *Leishmania major*-infected mice

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Cure of infections with *Leishmania* (*L.) major* is critically dependent on the activity of the inducible NO synthase (NOS2) that produces high levels of NO in the presence of ample oxygen. Therefore, we hypothesized that the tissue-oxygenation in cutaneous lesions may be an important factor that affects the leishmanicidal activity of macrophages.
When *L. major* skin lesions reached their maximum size, the infected tissue oxygen levels dropped to very low levels (O_2 ~2.8% (21 Torr)). Normalization of skin oxygen levels correlated with clinical healing. Subjecting mice to systemic hypoxia blocked the normalization of tissue oxygen levels in *Leishmania*-lesions and impaired the ability of infected animals to clear *L.major*. At an O_2-level below 4% (40 Torr), macrophages activated *in vitro* by IFN-gamma plus LPS were unable to produce NO and to clear intracellular *L. major*. However, upon reoxygenation these activated macrophages produced NO again and efficiently cleared the parasites. Using NO-donors we were able to rescue the defective leishmanicidal activity observed in hypoxic macrophages.Infection of activated *Nos2*$^{-/-}$ macrophages under normoxic conditions completely mimicked the defective leishmanicidal activity observed in infected wild-type macrophages under hypoxic conditions. Finally, using *Nos2*-deficient mice we were able to demonstrate that systemic hypoxia impaired the leishmanicidal activity of infected mice in a *Nos2*-dependent manner, too.
In summary, this study demonstrates that *Leishmania*-lesions are transiently hypoxic. Since hypoxia blocked the leishmanicidal activity macrophages, we propose that hypoxia may be a hitherto underestimated local milieu factor which contributes to the persistence of *Leishmania* even in immunocompentent hosts.

Abstracts

of the

Poster Presentations

A05

Selective activities of the aryl hydrocarbon receptor in M1- and M2-polarized human macrophages.

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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor implicated in diverse physiological processes. It is expressed in several cell lineages, including lymphocytes, dendritic cells and macrophages (Mϕ). The antigen presenting cells from AhR-deficient mice have exacerbated inflammatory responses; therefore AhR appears to play a critical role in anti-inflammation. Previous data from our laboratory showed that AhR is expressed in pro- (M1) and anti-inflammatory (M2) Mϕ generated from human monocytes cultured with GM-CSF and M-CSF, respectively. In the present study we sought to characterize some of the Mϕ functions where AhR is involved. AhR is not expressed in monocytes, and was detected in differentiating M1 and M2 Mϕ from the first day of culture. AhR was constitutively active in both M1 and M2 Mϕ. The activity of AhR on Mϕ effector functions was evaluated by adding the AhR antagonist α-naftoflavone (α-NF) during the differentiation process. AhR was positively involved in the production of IL-6 only in M1 Mϕ, and of IL-10 in both Mϕ subtypes. M2 had higher ability to phagocytose zymosan than M1 Mϕ, and this property was not affected by the AhR activity. Additionally, the growth suppressive activity on cancer cell lines of M1 Mϕ-conditioned medium was reverted to the values of M2 Mϕ when M1 Mϕ were grown in the presence of α-NF. This reversion seemed to be independent on activin A production, a mayor intermediary of the tumor-resistance ability of M1 Mϕ. Thus, AhR has different activities in Mϕ according with their polarization status.

A06

One-time local low-dose irradiation of joints of human TNF-α transgenic mice reduces bone-loss and osteoclast numbers *in vivo*

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Background: Human TNF-α tg (hTNF-α tg) mice develop a Polyarthritis (PA) that closely resembles clinical manifestations in Rheumatoid Arthritis (RA) patients, including osteoclast-mediated bone-loss in affected joints. Osteoclasts are an extremely specialized form of macrophages and the only type of cells capable of digesting bone. For decades, low-dose radiation-therapy (LD-RT) has been successfully applied in the treatment of inflammatory degenerative diseases, however, the underlying mechanism are rarely understood. We therefore examined the capability of LD-RT to avoid bone-loss in hTNF-α tg mice.
Methods: Inflamed joints of the mice were locally irradiated (left hind paw) with a single dose of 0.5Gy of X-ray. Grip strength and swelling of irradiated and non-irradiated paws was monitored. One month after the irradiation, paw sections were taken and analyzed for bone-loss and osteoclast number.
Results: Mice treated with a single dose of 0.5Gy showed higher grip strength and less swelling in the treated hind paws than control mice and untreated joints. Histomorphologic analyses showed that bone-loss and osteoclast numbers in both hind paws, treated and untreated ones, of the irradiated mice were reduced when being compared to control animals.
Conclusion: These data suggest an important role of LD-RT in the clinical treatment of patients with chronic inflammatory diseases. Since a reduced number of osteoclasts in treated mice was observed in irradiated and non-irradiated paws, we suggest that immune mediated abscopal effects might contribute to the anti-inflammatory effects exerted by LD-RT.
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A07

Network engineering on transcriptional level reveals a continuum of human macrophage differentiation

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Macrophages respond to a wide array of environmental signals and their activation is associated with significant transcriptional reprogramming. While much progress has been made in the understanding of macrophage activation, polarization and function, the transcriptional programs that regulate these processes remain poorly characterized. Based on these current concepts, we were interested to answer the following questions. (1) is there an underlying, stable and lineage-specific transcriptional program in human macrophages clearly distinguishing these cells from closely related cell types, e.g. dendritic cells (DC), (2) is macrophage polarization into classical M1- and alternative M2-macrophages the only outcome of activation, (3) is there a central activation signature independent of differentiation and (4) can we link findings obtained in the murine system to human macrophages? To address these questions, we have stimulated human macrophages with a large and diverse set of activation signals and acquired 299 human macrophage transcriptomes. Our analysis revealed a continuum model of macrophage differentiation extending the current M1- and M2-polarization model. Reverse network engineering identified transcriptional regulators associated with macrophage activation which was complemented by sets of regulators leading to specific macrophage differentiation programs. These programs were used to characterize human tissue alveolar macrophages in the context of specific differentiation signatures that operate in vivo. Finally, integrating our human dataset with the murine data derived from the ImmGen project allowed us to refine an activation- and differentiation-independent core signature for human and murine macrophages leading also to a better understanding of general mechanisms of transcription control and macrophage biology.

A08

NF-κB-activation as key event of dendritic cell activation is suitable to generate designer DC of superior T cell activation capacity

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Cancer vaccines should induce robust, long-lasting and effective tumor-specific T cell responses. Monocyte-derived, cytokine-cocktail-matured Dendritic Cells, loaded with tumor-antigen, were shown to induce both helper and cytotoxic T cells in numerous clinical trials, but there is clearly room for improvement. Several studies indicate that cytokine-maturatured, DC need additional activation stimuli to be most effective. All these stimuli intersect in the activation of the NF-κB signaling pathway. Here we show that transfection of constitutively active IκB kinases (caIKK) into cocktail-matured DC further significantly enhances – both in quantity and quality – their T cell-stimulatory capacity. The transfection of mRNA encoding for caIKK resulted in a further up-regulation of co-stimulatory surface markers such as CD70, as well as pro-inflammatory cytokines, especially the T_H1-response-inducing IL-12p70. Most importantly, the (wild type) MelanA-specific cytotoxic T cells induced by caIKK-transfected DC, combined high CD27 expression, indicating a more memory-like phenotype, and a markedly enhanced secondary expandability with a high lytic capacity. In contrast, CTL primed and expanded with unmodified “standard” cocktail-matured DC could not maintain their proliferative capacity upon repetitive stimulations.

Our data show that the activation of NF-κB enabling DC not only to prime CTL but to imprint on the T cells the capacity for enhanced expansion upon antigenic rechallenge. We hypothesize that such designer DC expressing constitutively active IkB kinases will prove highly immunogenic also in vivo and possibly turn out as a new strategy to improve the clinical efficacy of therapeutic cancer vaccinations.

A09

A concurrent interaction of DC with $CD4^+$ and $CD8^+$ T cells is necessary for an effective secondary CTL expansion

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$CD4^+$ T-cell help is crucial for an effective cytotoxic T lymphocyte (CTL) memory formation. However, it is still not satisfyingly resolved whether the presence of the $CD4^+$ T-helper cell is required during the dendritic cell (DC) / $CD8^+$ T-cell encounter (*concomitant three-cell interaction* model), or whether the DC will "memorize" the helper signal after the $CD4^+$ T cell has already left (*sequential two-cell interaction / DC licensing* model). Hence, we established a human autologous Ag-specific *in-vitro*-system to analyze the interaction between monocyte-derived (mo) DC and TCR-transfected $CD4^+$ T cells, and to subsequently compare both T-cell-help models.
The Ag-specific cross-talk of T-helper cells and DC resulted in Th1-cytokine secretion, T-cell activation, and DC activation of cocktail-matured moDC. Contradictory to common belief, emulation of a sequential interaction of the DC with the T-helper cell and afterwards of the licensed DC with $CD8^+$ T cells, barely improved CTL priming and expansion compared to the non-licensed DC conditions. In sharp contrast, the *concurrent* encounter of helper cells and CTL with the *same* DC was demonstrated to be a prerequisite to prime $CD8^+$ T cells for optimal secondary expansion. This $CD4^+$ T-cell help was obligatory during priming, but dispensable for subsequent expansion.
In conclusion, our human *in-vitro*-system revealed that the concomitant interaction of all three cell types is mandatory during priming to elicit an effective Ag-specific $CD8^+$ T-cell expansion. This experimental human model can serve as a valuable tool to further investigate T-cell help mechanisms, which influences both cellular therapeutic approaches and basic scientific understanding.

A10

In vitro differentiation of functional osteoclasts using conditionally immortalized myeloid progenitors Hoxb8 cells

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Osteoclasts originate from monocyte/macrophage precursors and differentiate into multinucleated osteoclasts under the influence of M-CSF and RANK-L. In general, for studying osteoclasts physiology/pathology, murine bone marrow derived precursors are used. The goal of our study was to develop osteoclasts using immortalized Hoxb8 precursors myeloid cells that closely resemble the characteristics of primary osteoclasts differentiation and function. Hoxb8 cells are conditionally immortalized monocyte/macrophage murine progenitors cell line, with estrogen-dependent production of Hoxb8, a crucial transcription factor blocking myeloid differentiation. Hoxb8 cells are already described to efficiently differentiate in functional macrophages upon estrogen withdrawal.
Hoxb8 cells were differentiated on plastic and dentin sections in the presence of M-CSF and RANK-L and differentiation and functionality compared with bone marrow derived osteoclasts.
Hoxb8 cells differentiated into multinucleated osteoclasts, positive for TRAP activity, with morphology that was not different from BM derived osteoclasts.
Osteoclasts markers (NFATc1, DC-STAMP, TRACP, CTR, CTSK) were strongly upregulated in Hoxb8 osteoclasts compared to undifferentiated Hoxb8 and Hoxb8 macrophages. Kinetics of osteoclast markers expressed during differentiation resembled those of bone marrow derived osteoclasts. Development of resorption pits on dentin slides demonstrated that Hoxb8 osteoclasts are functionally active in bone degradation.
In conclusion, our results suggest that Hoxb8 cells are able to differentiate into functional osteoclasts. Our system represents a valuable tool that allows unlimited production of osteoclasts from WT or genetically modified mice which can be used for studying osteoclasts physiology/pathology.

A11

Transcription factor AP-1 as switch regulators of M1 into M2 macrophages

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Macrophages (MΦ) are central players in immune responses. Depending on the microenvironment and outer signals MΦ can acquire different functional phenotypes. Two well-established types of MΦ referred to as classical activated (M1) and alternative activated (M2) MΦ. Recent studies have shown that Fos proteins, cFos and Fra-1 are negative regulators of NF-κB-mediated stress responses in inflammatory models. However, the role of Fra-2 and cJun is not yet well defined. In blood samples from rheumatoid arthritis (RA) patients, mRNA levels of Fra-1, Fra-2 and cJun were decreased whereas interestingly no difference could be detected for cFos between healthy controls and RA patients, suggesting that AP-1 plays a role in autoimmune diseases. Furthermore, Fra-1, Fra-2 and c-Jun were found drastically activated in thioglycollate elicited mouse MΦ after LPS or apoptotic cell treatment. We could confirm that thioglycollate elicited Fra-1$^{-/-}$ peritoneal MΦ following incubation with LPS secreted less M1 cytokines such as TNF, IL-1β and KC. Moreover, we show for the first time that the incubation of thioglycollate elicited Fra-1$^{-/-}$ peritoneal MΦ with apoptotic cells also results in a switch into M2 MΦ with increased Arg-1 and decreased IL-6 expression. Our data suggest that AP-1 is an important regulator of M1 versus M2 differentiation, thereby affecting the pro- and anti-inflammatory potential of MΦ. By the generation of mouse genetically deleted for Fra-1, Fra-2 or cJun in MΦ, we are depicting the molecular mechanisms in autoimmune and inflammatory models.

A12

PKC-eta and NOS2 Phenotype Regulation: Effects of Anakinra and Infliximab treatment on PBDM from RA Patients.

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Nitric Oxide (NO) is implicated in the pathogenesis of rheumatoid arthritis (RA) and is elevated in the circulation in severe RA. Its production is associated with NOS2 expression by circulating monocytes and is effected by proinflammatory cytokines, in particular TNF-α and IL1β. We have previously shown that the expression of NOS2 by monocytes is linked to the appearance of PKC-η. The aim of this study was to determine how the expression of PKC-η and NOS2 by monocytes was affected by anti-cytokine treatments. Patients with severe RA (with greater than 10 active joints) were selected that no longer responded to traditional treatment and compared with healthy subjects and patients with Osteo Arthritis as controls. The severe RA patients were divided into 3 groups, RA-naïve, were untreated, and two received anti-cytokine treatment either Infliximab (an anti-TNF-α) or Anakinra (an IL-1R-antagonist). Treatment lasted for at least 30-60 days. Blood samples were collected and plasma NO determined and peripheral blood derived macrophages (PBDM) isolated. Two-colour confocal microscopy and RT-PCR was used to determine expression of PKC-η and NOS2. Both anti-cytokine therapies were effective in reducing joint involvement and ESR levels. However, there was a strong divergent effect of the anti-cytokine treatments on the PBDM phenotype and serum NO levels. As expected, levels of NO were found to be associated with joint pathology. We provide an explanatory hypothesis for how the two cytokines possibly regulate PKC-η and NOS2 phenotype and propose that PKC-eta may be an effective target in inflammatory conditions where NO is implicated.

A13

T cell derived IL-3 enhances the pro- and anti-inflammatory properties of human macrophages dependent of the activation status

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Macrophages play a critical role in regulating the local immune response, contributing to both the initiation and resolution of inflammatory processes. Depending on their activation mode, macrophages secrete a broad repertoire of pro- or anti-inflammatory cytokines and are able to interact and activate bystander immune cells. Conversely, macrophages activation is dependent on the cytokines produced by other leukocytes. One example is T cell derived IFN-γ driving classical macrophage activation. In addition, T cells are able to secrete IL-3 upon T cell receptor engagement. Here we demonstrate that activated human macrophages up-regulate the IL-3 receptor. Furthermore IL-3 treatment induces specific changes in the macrophage phenotype and differentially enhances its function depending on the pre-treatment activation status. In classically activated macrophages IL-3 promoted an inflammasome response measured by elevated IL-1β levels and increased the expression of the co-stimulatory molecule CD86. In contrast, non-activated resting macrophages displayed signs of alternative activation upon IL-3 treatment demonstrated by increased surface expression of CD206 and CD163 and high IL-10 production. Interestingly, IL-3 also enhanced the secretion of IL-12p40 which was more pronounced in resting and alternatively activated macrophages. In conclusion, we demonstrate that T cell derived IL-3 is an important regulator of macrophage function and that this regulation is highly dependent on the preexisting activation status of the macrophage. Overall, these findings suggest a role for IL-3 being part of a positive feedback loop from T cells to macrophages regulating the course of adaptive immune responses.

A14

Transcription and enhancer profiling in human monocyte subsets

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Human blood monocytes comprise at least three subpopulations that differ in phenotype and function. Here we present the first in-depth regulome analysis of human classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{+}CD16^{+}$), and nonclassical ($CD14^{dim}CD16^{+}$) monocytes. Cap Analysis of Gene Expression (CAGE) adapted to Helicos single molecule sequencing was used to map transcription start sites throughout the genome in all three subsets. In addition, global maps of H3K4me1 and H3K27ac deposition were generated for classical and nonclassical monocytes defining enhanceosomes of the two major subsets. We identified differential regulatory elements (including promoters and putative enhancers) that were associated with subset-specific motif signatures corresponding to different transcription factor activities and exemplarily validated novel downstream enhancer elements at the *CD14* locus. In addition to known subset-specific features, pathway analysis revealed marked differences in metabolic gene signatures. While classical monocytes expressed higher levels of genes involved in carbohydrate metabolism priming them for anaerobic energy production, nonclassical monocytes expressed higher levels of oxidative pathway components and showed a higher mitochondrial routine activity. Our findings describe promoter/enhancer landscapes and provide novel insights into the specific biology of human monocyte subsets.

A15

Differentiation-dependent binding site selection of the hematopoietic master transcription factor PU.1 in macrophages

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PU.1 is a master transcription factor crucial for the development of many hematopoietic lineages.Its binding patterns significantly change during differentiation of progenitor cells into mature blood cell types, but the 'rules' for binding or not-binding of potential binding sites are only partially understood. To unveil basic characteristics of PU.1 binding site selection in different cell types, we studied the binding properties of PU.1 during human macrophage differentiation in comparison with other hematopoietic lineages (B cells, granulocytes, dendritic cells & various cell line models). Using in-vivo and in-vitro binding assays as well as computational prediction, we show that PU.1 selects its binding sites primarily based on sequence affinity which results in the frequent autonomous binding of high affinity sites in DNase I inaccessible regions (25-45% of all occupied sites). Increasing PU.1 concentrations and the availability of cooperative transcription factor interactions both decrease affinity thresholds for in-vivo binding and fine-tune cell type-specific PU.1 binding. Correspondingly, lineage- or cell type-specific binding sites were strongly associated with sequence motifs for transcription factor combinations that were unique for each lineage or cell type. Our data supports a model of PU.1 binding control that involves motif binding affinity, PU.1 concentration, cooperativeness with neighbouring transcription factor sites and chromatin domain accessibility, which explains the large majority of differential binding events between blood cell lineages.

A16

The role of nicotinamide as an immunmodulator of two human macrophage subsets

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Nicotinamide (NAM) a component of vitamin B3 is produced by several enzymes (sirtuins, ARTs, PARPs, CD38) that use NAD as a substrate. As the endproduct of these reactions it may act as a specific inhibitor. To sustain enzyme activity, NAM is recycled back to NAD by a salvage pathway. Here we asked in how far these nicotinamide mediated processes have an impact on the differentiation and immunological properties on the 2 monocyte derived macrophage subsets termed M1 and M2.
Monocytes were differentiated into M1 and M2 macrophages in the presence and absence of NAM. After 7 days in culture the cells were incubated in the presence and absence of LPS. After 16h following parameters were measured: intracellular NAD/NADH concentrations, expressions of cell surface markers and NAD-dependent enzymes as well as the cytokine production.
We found that some surface markers differentiating between M1 and M2 macrophages were downregulated in response to NAM treatment whereas other surface antigens, in part of unknown function, showed an increased expression.
Furthermore we identified nicotinamide as a compound capable of inhibiting LPS-induced cytokine production of M1 but not M2 macrophages.
Changes of intracellular NAD concentrations observed under different experimental conditions may affect the activity of NAD-dependent enzymes. Among these enzymes we concentrated on sirtuins as they regulate transcription factors known to modulate some of the here described immune responses.
Taken together vitamin B3 has the potential to interfere with important immunological properties of M1 and M2 macrophages.

A17

Development of human monocyte subsets and its modulation by immunosuppressants

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Distinct human monocyte subsets contribute to atherosclerosis as an inflammatory disease. Human monocyte subset development and its potential modulation by routinely used drugs remains poorly understood. Hematopoietic stem cell transplantation provides a unique glimpse into these issues.
Therefore, we analyzed monocyte subsets in patients following autologous and allogenic stem cell transplantation. *In vivo* $CD14^{++}CD16^{-}$ monocytes were the first to arise, followed by $CD14^{++}CD16^{+}$ and later by $CD14^{+}CD16^{++}$ monocytes. Monocyte subset distribution did not differ significantly in stable patients after allogenic compared to autologous transplantation ($P > 0.05$). Corticosteroids considerably depleted $CD14^{++}CD16^{+}$ and $CD14^{+}CD16^{++}$ cells *in vivo*, but left $CD14^{++}CD16^{-}$ monocytes unaffected. Calcineurin inhibitors, mycophenolic mofetil and methotrexate did not influence monocyte subset development, but modified surface receptor expression (CCR2, HLA-DR, ENG, TEK and TLR4). Furthermore, human monocytes were generated *in vitro* from $CD34^{+}$ progenitor cells. The impact of conventional immunomodulators – steroids, rapamycin, calcineurin inhibtors – and of the aryl hydrocarbon receptor (AHR) activator benzo(a)pyrene upon monocyte subsets was studied. Only steroids, rapamycin and benzo(a)pyrene significantly affected CD16-positive monocyte counts.
In summary, we report *in vivo* the developmental relationship of all three monocyte subsets and the effects of established and experimental immunomodulators *in vivo* and *in vitro*.

A18

Hierarchical and Stochastic Manipulation of Macrophage Phenotype – M1 and M2 do not compete on equal terms

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Macrophages can exhibit both M1 pro-inflammatory and M2 anti-inflammatory properties, depending on the disease state and the signals they receive. The pathogenesis of autoimmune diseases is associated with a conjectured imbalance in immune responses, primarily due to infiltration of M1 macrophages. Adoptive transfer of M2 macrophages to re-adjust the imbalance in autoimmune diseases has been pioneered in recent years. However, it is still unknown how M2 macrophages behave in an M1-dominated–environment and how many cells will be required in order to efficiently achieve local immune modulation. In the current study we addressed these questions in *in vitro* studies of M1 and M2 macrophages by analysing the phenotype of the macrophages after co-culture or exposure to conditioned media. Furthermore, we assessed functional properties of macrophages in T-cell co-culture assays and in an *in vivo* air pouch model. We demonstrate that M2 macrophages have distinct morphological, surface marker and cytokine profiles compared to M1 cells. Importantly, an activation hierarchy was observed with M2 cells dominating M1 cells in co-culture and conditioned media assays, as well as in functional assays. M2 down-regulation of M1 phenotype and function was observed, together with stability of M2 phenotype and enhancement of M2 function in the presence of M1 cells. We conclude that manipulation of macrophage activation phenotypes is an activation-specific and stochastic process, and that further development of adoptive M2 transfer as a potential therapy for inflammatory disorders is warranted.

A19

Inflammatory human macrophages can kill *Leishmania* in a LL-37 specific manner

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Human primary macrophages (hMDM) can both host *Leishmania* parasites, as well as kill them. Depending on the microenvironment, hMDM can have an inflammatory M1 phenotype, as well as anti-inflammatory M2 phenotype. In leishmaniasis the contribution of the hMDM phenotype to parasite propagation is not known. Moreover the mechanism of parasite killing in hMDM is not fully understood. We hypothesize that the inflammatory M1 phenotype can kill parasites better as compared to the anti-inflammatory M2 phenotype.
In this study we searched for hMDM phenotype specific killing mechanisms for both the promastigote and amastigote life stages of *L. major* parasites. We found that M1 hMDM are more resistant to *L. major* infection as compared to M2 hMDM. Searching for parasite killing mechanisms, we found that the antimicrobial peptide cathelicidin (LL-37) was specifically up regulated in the inflammatory M1 phenotype. Moreover, we could show that recombinant human LL-37 can kill extracellular *L. major* promastigotes but not amastigotes. Subsequently, we established a siRNA knockdown for the LL-37 peptide in both M1 and M2 hMDM. We found a significant higher promastigote survival in the inflammatory M1 hMDM when LL-37 was silenced. Silencing had no significant effect on *L. major* amastigote survival. Our data suggest that the inflammatory M1 phenotype can contribute to control of a *Leishmania* infection in a LL-37 dependent manner. To assess the hMDM phenotype and its consequence for disease further, we are currently collecting LL-37 expression levels from patients suffering from either visceral or cutaneous *Leishmania* infection in Ethiopia.

A20

Complement receptor usage of *Leishmania* parasites in different phenotypes of primary human macrophages

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Leishmania parasites can infect both inflammatory (M1) as well as anti-inflammatory (M2) human macrophage (hMDM) phenotypes. Host cell entry can be mediated by either complement receptor 1 (CR1) or complement receptor 3 (CR3). CR1 mediated uptake causes inflammation whereas CR3 ligation silences hMDM. In this study we focus on the complement receptor usage of different *Leishmania* species as agents for cutaneous or visceral Leishmaniasis in both M1 and M2 hMDM.
We found CR1 to be an inflammatory M1 hMDM marker, downregulated on M2 hMDM. The anti-inflammatory CR3 was equally expressed on both hMDM phenotypes. With specific blocking antibodies against CR1 and CR3 we investigated species-specific uptake of *Leishmania* parasites into M1 and M2 hMDM. We found that *L.major* promastigotes uptake was significantly blocked via CR1 on M1 cells but *L.donovani* uptake was not influenced by CR1 blocking. Moreover for CR3 blocking no species-specific uptake was observed. Interestingly, blocking of CR3 resulted in a significant reduced uptake of both *L.major* and *L.aethiopica* amastigotes in M1 hMDM. Whereas in M2 hMDM the uptake of *L.major* and *L.aethiopica* promastigotes was reduced.
Subsequently, we silenced CR1 and CR3 on both hMDM phenotypes using siRNA knock-down techniques. The levels of mRNA, as well as surface expression of the complement receptors, were successfully reduced. Preliminary experiments with these knock-down cells suggest a *Leishmania* species- and parasite stage-specific uptake dependent on the different complement receptors.
Our data indicate that the hMDM entry mechanism as well as the parasite species and stage are important for infection outcome.

Myeloid cell development in humanized NSG mice

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In an attempt to study different human antigen-presenting cells (APC) in vivo, NSG mice were repopulated with human hematopoietic stem-cells (HSC). Six weeks after humanization, we found engrafted cells in different organs. B cells were the predominant population, but we also observed conventional dendritic cells (DC) and plasmacytoid DC next to myeloid precursors. The myeloid cell-lines were detectable for up to 36 weeks post transplantation. In parallel with DC in hematopoietic organs, we also found human Langerhans cells in murine epidermis in approximately 1/3 of our models. For optimizing the reconstitution of myeloid cells, we applied Fc-FLT3L and detected an increase of the myeloid compartment along with a marked reduction in the frequency of B cells.
To target APC in vivo, allo-reactive T cells from a third party donor were generated by mixed-lymphocyte reaction and injected into humanized mice. Proliferation of injected T cells was increased in the presence of human hematopoiesis when compared to that in control mice. Among APC, B cells seemed to be the main target. In first experiments using mice beyond week 12 after humanization, we also found evidence that myeloid cells were also depleted. This might indicate that DC obtain their full functionality later during humanization.
Further experiments are needed to evaluate the biology of the engrafted DC with regard to their human counterparts. Factors that are essential for differentiation of myeloid cells need to be explored further. Our model will be further improved for investigating development, differentiation and function of myeloid cells *in vivo*.

B05

Unique characteristics of murine neonatal macrophages

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Introduction: Neonates, preterms in particular, are vulnerable to infections because they rely primarily on innate immunity. Macrophages (MΦ) are key players as innate immune cells. The aim of our study was to characterize neonatal tissue MΦ and to compare them to their adult counterparts.
Methods: MΦs were isolated by peritoneal lavages form neonatal (<24h) and adult (42d) C57BL6/J mice. Transcriptomes were analyzed by microarrays, phenotypes were characterized by Chipcytometry using a panel of 22 extra- and intracellular markers. Functional assays included cytokine release upon toll-like receptor stimulation (multiplexed bead assay) and T cell proliferation assays. In some experiments MΦs isolated for fetal mice were included as additional controls.
Results: We observed a distinct neonatal phenotype with low expression of classical MΦ markers (F4/80, CD14, CD11b), Toll-like receptors (TLR 2, 4, 9) and antigen presentation markers (MHCII, CD80, CD86). MΦs isolated from fetal mice showed a similar phenotype.
Furthermore, transcriptome analysis revealed significant differences between neonatal and adult peritoneal MΦs (Principal component analysis). Upstream analysis revealed, i.e. a lack of IFNγ induced transcription in neonates ($p<0.005$).
Cytokine (IL6, IL1-α) and chemokine (ccl2, ccl3) release of neonatal MΦs upon LPS stimulation differed significantly compared to adults. Moreover, neonatal MΦs were unable to induce T cell proliferation.
Conclusions: Neonatal MΦs express a distinct phenotype which appears not to be related to the stress of birth. The differences in gene expression, cytokine release and their lacking ability to induce T cell proliferation could help to explain neonatal immune reactions.

B06

Splenic CD169+ macrophages deliver antigens to follicular dendritic cells and stimulate T cell dependent germinal center B cell responses

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CD169+ macrophages are strategically located in secondary lymphoid organs to capture antigens, activate innate immune responses and prevent further spreading of infectious agents. Additionally CD169+ macrophages have been shown to transfer antigens to follicular B cells, although their role in the activation of adaptive humoral immune responses is not clear. Here we show that antigen capture by splenic CD169+ macrophages strongly stimulates T cell-dependent high affinity germinal center B cell responses. Furthermore, we observed that CD169+ macrophages migrated into B cell follicles and deposited antigens on follicular dendritic cells (FDC). Upon activation CD169+ macrophages increased surface expression of costimulatory molecules. We also observed that cytokines that promote B cell responses were induced in a macrophage dependent manner. Our results indicate that antigen capture by CD169+ macrophages and the delivery to FDC is an important mechanism to activate high affinity humoral immune responses. Together with our previous observation that CD169+ macrophages transfer antigen to CD8+ dendritic cells and thereby facilitate CD8+ T cell responses, our studies demonstrate an important role for CD169+ macrophages in the activation of adaptive immune responses, which could be exploited for vaccination strategies.

B07

Analysis of Dendritic Cells in human lymphoid organs

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Dendritic Cells (DCs) are important regulators of immune responses. In our previous studies we found differential antigen presentation capacities of murine DC subpopulations using an in vivo antigen targeting system. In contrast to murine DCs, the functional role of human tissue DCs is largely unknown. We are focussing on the characterization of DC subpopulations directly isolated from human lymphoid tissues to understand their functional role in the human immune response. Human tissues (thymus, spleen, bone marrow, tonsils, cord blood, peripheral blood, together around 300 samples) were received from otherwise healthy individuals. For our studies we performed 6 color confocal immunofluorescence analyses, and up to 15 color FACS and cell sort analyses for the study of 284 cell surface molecules (Lyoplate assay). We further investigated the DC's antigen uptake properties and analyzed the RNA expression by microarrays. The percentage of the three main DC subpopulations of mDC1, mDC2, and pDCs was varying depending on the tissue analyzed, indicating different functional roles of the DC subpopulations. Only very few cell surface molecules were uniquely expressed on the different DC subpopulations. Further, future potential antigen targeting receptors of the C-type lectin and Fc receptor family were investigated. Depending on the targeting antibody CD4 or CD8 T cell responses could be initiated. Our microarray data together suggest differential antigen presentation capacities of pDCs, mDC1, and mDC2 cells.
With cutting edge technologies we have characterized directly isolated human tissue DC subpopulations.

B08

Do alveolar macrophages initiate aseptic, nanoparticle induced acute lung inflammation?

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Inhalation of carbonaceous nanoparticles (CNP), a main constituent of urban air pollution, can trigger pulmonary inflammation. While alveolar macrophages initiate inflammation upon airway infection, their contribution to inflammation caused by the sterile CNP stimuli is unclear.Here we ask whether alveolar macrophages initiate CNP-induced, aseptic lung inflammation.
C57BL/6N mice were intratracheally instilled with 20µg CNP (diameter: 7-12 nm), or 50µl vehicle (endotoxin free water). 3hrs to 7 days after treatment the course of pulmonary inflammation was monitored by bronchoalveolar lavage (BAL) analysis. To narrow down the source of inflammatory mediator production, pro-inflammatory genes were analyzed from total BAL cells, from BAL purified alveolar macrophages, and $CD45^+$ or $CD45^-$ cells isolated from lung homogenates.
CNP instillation caused acute neutrophil accumulation at 18 to 24hrs, highest BAL CXCL cytokine concentrations at 12 to 18hrs, and highest Cxcl lung mRNA levels at 12hrs. Profiling cellular subsets surprisingly revealed no CNP impact on the inflammatory status (Nos2, Tnf, Il1b, Cxcl2) of BAL macrophages 6 and 12hrs after treatment. In contrast, at the 12hrs time point Cxcl1, -5, and Csf2 expression was up to 50 fold induced in pulmonary $CD45^-$ cells (mainly alveolar epithelial cells). Non-lavageable $CD45^+$ leukocytes possessed the strongest Tnf and Cxcl2 signals, but the induction was much less pronounced (~2fold) and did not precede Cxcl1/5 expression, thereby precluding these population from initiating the chemokine release.
Our data suggests that alveolar epithelial cell derived chemokines (Cxcl1 and Cxcl5) initiate CNP triggered neutrophil recruitment to the airspace, with no obvious role for alveolar macrophages.

B09

Understanding migratory capacities of human Dendritic Cell subsets in different tissues

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Purpose/Objectives: Dendritic cells (DCs) are very important antigen presenting cells of the immune system. In humans and mice several DC subsets have been identified. For the interaction of DCs and T cells the expression of chemokine receptors (CCRs) and the secretion of chemokines are essential. However, in humans it is still not clear whether the DC subsets differ in the expression of CCRs and Toll-like receptors (TLRs) and whether this potential difference might be responsible for a DC subset-specific recognition of distinct classes of pathogens (e.g. viruses, bacteria, etc.). Therefore, we analyze the CCR and TLR expression profile on different immature and activated DC subsets in a variety of human lymphoid tissues (blood, spleen, thymus, tonsils).
Materials and Methods: Leukocytes were enriched from patient material and expression of CCRs and TLRs was examined with Multicolor-FACS- and confocal immunofluorescence-analyses.
Results: Myeloid DCs type 1 (mDC1) and plasmacytoid DCs (pDC) show a broad expression of CCRs, whereas Myeloid DCs type 2 (mDC2) are negative for most of the CCRs. Interestingly, the subsets-specific expression of certain CCRs differs between different tissues. The mDC1 express most of the extracellular TLRs, whereas mDC2 and pDCs mainly express intracellular TLRs.
Conclusions: The specific expression of CCRs and TLRs could refer to a specialization in pathogen recognition or immune cell interactions. In further experiments we want to determine how the expression profile changes after activation and which CCRs are important for interactions with other immune cells and for the homing to different tissues.

B10

Dendritic cells control CNS autoimmunity by induction of Tregs through PD-L/PD-1 interaction

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Experimental autoimmune encephalomyelitis (EAE) is an autoimmune disease of the central nervous system (CNS) induced by priming $CD4^+$ T cells using CNS derived myelin antigens, which are normally sequestered behind the blood-brain barrier. DCs are sparse in a healthy CNS and are primarily found in vessel-rich areas. CNS inflammation is accompanied by entry of DCs into the CNS. These DCs, but not other resident or infiltrating cells, are the most efficient APCs in driving the reactivation of transferred myelin-specific $CD4^+$ T cells.
Steady-state DCs are known to promote immune homeostasis by inducing and maintaining peripheral T cell tolerance whereas mature DCs are established as unrivaled APCs, initiating immune responses by exerting their proinflammatory role. Using various genetic approaches, we depleted $CD11c^+$ DCs in mice and induced autoimmune CNS inflammation. Unexpectedly, mice lacking DCs developed aggravated disease compared to control mice. When DCs were engineered to present MOG, a CNS autoantigen, in an induced manner, we found robust tolerance that prevented disease. This tolerogenic effect was maintained even when MOG presentation was induced 7 days post immunization, and coincides with an upregulation of the PD-1 on antigen-specific T cells. Strikingly, induction of MOG presentation at the peak of disease (once the mice developed profound paralysis) reverted disease outcome and restored motoric function.
Our results show that DC-depletion, either from birth or later in adulthood, did not prevent EAE induction, but instead led to a lower state of tolerance and stronger inflammatory responses. We also show that DCs are responsible for the upregulation of PD-1 on antigen-specific T cells and subsequently induce de novo conversion of Treg cells from naive T cells during immune responses. Finally, we show that disease can be reverted by autoantigen presentation at the site of inflammation, thus serving as a potential future therapy.

C04

A missense mutation in the SH2 domain of SLP-76 regulates the formation of the immune synapse between myeloid and natural killer T (NKT) cells

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The intracellular adaptor protein SH2 (Src homology 2)-domain-containing leukocyte phosphoprotein of 76 kDa (SLP-76) is expressed in many hematopoietic cell types, except mature B cells. SLP-76 is also critical for the formation of the immunological synapse between myeloid and lymphoid cells. While T cell including natural killer T (NKT) cell development in SLP-76 knock-out mice is arrested at the double-negative stage, myeloid cell populations are still present, but exhibit selective activation deficits. In order to study the role of SLP-76 for the formation of the immunological synapse, we utilized a N-ethyl-N-nitrosourea (ENU) mutagenesis approach and generated a mutant mouse carrying a missense mutation in the SH2 domain of SLP-76.

Mutant mice exhibited reduced SLP-76 protein expression in different myeloid and lymphoid cell compartments. While the development of conventional T cell and myeloid cell populations was not affected, there was a striking redistribution of NKT cells from the spleen and liver into peripheral lymph nodes and the lung. Furthermore, NKT cells, but not conventional T cells exhibited a pronounced Th17 expression profile. Vav1 and Nck that form with SLP-76 a complex that is critical for the formation of the immunological synapse were not properly recruited to the NKT cell-antigen presenting cell (APC) interaction site. Furthermore, Toll-like receptor or Fc-gamma receptor signaling in myeloid cell populations did not restore the Th1/Th2 phenotype in NKT cells. Thus, altered lipid presentation pathways and/or interactions with different APCs might skew the cytokine profile of NKT cells identifying SLP-76 as interesting target for therapeutic intervention.

C05

Extracellular vesicles modulate host-microbe responses by altering TLR2 activity

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Lactic-acid-bacteria (LABs), including *Bifidobacterium* and *Lactobacillus* genera, have been proven beneficial in the maintenance of intestinal homeostasis. Ligation of Toll-like receptors (TLRs) expressed by intestinal epithelial cells (IEC) and resident dendritic cells (DC) to cell wall components expressed by LABs contributes to this mechanism of action.

Extracellular vesicles (EV), important in cellular communication, originate from a broad range of cell types (including IEC and DCs) and can be found in virtually any body fluid. The reported presence of pattern-recognition receptors (including TLRs) on EVs, triggered the hypothesis that EVs can intervene with TLR activity.

Heat-inactivated serum-derived EVs were collected using ExoQuick®. Intact human serum (HS), depleted serum (HS-D) and vesicle-containing pellets, reconstituted to the original volume with medium, (HS-EV) were collected.

Monocyte-derived dendritic cells (moDC), THP-1 or HEK cells stably transfected with TLR2/TLR6, expressing an NFkB reporter construct were seeded in the presence of HS, HS-D or HS-EVs and stimulated with bacteria, TNFα or specific TLR2 ligands. After 16H NFkB activity (HEK-transfectants, THP-1) or cytokine release (moDC) was measured.

Bifidobacterium, in contrast to *Lactobacillus* strains, induced TLR2 activity which was inhibited by HS or HS-EVs. EVs depletion rescued TLR2 activity.

TLR2-heterodimer specific ligands showed that HS-EVs inhibition was TLR2/6 specific. Incubation of bacteria in the presence of HS and HS-EV, in contrast to medium or EV depleted serum, resulted in bacterial aggregation. Both Bifidobacteria and Lactobacilli induced dendritic cell IL-6 and TNFα release, which was either enhanced (Bifidobacteria) or reduced (Lactobacilli) upon EV depletion.

Concluding, EVs modulated TLR2 and moDC responses strain and ligand dependently. Attachment of EVs to bacteria induced bacterial aggregation and either enhanced (Lactobacilli) or reduced (Bifidobacterium) cellular responses.

C06

The Importance of Experimental Milieu in Regulation of Macrophage Activation

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Background: Macrophages are important source of cytokines and other compounds, which recruit additional cells to sites of infection or tissue injury. Importantly, their functions might be directly controlled by the composition of milieu in their immediate vicinity. Therefore, the analysis of their function under different in vitro as well as “simulated” in vivo condition is of particular interest.
Materials and methods: Mice peritoneal macrophages were stimulated with different activators (e.g. LPS, IFN-γ, TNF-α and interleukins or their combination) under different experimental conditions (in vitro and “simulated” in vivo conditions). Consequently, the physiological functions of macrophages were assessed using different luminometric, spectrophotometric, molecular, and immunohistochemical methods.
Results: Our data demonstrate that there exists crucial difference in activation of macrophages under classical in vitro and “simulated” in vivo conditions. These abnormalities are accompanied by significant changes in inflammatory response of macrophages as well as in activation of intracellular signaling pathways.
Conclusions: The activation of mice peritoneal macrophages is dependent on the composition of milieu in their immediate vicinity and the “classically used” in vitro systems seems to be not suitable for correct analysis of their functions.

D04

Histone acetylase inhibitors induce caspase-1 independent IL-1β secretion

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In the course of the last years evidence has accumulated showing that histone deacetylase inhibitors (HDACi) have important immune modulatory activity. In the present work it is shown that in human and murine dendritic cells and murine macrophages HDACi are strong activators of LPS induced IL-1β processing and secretion. Strikingly, this IL-1β secretion was independent of the inflammasome components NLRP3, ASC and even caspase-1 and activation kinetics differed completely from that observed after inflammasome activation. Inhibition studies showed that the histone deacetylase HDAC6 is responsible for this HDACi/LPS induced IL-1β secretion. Mechanistically, HDACi/LPS induced IL-1β secretion was strictly dependent on Trif and was associated with a functional impairment of autophagic processes. Importantly, these data demonstrate that besides the conventional inflammasome dependent IL-1β cleavage, dendritic cells and macrophages are capable of activating IL-1β by a novel, alternative mechanism. Treatment of mice with HDACi during the induction of a dextran sulfate sodium-induced colitis resulted in a strong increase of intestinal IL-1β. As naturally occurring HDACi like butyric acid are physiologic components of the intestinal milieu, HDACi induced IL-1β may have a physiological function in intestinal homeostasis.
Altogether the data demonstrate that in addition to the conventional inflammasome dependent IL-1β activation dendritic cells and macrophages are capable to activate IL-1β by a new until now unknown additional mechanism.

D06

Neutrophils Interact with NK Cells in Visceral Leishmaniasis

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Neutrophils serve as host cells for *Leishmania* parasites during the early phase of infection. On the one hand, they are thought to exert leishmanicidal effector functions. On the other hand, the parasites utilize neutrophils as “Trojan horses” to silently invade macrophages. Besides their phagocytic and anti-microbial activity, neutrophils also function as regulators of other immune cells. Recently, neutrophils were reported to be required for NK cell development and maturation under homeostatic conditions.
We previously showed that NK cells were rapidly activated during *Leishmania* infection, which led to the expression of protective IFN-γ and cytolytic activity. Here, we newly identified neutrophils as critical accessory cells to fully activate the NK cell response upon *Leishmania* infection. Depletion of neutrophils significantly reduced NK cell effector functions in *Leishmania infantum*-infected C57BL/6 mice. Additionally, confocal microscopic analysis revealed direct interaction between the two cell populations. When we analyzed the potential involvement of NK cell-activating cytokines, we found that neutrophils were neither a source of IL-12 or IL-18 (both of which are essential for full NK cell activity in leishmaniasis) nor did they influence the IL-12 release by dendritic cells. Although IFN-α/β inducible gene 15 (ISG15), described as a neutrophil-derived signal for NK cell-priming, was up-regulated in neutrophils purified from the spleen of *Leishmania*-infected mice, the NK cell activity of WT and ISG15$^{-/-}$ mice was comparable. Thus, the soluble factors IL-12, IL-18 and ISG15 do not seem to account for the neutrophil-dependent activation of NK cells in leishmaniasis.

D07

CD103$^+$ DCs play a crucial role during the manifestation of Citrobacter rodentium induced colitis

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Dendritic cells (DCs) are crucial components of the immune response during mucosal inflammatory and infectious conditions. Interestingly, certain pathogens evolved strategies to specifically target subgroups of DCs to gain access to the host. For example, i.v. administered Listeria monocytogenes specifically infect CD8α^+ DCs that transport the bacteria into the T cell zone of the white pulp. Hence, Batf3$^{-/-}$ mice that lack lymphoid CD8α^+ and related, non-lymphoid CD103$^+$CD11b$^-$ DCs are protected against Listeria infection.
In this study, we seek to investigate the role of mucosal CD103$^+$CD11b$^-$ DCs during infectious colitis exploring the Citrobacter rodentium (C.r.) model. C.r. is related to EHEC and provides an excellent in vivo model to investigate host-pathogen interactions. After oral infection of Batf3$^{+/+}$ and Batf3$^{-/-}$ mice with a bioluminescent strain of C.r., the course of infection was monitored in vivo by bioluminescent imaging. While in Batf3$^{+/+}$ mice bacterial counts increased and peaked around days 7-10, Batf3$^{-/-}$ mice displayed significantly reduced bacterial burden throughout the course of infection. To separate the contribution of the adaptive from the innate immune system, we crossed Batf3$^{-/-}$ mice to the Rag1$^{-/-}$ background. C.r. infected Rag1$^{-/-}$Batf3$^{+/+}$ mice showed a progressive course of infection and died within 28 days (median 23d). In contrast, Rag1$^{-/-}$Batf3$^{-/-}$ mice displayed diminished bacterial burden and prolonged survival (median 34d) suggesting independence of the protection of Batf3$^{-/-}$ mice from the presence of the adaptive immune system. Together, CD103$^+$ DCs seem to play a crucial role during the establishment of the C.r. colitis.

D08

Crucial Role of MIF in Trypanosomiasis-elicited Inflammation-associated Immunopathology.

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The major pathological parameter in African trypanosomiasis is anemia of chronic disease (ACD), whereby iron homeostasis is skewed towards iron accumulation within the reticuloendothelial system. Hereby, a sustained type-1 cytokine-mediated inflammation and hyperactivation of myeloid cells contributes to the development of trypanosomiasis-associated immunopathology (anemia and liver injury). Furthermore, the polarization of myeloid (M) cells into distinct activation states (M1, M2) may contribute to trypanosusceptibility or tolerance. Reprogramming macrophages from M1 towards M2 alleviates ACD and normalizes iron homeostasis and erythropoiesis. A comparative gene analysis between a trypanosusceptible and tolerant model identified MIF (macrophage migrating inhibitory factor) as a potential candidate involved in inflammation-associated pathology.
The contribution of MIF in inflammation-associated pathology was evaluated using MIF-deficient mice (MIF$^{-/-}$) as well an anti-MIF neutralising antibody. In addition, a comparative gene expression study, focussing on genes involved in iron homeostasis and erythropoiesis, was performed using myeloid cells from wild-type and MIF$^{-/-}$ mice. The results revealed that MIF plays an important role in maintaining a prominent pro-inflammatory immune response. Furthermore, trypanosome-infected MIF$^{-/-}$ mice exhibited reduced anemia development which coincided with a restored iron-homeostasis and an increased erythropoiesis compared to wild-type mice. Besides, MIF deficiency resulted in reduced liver injury as evidenced by serum AST/ALT levels, which was associated with reduced infiltration of inflammatory (CD11b$^+$Ly6c^{high+}) cells expressing CD74, i.e. the cell surface MIF receptor mediating MIF's inflammatory and proliferative responses.
Therefore, MIF plays a crucial role in trypanosomiasis-elicited inflammation-associated immunopathology. Hence, targeting the MIF/CD74 axis might be an attractive approach to block inflammation-associated pathologies.

D09

Effects of soluble helminth components on human monocytes and macrophages in neuroinflammation

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Clinical trials show that infection with the helminth (worm) *Trichuris suis* can strongly reduce the severity of inflammatory diseases such as Crohn's disease and multiple sclerosis (MS). Helminths suppress pro-inflammatory immune responses, and enhance regulatory responses in their hosts, which allow the parasites to survive. These immunomodulating properties are thought to be responsible for the helminth-induced resistance to inflammatory diseases.
We recently set out to investigate whether a helminth-based therapy may be beneficial for the treatment of MS without having to use live worms. Our studies showed that treatment with soluble compounds of *T. suis* strongly reduced the disease severity in murine experimental autoimmune encephalomyelitis, an animal model for MS. To translate these promising results to the human system, we aim to define the molecular mechanisms by which the worm components modulate the function of human innate immune cells, which are crucial for the induction of actual damage in the CNS during neuroinflammation.
We here investigated the effects of soluble *T. suis* components on LPS induced TNFα production by human macrophages, and on the migratory properties of human monocytes across brain endothelial cells.
Results show that soluble products of *T. suis* substantially suppress LPS induced TNFα production by human macrophages and that this suppression is lasting for a prolonged period of time. Furthermore incubation of monocytes with *T. suis* components reduces their potential to migrate across brain endothelial cells by 50%.
The data indicate a potent anti-inflammatory effect of soluble products of *T. suis* on human myeloid cells.

D10

NO-independent Mechanism of IL-1β Inhibition by IFN-γ

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Background: The pro-inflammatory cytokine IL-1β is initially transcribed as an inactive precursor protein, pro-IL-1β, and requires cleavage by caspase-1 within the inflammasome complex for activation. IFN-γ has been reported recently to inhibit inflammasome activation in a nitric oxide (NO) dependent manner through S-nitrosylation of Nlrp3, thus suppressing generation of active IL-1β. However, generation of NO requires prolonged stimulation with IFN-γ. We describe here that IFN-γ inhibits IL-1β production by an additional, very rapid mechanism that is independent of NO.
Results: Co-stimulation of murine BMDM and DC with IFN-γ specifically suppressed bacterial RNA and LPS induced secretion of active IL-1β without affecting production of other pro-inflammatory cytokines including TNF, IL-6 and IL-12p40. Despite minor effects on inflammasome activation, the most prominent regulation occurred on the level of IL-1β transcription and was detectable as early as 30 min after stimulation. Of note, using iNOS-deficient cells, it could be demonstrated that this rapid inhibition was independent of NO. Mechanistically, co-stimulation with IFN-γ impaired binding of NF-κB p65 to the IL-1β promoter, thus representing a novel mechanism of IL-1β suppression by IFN-γ. Moreover, IFN-γ dependent inhibition of IL-1β attenuated effector functions of this cytokine as demonstrated by impaired differentiation of Th17 cells *in vitro* and decreased production of neutrophil chemotactic factor CXCL1 in target cells.
Conclusion: We describe here a novel, very rapid and NO-independent mechanism of IL-1β inhibition by IFN-γ. These findings provide evidence for a rapid immune modulating effect of IFN-γ independent of NO.

D11

S1P receptor 4 as a pharmacological target to block interferon alpha production in human plasmacytoid dendritic cells

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Plasmacytoid dendritic cells (pDCs) are rare but highly specialized cells that secrete large amounts of type I interferons (IFNα/ß) in response to viral infection. The production of IFNα/ß in human pDCs is triggered by toll-like receptors (TLR) 7 and 9 which are localized within the endosomal compartments. Besides their anti-viral activity, pDCs are also able to take up and present antigens to T cells and therefore play a role in both innate and adaptive immunity. Therefore, pDCs play a crucial role in several autoimmune diseases and cancer. Sphingosine-1-phosphate (S1P) is a lipid mediator, which signals through 5 G-protein coupled receptors (sphingosine-1-phosphate receptor; S1PR1-5) and induces immune cell migration, but also modulates immune cell activation. Our recent studies show that human, primary pDCs express the S1P receptors 1, 4 and 5 and S1P stimulation leads to a robust decrease in IFNα production after TLR9 activation with CpG in an S1PR4-dependent manner. Unexpectedly, attenuated IFNα levels are not translated into antigen-driven T cell proliferation by human pDCs in T-cell/pDC cocultures, but rather shift cytokine production of cocultured T cells from a Th1 (IFN-γ) to a regulatory profile (IL-10). pDCs express several inhibitory receptors, such as immunoglobulin-like transcript 7 (ILT7), to avoid an overshooting TLR response. Mechanistically, S1PR4 signalling rescues the CpG-dependent decrease in ILT7 expression, thereby restricting IFNα production and keeping pDCs in a more tolerogenic state. S1PR4 agonists are the first agents that maintain ILT7 expression in human pDCs and are therefore a promising tool to restrict pathogenic IFNα production.

D12

From mouse to man: Mincle expression in human myeloid cells and recognition of mycobacterial cord factor

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Induction of a robust cellular immune response is essential if vaccinating against intracellular pathogens such as *Mycobacterium tuberculosis.* The glycolipid Trehalose-dibehenate (TDB), a synthetic analog of the mycobacterial cord factor, induces a strong Th1/Th17 cell-mediated immune response in animal models and has entered Phase I clinical studies.
The macrophage-inducible C-type Lectin receptor Mincle has recently been identified as a FcRγ-dependent receptor for cord factor and TDB by us and others. Yet it is unknown whether human Mincle is able to recognize TDB and if the same signaling pathway via Syk/Card9 is induced in primary human antigen presenting cells (APC).
To address these questions we first analyzed the expression of Mincle and related C-type lectins in different human antigen presenting cells by qRT-PCR. Mincle is highly expressed in human APC. Highest basal levels of Mincle mRNA were detected in purified granulocytes followed by CD14+ monocytes.
Purified PBMC and monocytes obtained from healthy donors as well as monocyte-derived M1 and M2-like macrophages and dendritic cells were stimulated with glycolipids in vitro and analyzed for their inflammatory cytokine production. Performing ELISA and CBA we measured a significant increase of several cytokines following TDB stimulation.
Furthermore, retroviral transduction of murine Mincle-deficient DC with human Mincle receptor restored cytokine production in response to TDB.
Our data indicate that human APC are able to respond to TDB stimulation and emphasize a similar role of the C-type Lectin receptor Mincle for cord factor recognition in mouse and man.

D13

Modulation of macrophage and dendritic cell functions by pharmaceutical sodium chlorite (DAC-N-055), a novel compound for the treatment of cutaneous leishmaniasis

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Cutaneous leishmaniasis (CL) is caused by different species of the protozoan parasite *Leishmania* and characterized by chronic, frequently ulcerating and slowly healing skin lesions. An efficient, cost-effective, and well-tolerated local therapy is still missing. In clinical trials with CL patients in Afghanistan we observed that application of pharmaceutical sodium chlorite (DAC-N-055) after removal of necrotic tissue accelerated the wound healing. Here, we investigated possible anti-parasitic and immunomodulatory effects of DAC-N-055.
DAC-N-055 was cytotoxic against extracellular *Leishmania* promastigotes, whereas amastigotes within murine bone-marrow macrophages (BMM) remained unaffected. Using quantitative mRNA and protein expression analyses, DAC-N-055 enhanced the expression of inducible NO synthase (iNOS) and the production of NO as well as the release of TNF, IL-6, IL-10 and TGF-□ by IFN-g-stimulated BMM. In contrast, expression of IL-12 by bone-marrow dendritic cells (BMDC) was not altered by DAC-N-055. In both BMDC and BMM production of IFN-alpha/beta, which is required for the early iNOS expression in murine CL, was increased by DAC-N-055 upon stimulation with *Leishmania*, IFN-gamma or LPS. Expression of IFN-alpha/beta in DAC-N-055-stimulated BMM was not associated with the activation of nuclear factor kappa B (NfkappaB), interferon regulating factor (IRF)-3 or IRF-7, but was paralled by an increased phosphorylation of the mitogen-activated protein kinases cJun N-terminal kinase, p38 and extracellular signal-regulated kinase.
Together, these data suggest that DAC-N-055 is a leishmanicidal and immunomodulatory compound, which enhances the production of NO and of several pro- or anti-inflammatory cytokines that might contribute to a balanced immune response and an improved wound healing.

D14

Clade Specific Virulence Patterns of *M. tuberculosis* Complex Strains in Human Primary Macrophages and Aerogenically Infected Mice

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In infection experiments with genetically distinct *Mycobacterium tuberculosis* complex (MTBC) strains we identified clade specific virulence patterns in human primary macrophages and aerosol infected mice, both reflecting relevant model systems. Exclusively human adapted *M. tuberculosis* lineages, also termed clade I, comprising "modern" lineages such as Beijing and Euro-American Haarlem strains showed a significantly enhanced capability to grow compared to clade II strains, which include “ancient” lineages such as e.g. East African Indian or *M. africanum* strains. However, a simple correlation of inflammatory response profiles with strain virulence was not apparent. Overall, our data reveal three different pathogenic profiles: 1) strains of the Beijing lineage are characterized by low uptake, a low cytokine induction and a high replicative potential, 2) strains of the Haarlem lineage by high uptake, high cytokine induction and high growth rates and 3) EAI strains by a low uptake, low cytokine induction and low replicative potential. Our findings have significant implications for our understanding of host - pathogen interaction and factors that modulate the outcome of infections. Future studies addressing the underlying mechanisms and clinical implications need to take into account the diversity of both, the pathogen and the host.

D15

The IDO1-induced kynurenines play major role in the antimicrobial effect of human myeloid cells against *Listeria monocytogenes*

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Induction of indoleamine 2, 3-dioxygenase (IDO1) is an established cellular response to infection with numerous pathogens. Several mechanisms such as IDO1-mediated tryptophan (Trp) depletion, but also accumulation of Trp catabolites have been associated with the antimicrobial effects of IDO+ cells. Recent findings of IDO1 as an immunoinhibitory and signaling molecule extended these previous observations.
Using infection of professional phagocytes with *Listeria monocytogenes (L.m.)* as a model, we were interested to answer the following questions: (1) are human as well as murine myeloid cells capable of IDO induction upon *L.m.* infection, (2) does Trp excess lead to antimicrobial potential, (3) are there certain Trp catabolites with bactericidal activity and (4) can we translate our findings to other bacteria species.
We illustrate that IDO1 induction is a species-specific event observed most exclusively in human but not murine myeloid cells. Knockdown and inhibition experiments indicate that IDO1 enzymatic activity is required for the anti-*L.m.* effect. Surprisingly, the IDO1-mediated antimicrobial effect is less prominent when Trp is depleted but can be significantly amplified by tryptophan excess leading to increased accumulation of catabolites which promote enhanced bactericidal activity. We observed a pathogen-specific pattern with kynurenine (Kyn) and 3-hydroxy-kynurenine (3HK) being most potent against *L.m.* but not against other bacteria. Hence, apparent discrepant findings concerning IDO1-mediated antimicrobial mechanisms can be reconciled by a model of species and pathogen-specificity of IDO1 function. Our findings highlight the necessity to consider species-, and pathogen-specific aspects of host-pathogen interactions when elucidating the individual role of antimicrobial proteins such as IDO1.

D16

MicroRNA-223 controls susceptibility to tuberculosis by regulating lung inflammation

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The molecular mechanisms that control innate cell trafficking during chronic infection and inflammation, such as tuberculosis (TB), are incompletely understood. During active TB, myeloid cells infiltrate the lung and sustain local inflammation. While the chemoattractants orchestrating these processes have been recognized, less is known about the post-transcriptional events that dictate their availability. We identified microRNA (miR)-223 as one of the most differentially regulated small non-coding RNAs in lung parenchyma of patients and susceptible mice during active TB. MiR-223 controlled lung recruitment of myeloid cells, and consequently, neutrophil-driven lethal inflammation, by directly targeting the chemoattractants CXCL2, CCL3 and IL-6. Our study reveals an essential role for a single miR in TB. Moreover, we identify new targets for and assign novel biological functions to miR-223. By regulating leukocyte chemotaxis via chemoattractants, miR223 is critical for control of TB and probably other nonresolving inflammatory diseases.

D17

Role of dendritic cells in the invasion and dissemination of *Yersinia enterocolitica*

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Yersinia enterocolitica (Ye) is an extracellular bacterium that enters the host via contaminated food and causes acute and chronic gastrointestinal diseases. After colonisation of the small intestine the bacterium invades the peyer's patches (PP) via M cells and disseminates to the mesenteric lymph nodes (MLN), spleen and liver.
We assume that Ye uses DCs to disseminate from the intestine. By means of an oral mouse infection model and various transgenic mice we analysed the different invading and dissemination routes via PP, CX_3CR1^+ cells and $CD103^+$ DCs in the lamina propria (LP). Oral infection of lymphotoxin beta receptor knockout ($LT\beta R^{-/-}$) mice (lacking PP and MLNs) with Ye revealed similar bacterial burden in the spleen 1 h post infection as wild type mice, demonstrating an alternative PP independent dissemination route for Ye. Histological analysis of the small intestine showed Ye in close contact to CX_3CR1^+ as well as $CD103^+$ DCs 30 minutes after infection indicating phagocytosis by both DC populations. This finding was confirmed by flow cytometry analysis of LP leukocytes showing Ye predominantly associated with $CD103^+$ DCs and CX_3CR1^+ cells. Furthermore, Ye were found most frequently associated with $CD103^+$ DCs in the MLNs from wild type mice, whereas no Ye^+ DCs could be found in the MLNs of $CCR7^{-/-}$ mice, indicating a CCR7 dependent transport of Ye from LP to MLNs by $CD103^+$ DCs. In contrast, dissemination of Ye to the spleen was dependent on the uptake by $CXCR1^+$ DCs in the LP as significantly less Ye could by recovered from the spleen of $CX_3CR1^{GFP/GFP}$ mice (impaired dendrite formation) compared to wild type mice. Altogether these data indicate that uptake and dissemination of Ye takes place by alternative routes independently of PP most likely via the involvement of CX_3CR1^+ and $CD103^+$ DCs.

D18

DC don't seem to mind RNA electroporation

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Monocyte-derived dendritic cells (Mo-DC) loaded with tumor antigen (Ag) are frequently used in vaccination strategies. To deliver Ag into DC, transfection with mRNA is an attractive method. However, it was reported that exogenously delivered mRNA could induce DC activation resulting in the up-regulation of activation markers and cytokine production, which could have important implications for immunotherapy.
Therefore, we examined for 15 different mRNAs whether their electroporation had an influence on cocktail-matured Mo-DC. No difference greater than 1.5-fold in the expression density of any of the DC maturation markers CD25, CD40, CD83, CD86, and CD70 between the mock- and the RNA-electroporated DC was detected for all 15 mRNAs. Moreover, none of the 15 mRNA altered the secretion of IL-8, TNF, and IL-6 more than 30% compared to mock-electroporation.
Furthermore, we performed microarray analyses to explore the effect of MelanA-mRNA electroporation on the DC's transcriptome. Between mock-electroporated DC and Melan-A-electroporated DC only 3 significantly differentially expressed genes (DEGs) (i.e., interferon-induced protein with tetratricopeptide repeats 3, interferon-induced protein 44, XIAP associated factor 1) were found.
From these data we can conclude that in our case the introduction of mRNA into human cocktail-matured Mo-DC by electroporation does not result in a difference in phenotype, cytokine production, and the whole transcriptome of these cells.

D19

A new role for Fc gamma receptors: Th17 induction in response to bacteria via synergy with Toll-like receptors on human dendritic cells

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Dendritic cells (DCs) are essential in inducing adaptive immune responses against bacteria by expressing cytokines that skew T cell responses towards protective Th17 cells. Although it is widely recognized that induction of these cytokines by DCs involves activation of multiple receptors, it is still incompletely characterized which combination of receptors specifically skews Th17 cell responses. Here we have identified a novel role for Fc gamma receptors (FcγRs) in promoting human Th17 cells. Activation of DCs by bacteria opsonized by serum IgG strongly promoted Th17 responses, which was FcγRIIa-dependent and coincided with enhanced production of selected cytokines by DCs, including Th17-promoting IL-1β and IL-23. Notably, FcγRIIa stimulation on DCs did not induce cytokine production when stimulated individually, but selectively amplified cytokine responses through synergy with Toll-like receptor (TLR) 2, 4 or 5. Importantly, this synergy is mediated at two different levels. First, TLR-FcγRIIa co-stimulation strongly increased transcription of pro-IL-1β and IL-23p19. Second, FcγRIIa triggering induced activation of caspase-1, which cleaves pro-IL-1β into its bioactive form and thereby enhanced IL-1β secretion. Taken together, these data identified cross-talk between TLRs and FcγRIIa as a novel mechanism by which DCs promote protective effector Th17 cell responses against bacteria.

D20

Effect of secreted phosphatases of *Leishmania* parasites on the immune systems of infected hosts

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Leishmania major is the causative agent of cutaneous leishmaniasis, a worldwide prevalent parasitic disease. In its insect vector, the parasite resides in an extracellular, highly infective, promastigote form. Once in its mammal host, the parasite enters myeloid host cells with a high prevalence for macrophages. There, they transform into intracellular amastigotes, which facilitate the spread in the host organism. *Leishmania* parasites, like other intracellular pathogens, manipulate host cell functions by producing diverse virulence factors. However, only few *Leishmania* virulence factors have been characterized to date. Here, we identified two homologous tyrosine phosphatases which were shown to be partly secreted by *Leishmania major*. Interestingly, these phosphatases have a strong structural homology with the human phosphatase PRL-1 (Phosphatase of Regenerating Liver 1) that is involved in the regulation of numerous cellular features such as growth and cell motility. Combining a biochemical characterization of the catalytic activity of these phosphatases and a functional study based on ectopic expression vectors for Leishmania parasites, we are aiming to determine the nature and the localization of their molecular target(s) in macrophages.

The study of these two new putative virulence factors should allow us to better understand the mechanisms developed by *Leishmania* parasites to escape the immune response, but also help to define a novel class of *Leishmania* virulence factors which might also be suitable as drug targets.

D21

Infection of bone marrow-derived macrophages and dendritic cells from cathepsin B and cathepsin L deficient mice with *Leishmania major*: a tale of susceptibility and resistance

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Leishmaniasis affects worldwide 12 million people in 89 countries. In BALB/c mice, infection with *L. major* triggers a Th2-mediated lethal manifestation, whereas in C57BL/6 mice, a Th1-mediated self-healing form takes place. Cysteine proteases of the papain family found in *Leishmania* have been described as essential for the parasite pathogenicity, virulence and survival, particularly cathepsins B-like and L-like. Therefore, both cathepsins are promising targets for the development of new drugs. However, these enzymes are also expressed in mammals, and their role in the immune response is currently under extensive research. Experiments with the cathepsin B (Ctsb) inhibitor CA074 and a cathepsin L (Ctsl) inhibitor CLIK148 showed that these compounds could direct the immune response of *L. major*-infected mice towards a Th1 or Th2 profile, respectively. However, the mechanisms by which the polarization of naïve Th0 cells was modulated were not further investigated.
We therefore investigated the response of bone marrow-derived macrophages and dendritic cells from *Ctsb*-/- and *Ctsl*-/- mice to *L. major* infection. Our results indicate that macrophages from *Ctsl*-/- mice are more susceptible to infection in comparison with WT mice, and dendritic cells from *Ctsl*-/- and *Ctsb*-/- mice present differences in maturation after infection with *L. major* promastigotes, as well as in cytokine production under different stimuli. All together, our results confirm that host cathepsins play a role in the immune response to *L. major* infection and future work will focus on the mechanisms of the observed effects.

D22

Iron Homeostasis and Immune Function in Macrophages infected with different Intracellular Pathogens

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Background: Following an infection with intracellular bacteria a struggle for the essential nutrient iron between host phagocytes and the invading pathogens ensues. Consequently, competitive interactions between macrophage iron transporters and microbial iron acquisition systems form a central battlefield that determines the course of disease. While *Salmonella typhimurium* is mainly contained in the phagolysosome, *Listeria monocytogenes* is able to gain access to the host cell cytosol. Therefore it is possible that different defense strategies of macrophages on the one side and iron acquisition abilities of the pathogens on the other side are present.
Methods: We used the murine macrophage cell line RAW264.7 either stably transfected with a functional allele of a cation transporter present on the late phagolysosome, Nramp1 (RAW-37), or a non-functional control of the same transporter (RAW-21). Further experiments were performed with bone marrow derived macrophages of wild type and hemochromatosis (Hfe -/-) C57BL/6 mice.
Results: We found varying intramacrophage survival of *Listeria* and *Salmonella* when stimulated with different iron sources, i.e. iron salts versus iron isomaltoside. This effect was reproducible in the RAW-21 and RAW-37 cell line with significant lower bacterial load in the latter, indicating an important function of Nramp1 in host defense against *Salmonella*, but interestingly also for *Listeria*, which is able to escape the phagolysosome. In addition Hfe(-/-) bone marrow derived macrophages showed an improved control of infection with both intracellular bacteria which could be traced back to limitation of intracellular iron concentrations.
Conclusion: The ability of bacteria to use external iron sources depends on their uptake, compartmentalization and utilization by macrophages. Nramp1 and Hfe exert anti-bacterial activity by limiting the access to iron for intracellular microbes.

D23

Glucocorticoid-induced leucine zipper GILZ as a modulator of macrophage functions and macrophage desensitization

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Induction of glucocorticoid-induced leucine zipper (GILZ) by glucocorticoids plays a key role in their anti-inflammatory action. GILZ mRNA and protein levels are actively downregulated in primary human macrophages *via* a destabilization of GILZ mRNA. We aimed to investigate effects of GILZ downregulation in the inflammatory activation of macrophages *via* Toll-like receptor ligands by studying wild-type and GILZ-deficient murine bone-marrow derived macrophages.
As previously shown for human macrophages, GILZ was downregulated in murine macrophages from wild-type animals upon treatment with diverse inflammatory stimuli, including LPS, the TLR2 ligands Pam3CSK4 or LTA, heat-killed *Staphylococcus aureus*, and IFN-γ.
The expression of TNF-α and IL-1β upon MyD88-dependent and MyD88-independent TLR activation was significantly increased in macrophages derived from GILZ deficient animals. This effect most likely is due to an activation of ERK, which is critical for TLR-induced macrophage activation, and which was significantly amplified in GILZ knockout cells. Accordingly, ERK inhibitors abrogated the inflammatory expression profile in GILZ deficient macrophages.
The LPS-induced infammatory activation of macrophages is heavily decreased upon pre-treatment of macrophages with low dose LPS, an effect termed endotoxin tolerance. In endotoxin tolerant wild-type cells GILZ levels were not downregulated, while ERK activation was strongly decreased. *Vice versa*, GILZ knockout macrophages exhibited reduced desensitization coupled with elevated ERK activation.
In conclusion, our data provide evidence that GILZ plays an important role as a regulator of macrophage functions.

D24

HCMV preferentially triggers pDC and M2 macrophages, but not M1 macrophages, to mount type I IFN responses

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We and others found that human cytomegalovirus (HCMV) can trigger plasmacytoid dendritic cells (pDC) to mount type I interferon (IFN-I) responses. Here we studied whether HCMV also induces macrophages to produce IFN-I. To this end, we isolated monocytes from peripheral blood mononuclear cells and differentiated them to classically activated M1 or alternatively activated M2 macrophages. As expected, upon poly(I:C) stimulation M1 macrophages showed enhanced IFN-I responses compared with M2 macrophages. Interestingly, upon HCMV-GFP infection at a multiplicity of infection of 3 30% to 50% of M2 macrophages were GFP positive, whereas among M1 macrophages less than 10% GFP positive cells were found. Of note, HCMV stimulated M2 macrophages mounted significantly stronger IFN-I responses than M1 macrophages. This was illustrated by ELISA analysis of supernatants as well as intracellular IFN-I staining. To study whether infected macrophages produce IFN-I, or whether infected cells are taken up by uninfected macrophages which then are triggered to mount IFN-I responses, M2 macrophages were infected, fluorescently labeled and co-cultured with uninfected macrophages. Under such conditions, only labeled cells showed intracellular IFN-I staining. In conclusion, our observations suggest that upon HCMV infection pDC and M2 macrophages are strong IFN-I producers. In contrast to pDC, M2 macrophages are readily HCMV infected and then mount IFN-I responses. These insights into the interactions of macrophage subsets with HCMV may pave the road to develop new innovative therapeutic approaches in patients with detrimental HCMV infection.

D25

TAP fate and function in human monocyte-derived dendritic cells

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Even though in the human system BDCA3$^+$ dendritic cells (DC) have recently been proposed to be the major cross-presenting DC subset, the molecular mechanism underlying cross-presentation still has not been conclusively elucidated. Nevertheless, monocyte-derived DC are currently being tested as immunotherapeutics in multiple clinical trials. Here we studied the subcellular localization of the transporter associated with antigen processing (TAP), a key player of the canonical and cross-presentation pathway. Interestingly, TAP expression is detected throughout DC differentiation from monocytes to mature DC (mDC) with even enhanced protein levels found in immature DC (imDC) and mDC. In contrast, monocytes showed the most efficient TAP mediated peptide translocation. Extensive confocal laser scanning microscopy studies of the subcellular localization of the TAP complex indicated a massive redistribution during DC maturation. While throughout the differentiation the majority of the TAP complex colocalized with calnexin and thus was associated with the ER membrane, in monocytes it largely colocalized with the early endosomal markers EEA1 and Rab5. Upon differentiation to imDC and mDC the TAP complex was primarily found in LAMP-1$^+$ lysosomal compartments, whereas the association with endosomal compartments was no longer found. In conclusion, these results indicate that the majority of TAP is localized within the ER, while in monocytes a minor proportion of TAP is shuttled to early endosomes, which during DC maturation relocates to the lysosomal compartment. Thus, during DC maturation antigen presentation is diminished not by regulation of TAP expression, but by relocalization of the peptide translocation machinery.

D26

CD14-dependent monocyte isolation enhances phagocytosis of *Listeria monocytogenes* by proinflammatory, GM-CSF-derived macrophages

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Macrophages are an important line of defence against invading pathogens. Human macrophages derived by different methods were tested for their suitability as models to investigate *Listeria monocytogenes* (*Lm*) infection. Human primary monocytes were isolated by either positive or negative immunomagnetic selection and differentiated in the presence of granulocyte macrophage colony-stimulating factor (GM-CSF) or macrophage colony-stimulating factor (M-CSF) into pro- or anti-inflammatory macrophages, respectively. Regardless of the isolation method, GM-CSF-derived macrophages (GM-Mφ) stained positive for CD206 and M-CSF-derived macrophages (M-Mφ) for CD163. Upon infection with *Lm*, all primary macrophages showed good survival at high multiplicities of infection. M-Mφ generally showed high phagocytosis of *Lm*. Strikingly, phagocytosis of *Lm* by GM-Mφ was markedly influenced by the method used for isolation of monocytes. GM-Mφ derived from negatively isolated monocytes showed low phagocytosis of *Lm* whereas GM-Mφ generated from positively selected monocytes displayed high phagocytosis of *Lm*. Moreover, incubation with CD14 antibody was sufficient to enhance phagocytosis of *Lm* by GM-Mφ generated from negatively isolated monocytes. By contrast, non-specific phagocytosis of latex beads by GM-Mφ was not influenced by treatment with CD14 antibody. Furthermore, phagocytosis of *Lactococcus lactis*, *Escherichia coli*, human cytomegalovirus and the protozoan parasite *Leishmania major* by GM-Mφ was not enhanced upon treatment with CD14 antibody indicating that this effect is specific for *Lm*. Based on these observations, we propose macrophages derived by *ex vivo* differentiation of negatively selected human primary monocytes as the most suitable model to study *Lm* infection of macrophages.

D27

Peritoneal macrophages inhibit dendritic cell-derived cytokine synthesis through Prostaglandin E2 during polymicrobial sepsis

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Polymicrobial sepsis induced by cecal ligation and puncture (CLP) rapidly causes the failure of dendritic cells (DC) to secrete IL-12 and TNF-α and to induce Th cell activation, summarized as "DC dysfunction". So far it is unclear whether DC dysfunction reflects exhaustion of DC after exposure to bacteria or whether DC are indirectly affected by a suppressive mediator that is released upon induction of sepsis. To address this issue, peritoneal lavage was prepared from mice 1 h after CLP and was added to BMDC. Sterile lavage from CLP mice induced the secretion of TNF-α from BMDC in a TLR2/MyD88-dependent but TLR4-independent manner. Moreover, the CLP-lavage reduced the CpG-induced IL-12 and TNF-α secretion from BMDC *in vitro* as well as from splenic DC *in vivo* after transfer of lavage into naïve mice. As *in vitro* model of CLP, peritoneal exudate cells (PEC) were stimulated with gut content equivalent to the amount used for the induction of sepsis *in vivo*. The sterile supernatant of PEC cultured with gut-derived bacteria for 1 h but not supernatants from PEC or bacteria alone suppressed the release of IL-12 and TNF-α from BMDC. The suppressive factor in the supernatant was produced by macrophages largely independent from TLR2, TLR4, and MyD88. We identified PGE_2 released by the macrophages as the factor that inhibited the TNF-α/IL-12 secretion from BMDC. We suggest that PGE_2 released by peritoneal macrophages immediately after induction of sepsis causes dysfunction of splenic DC. Therefore, DC dysfunction early after CLP does not reflect DC exhaustion.

D28

A role for the long-tailed myosins Myo1e and Myo1f in macrophage responses to TLR4 activation

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Investigation of the phosphoproteome of LPS-stimulated primary macrophages in previous work highlighted the cytoskeleton as one cell compartment with an enriched protein phosphorylation. In total we could detect 43 cytoskeleton-associated proteins which were regulated by this posttranslational modification. Among this group the two "long-tailed" Myosins 1e and 1f (Myo1e, Myo1f) were strongly phosphorylated. Because of their ability to simultaneously bind to actin and cell membrane or membrane-associated proteins they might play an important role in key macrophage functions like cell migration, spreading, phagocytosis, antigen presentation. To address the role of Myo1e and Myo1f in macrophage biology, we validated cytoskeleton-associated cell functions like spreading and phagocytosis as measurable readouts. Using primary Myo1e KO macrophages combined with a Myo1f siRNA KD we generate "double-deficient" cells to reduce redundancy effects due to their structural homology. Knockdown experiments demonstrated a requirement of Myo1f for efficient LPS-induced macrophage spreading, which was not affected in Myo1e KO cells. Significant effects on phagocytosis capacity and bacteria killing couldn't be observed. However, Myo1e plays a role in chemokine secretion and antigen presentation processes. We detected an increased MCP1 (CCL2) release in Myo1e-deficient macrophages and DC in response to LPS. Furthermore, Myo1e-deficient macrophages and DC had lower basal levels of MHC-II on the cell surface. Together, our data so far suggest a possible role for Myo1e in the transport of selected chemokines and MHC-II molecules to the cell surface. Whether Myo1e is also involved in MHC-II surface expression in other antigen presenting cells, namely B cells, will be the subject of ongoing experiments.

D29

LPS and glucocorticoids cooperate to limit inflammatory response in acute lung inflammation

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Glucocorticoids (GCs) are potent anti-inflammatory agents that have profound effects to suppress activation of macrophages during inflammatory disease. Suppression of cytokine expression via the glucocorticoid receptor (GR) monomer was suggested as the major mechanism of the anti-inflammatory effects mediated by GCs, whereas GR dimerization-induced gene expression would mediate side effects.
In contrast to the prevailing view we demonstrate that the GC activated GR dimer in concert with lipopolysaccharide (LPS) trigger p38 activation and synergistically induce the sphingosine kinase 1 (SphK1) gene in macrophages. The synergistic induction is of importance for the suppression of inflammation in a model of acute lung inflammation (ALI).
GCs did not reduce ALI in mice with impaired GR dimerization (GR^{dim}) and mice lacking the GR in myeloid cells ($GR^{LysMCre}$) that both failed to up regulate SphK1. Moreover chemical inhibition of p38 and p38 deficient macrophages impairs synergistic up regulation of the Sphk1 gene by GCs and LPS. Treatment of ALI with GCs resulted in elevated serum levels of sphingosine 1-phosphate (S1P), the product of SphK1, in wild-type, but not in GR mutant mice. Chemical inhibition of SphK1 activity as well as myeloid-specific deletion of SphK1 abrogated anti-inflammatory actions of glucocorticoids in ALI.
Thus, we describe here a novel mechanism of anti-inflammatory actions of GCs, which requires cooperative induction of Sphk1 by the GR dimer and p38 signaling in myeloid cells to suppress ALI.

D30

Enhancing Tetanus Toxoid (TT) specific cellular immune responses through targeted delivery of TT-peptide containing Nanocapsules to human Dendritic Cells

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Targeted delivery of antigens to Dendritic Cells (DCs) is a promising approach to induce antigen specific cellular immune responses. Aim of the present study was to evaluate the properties of nanocapsules (NCs) with encapsulated Tetanus Toxoid (TT) peptide and functionalized with anti-CD40, MPLA and IFN-γ with respect to the overall uptake, the released cytokine profile, the influence on the phenotypic maturation of human DCs, and the investigation of DC-T cell interactions after phagocytosis of TT-NCs.
NCs consisting of hydroxyethyl starch (HES) served as a carrier for TT-peptide, anti-CD40, MPLA and IFN-γ. Cord blood was acquired from caesarean sections of healthy newborns. DCs were generated by isolation of PBMCs from healthy adult volunteers following culturing of $CD14^+$ cells in the presence of IL-4 and GM-CSF. Autologous T cells were co-cultured with DCs after loading the with various formulations of nanocapsules. Up-take of NCs by DCs was investigated by flow cytometry and confocal microscopy, cytokine response by ELISA, and the response of T cells by a proliferation assay.
NC uptake by DCs was significantly enhanced by functionalizing of NCs with anti-CD40, anti-DEC205 and MPLA, whereas a combined coating had the most pronounced effect. With respect to cytokine profile coating with MPLA and anti-CD40 evoked a T_H1 type profile. The administration of TT peptide induced enhanced TT specific immune responses.
HES-NCs are a promising delivery system which for targeting antigens to human DCs. Simultaneous delivery of antigen along with signals which promote T_H1 type immune responses, offers the opportunity to overcome tolerance and to induce antigen specific cellular immunity.

D31

Roquin Promotes TNF mRNA Degradation via a Novel Class of Conserved RNA Stem-Loop Motifs

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Tumor necrosis factor (TNF) is the most potent pro-inflammatory cytokine of the mammalian organism. Numerous posttranscriptional mechanisms control the expression of this potentially harmful cytokine, including an AU-rich element (ARE) and a constitutive decay element (CDE) in the 3'UTR. Whereas ARE-mediated mRNA decay is transiently blocked during macrophage activation, the CDE causes constitutive mRNA decay, thereby limiting the expression of this potentially harmful cytokine under pro-inflammatory conditions. Here we demonstrate that the CDE folds into an RNA stem-loop in its active conformation. By RNA affinity purification we identified Roquin (Rc3h1) and Roquin2 (Rc3h2) as CDE-binding proteins, and by EMSA we confirmed the high specificity of Roquin for the CDE stem-loop. Overexpression, knockdown and morpholino approaches showed that Roquin accelerates TNF mRNA deadenylation and decay, and thereby suppresses TNF protein production in both RAW264.7 and primary bone marrow-derived macrophages.
Using a bioinformatics approach, we then identified CDEs on a genome-wide scale. More than 50 highly conserved CDEs were discovered in the mouse transcriptome, many of which encode regulators of development and inflammation. RNA-Seq of Roquin-associated mRNAs confirmed that CDE-containing mRNAs are the primary targets of Roquin. Taken together, we demonstrate that Roquin proteins act as major mediators of mRNA degradation by recognizing CDEs, a novel class of stem-loop RNA degradation motifs.
Reference: Roquin Promotes Constitutive mRNA Decay via a Conserved Class of Stem-Loop Recognition Motifs. Leppek et al., 2013, Cell 153:869-81

D32

Gata1 has an important role in the intrinsic counteracting of DC activation

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Dendritic cells (DCs) are key initiators and regulators of the immune response. The transcription factor Gata1 is expressed in several hematopoietic lineages and recently shown to be important in DC development.
To dissect the role of Gata1 in DCs, we make use of a DC-specific mouse Gata1 knockout model. Gata1 KO BM-DCs have an increased MHC-II and CD86 surface expression upon LPS stimulation compared to WT. In addition, the mRNA expression of IL6, IL12 and IL1α showed at least a 15x fold increase upon LPS stimulation in KO DCs.
In vivo, we found a higher surface expression of MHCII in Gata1 KO DCs. In addition, in steady state KO mice have a reduction of $CD4^+$ DCs in the spleen. Upon LPS administration, we did not observe the expected DC influx in the lymph nodes of KO mice, as occurs in WT mice. This suggests an important role for Gata1 in the intrinsic counteracting of DC activation, which could affect the concomitant DC migration upon activation.
To determine whether the differences we found in the lymph nodes of Gata1 KO mice are caused by a defect in migration, we studied several migration markers, e.g. SIRPα, CXCR4, CCR7. All showed an increased mRNA expression in KO DCs upon LPS stimulation. How this explains the absence of DC influx to lymph nodes after LPS administration is currently being investigated. One of our hypotheses is that Gata1 KO DCs might migrate towards a different location than the lymph nodes, in an uncontrolled manner.

D33

The expression of Tim proteins on macrophages and their key role in erythrophagocytosis

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Background: The T cell immunoglobulin and mucin proteins (TIMs) are a novel gene family, which are expressed on immune effector cells and exert diverse functions in regulating immunity. In recent studies it has been demonstrated that Tim-1, Tim-3 as well as Tim-4 are expressed on human and mouse macrophages and dendritic cells, and specifically target phosphatidylserine (PtSer) expressed on the surface of apoptotic cells. The recognition of PtSer on eryptotic cells is critical for the efficient clearance of senescent erythrocytes and for the prevention of autoimmunity. In this study, we determined the contribution of TIMs in erythrophagocytosis *in vitro* and studied their expression during *Plasmodium berghei* ANKA *(PbA)*-induced experimental cerebral malaria (ECM) in susceptible (C57BL/6) and resistant (BALB/c) mice

Methods: Peritoneal macrophages, bone marrow derived macrophages and J774 cells were cultured in 24-well plates and co-incubated with labelled eryptotic cells. BALB/c mice and C57BL/ 6 mice were inoculation of 5×10^6 PbA-parasitized red blood cells. Spleens, livers and brains were removed for mRNA expression of Tim-3, Tim-4 and different T cell and macrophage cytokines.

Results: In vitro, we found an increased expression of Tim proteins on activated macrophages, which correlated with an increased uptake of eryptotic mouse cells. Silencing of Tim-3 with siRNA or a blocking antibody in J774 leads to a decrease in erythrophagocytosis. In *PbA* infected mice the expression of TIMs were significantly increased and correlated with increased erythrophagocytosis and development of anemia.

Conclusion: Our data indicate that up-regulation of TIMs on spleen and liver macrophages contributes to erythrophagocytosis and is likewise centrally involved in the development of anemia in *PbA* infection which thus emerges as the results of intravasal hemolysis of infected cells and TIM mediated erythrophagocytosis.

D34

The MAPK phosphatase Dusp9 is selectively expressed in mouse plasmacytoid dendritic cells and regulates IFNβ production

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Plasmacytoid dendritic cells (pDC) efficiently produce large amounts of type I interferon in response to TLR7 and TLR9 ligands, whereas conventional DCs (cDC) predominantly secrete high levels of the cytokines IL-10 and IL-12. The molecular basis underlying this distinct phenotype is not well understood, but may be related to differences in MAPK activation. Here, we identified the MAPK phosphatase Dusp9/MKP-4 by transcriptome analysis as selectively expressed in pDC but not cDC. We confirmed the constitutive expression of Dusp9 at the protein level in pDC generated *in vitro* by culture with Flt3L and *ex vivo* in sorted splenic pDC. Dusp9 expression was low in $B220^-$ bone marrow precursors and was up-regulated during pDC differentiation, concomitant with established pDC markers (e.g. Tcf4). Higher expression of Dusp9 in pDC correlated with impaired phosphorylation of the MAPK ERK1/2 upon TLR7/9 stimulation. In contrast, stronger phosphorylation of Stat1 was detected in pDC, likely caused by autocrine IFNβ signaling and demonstrating the selective impairment of ERK1/2 activation in pDC. Notably, Dusp9 was not expressed at detectable levels in human pDC, although these displayed similarly impaired activation of ERK1/2 MAPK compared to cDC. Enforced retroviral expression of Dusp9 in mouse GM-CSF-induced cDC increased the expression of TLR7/9-induced IL-12p40 and IFNβ, whereas IL-10 levels were diminished. Taken together, the species-specific, selective expression of Dusp9 in murine pDC contributes to the differential cytokine/interferon output of pDC and cDC.

Human dendritic cell subtypes interact specifically with the pathogenic mold *Aspergillus fumigatus*

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Dendritic cells (DCs) are key antigen-presenting cells, which represent different DC subtypes, heterogeneous in terms of origin, morphology and function. We explored how different DC subtypes (myeloid DCs (mDCs), plasmacytoid DCs (pDCs) and monocyte derived DCs (moDCs)) interact with and affect the fungal pathogen *A. fumigatus*.

mDCs, pDCs and monocytes were isolated from the peripheral blood of healthy volunteer donors using antibody bound magnetic MicroBeads. Monocytes were differentiated into moDCs over 6 days with IL-4 and GM-CSF. DC subtypes were co-incubated with resting conidia or germ tubes and afterwards analyzed by time-lapse video microscopy, scanning electron microscopy, plating assays, flow cytometry, transwell assays and multiplex ELISA.

Our data show that the different DC subtypes interact with *A. fumigatus* specifically, although, moDCs and mDCs display similar characteristics. mDCs and moDCs recognized and responded to fungal morphologies. Although, mDCs did not kill conidia or phagocytose fungal morphotypes as efficiently as moDCs, they matured and secreted comparable amounts of cytokines. In contrast, pDCs did not phagocytose any fungal cells; they did not mature and secreted only limited cytokines upon contact to *A. fumigatus*. Due to rare and random contacts of pDCs with fungal cells during live-imaging it seems that pDCs do not actively recognize *A. fumigatus*.

In conclusion, DC subtypes interact distinctly with *A. fumigatus*, which indicate their different roles in the pathogenesis of invasive aspergillosis. moDC's and mDC's specific properties are worth to be considered in analyzing the feasibility of DCs as tools for future antifungal immunotherapy.

E05

Spingosine-1-phosphate receptor 1 on tumor-associated macrophages is required for lymphangiogenesis-dependent metastasis in breast cancer

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Macrophages are involved in virtually each step of tumor progression, reaching from possible eradication of tumors to promoting invasiveness and metastatic spread. The latter requires, at least partly, macrophage-dependent recruitment of vasculature to the developing tumor. Enhanced sphingosine-1-phosphate (S1P) levels in tumors are associated with increased angiogenesis and metastasis as well, which may require signalling through S1P receptor 1 (S1pr1) on macrophages. We analyzed tumor growth in the transgenic *polyoma middle T oncogene* (PyMT) breast cancer mouse model combined with macrophage-specific S1pr1 deletion ($F4/80^{cre/+}$ $S1pr1^{fl/fl}$) compared to controls ($F4/80^{cre/+}$ $S1pr1^{wt/wt}$). In this model, model S1pr1 is specifically deleted in $F4/80^{high}$ macrophage populations, among them tumor-associated macrophages (TAMs). The presence of S1pr1-deficient TAMs was associated with slower tumor progression and had a remarkable negative impact on metastasis formation, even when analyzing animals with similar tumor burden compared to control animals. When investigating the underlying mechanism, we did not observe alterations in pre-metastatic niche formation, but noticed a strong reduction of tumor-associated lymphangiogenesis. This lymphangiogenesis phenotype was also observed in the non-metastasizing methylcholanthrene (MCA)-induced fibrosarcoma model. $F4/80^{cre/+}$ $S1pr1^{fl/fl}$ as well as $F4/80^{cre/+}$ $S1pr1^{wt/wt}$ TAMs were sorted from both PyMT and MCA tumors and analyzed using whole genome mRNA microarrays to identify S1pr1-dependent targets that regulate tumor-associated lymphangiogenesis. Prospective targets are validated in primary TAMs and tested for their involvement in macrophage-dependent lymphangiogenesis *in vitro*.

E06

Addition of the cell death modulator zVAD-fmk to standard combinatory melanoma treatments results in activation of macrophages and dendritic cells

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Background: Radiotherapy (RT), chemotherapy (CT) and hyperthermia (HT) induce and modulate cancer cell death and thereby the tumor microenvironment. The latter determines recruitment and activation of immune cells
Methods: B16 melanoma death forms after single and multimodal treatments in the presence or absence of the necroptosis inhibitor necrostatin-1 or the apoptosis inhibitor zVAD-fmk were analyzed by AnnexinA5-FITC/PI staining. The release of danger signals was determined by ELISA and the activation of macrophages (MΦ) and dendritic cells (DC) after contact with the supernatants (SN) of the treated tumor cells by multicolor flow-cytometry.
Results: We revealed for the first time that immunogenic necroptosis is inducible in melanoma cells. The release of the heat shock protein 70 by the tumor cells was significantly increased after treatment with HT, either alone or in combination. The release of the danger signal high mobility group box 1 protein was increased in each case when zVAD-fmk was used for cell death modulation. SN of zVAD-fmk treated melanoma cells induced an up-regulation of the activation markers CD86 and MHCII on MΦ. The same was seen on DC, when zVAD-fmk was added to multimodal tumor treatments including the chemotherapeutic agent dacarbazine. SN of zVAD-fmk treated melanoma cells further induced a new, inflammatory DC phenotype and increased the activation of naïve T cells.
Conclusion: Multimodal melanoma treatments including zVAD-fmk should now be tested in syngeneic animal models to get hints which treatment regime might lead to the strongest induction of anti-melanoma immune responses in the clinics.

E07

Impact of single and fractionated radiotherapy alone or in combination with temozolomide on the activation of dendritic cells by supernatants of U87MG glioblastoma cells

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Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults. Standard therapy includes surgery, followed by radiotherapy (RT) in combination with the alkylating agent temozolomide (TMZ). However, the prognosis for patients remains poor with an overall median survival of 14.6 months. One reason for this is the immune suppressive properties of GBM. Further, the brain as immune privileged area limits anti-GBM immune responses. Since radio(chemo)therapy induces glioma cell death, we hypothesized that distinct treatments may result in immunogenic tumor cell death characterized by the release of danger signals such as heat shock protein70 (HSP70) and consecutively in the activation of dendritic cells (DC).
P53 wild type U87GM cells were treated with a single high dose (10Gy) or with fractionated RT (5x2Gy), either alone or in combination with TMZ. Cell death, the release of HSP70 and the expression of activation markers on human monocyte derived DC after contact with supernatants (SN) of the tumor cells was analyzed.
Fractionated irradiation resulted in significant increased amounts of necrotic U87GM cells and in increased release of HSP70, compared to single irradiation with 10Gy. The addition of TMZ slightly further enhanced this. Only fractionated irradiation, in the presence or absence of TMZ, generated SN that induced an increased expression of CD83 and CD86 on DC.
We conclude that fractionated RT is the main trigger for the induction of immunogenic U87GM cell death. Current research focuses on p53 mutated cell lines and primary GBM tumor cells and aims to identify immune activating treatment regimens.

E08

Batf3$^{-/-}$ mice lacking CD103^{+} dendritic cells exhibit increased inflammation induced intestinal tumor development

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The key function of dendritic cells (DCs) is the presentation of antigens to T cells which leads context-dependent to the induction of highly equipped effector and regulatory T cell populations. In the context of malignancies DCs are considered to play a key role in inducing and maintaining antitumor immunity. Interestingly, tumor infiltration by certain DC subsets in patients suffering from colorectal adenocarcinoma is associated with improved survival, leading to novel therapeutic options.
Mucosal inflammation often leads to increased tumor formation as seen in the inflammatory model of chemical induced colon carcinoma in mice, utilizing the carcinogenic effects of azoxymethane (AOM) with the inflammatory effects of dextran sodium sulfate (DSS). To examine the contribution of non-lymphoid CD103^{+} DCs on the induction and establishment of inflammation induced colon carcinomas we compared wildtype mice with Batf3$^{-/-}$ mice lacking lymphoid-resident CD8α^{+} DCs and their migratory counterparts, CD103^{+} DCs.
Tumor bearing Batf3$^{-/-}$ mice showed higher tumor load in the AOM/DSS model compared to wildtype. Furthermore, under suboptimal inflammatory conditions, Batf3$^{-/-}$ mice showed a faster onset of tumorigenesis, higher tumor incidence and higher tumor burden. *Ex vivo* analysis of both colon infiltrating as well as splenic or mesenteric lymph node derived T lymphocytes revealed a striking reduction of IFN-gamma^{+} T cells in the absence of Batf3$^{-/-}$ while the numbers of regulatory T cells remained unaltered compared to wildtype mice.
These results imply an important role for Batf3-dependent DCs during intestinal tumorigenesis and set the cornerstone to further investigate the role of Batf3 in mucosal cancer.

E09

Enzymatically modified LDL (eLDL) and idiopathic Hyperlipidaemia modulate Mono- cyte Antigens CD14, CD16, HLDR, TLR2 & TLR4 - effect of antilipaemic Treatment

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We studied the possible effect of enzymatically modified low density lipoprotein (eLDL) compared to native LDL on monocyte antigens and the cellular ultrastructure after challenge with these lipids; in similar manner patients (n=7) suffering from idiopathic hypercholerol- emia (HCH) were assessed.
Peripheral blood monocytes (Mo) were isolated from healthy donors by gradient centrifugation (Nycoprep), and cultured in RPMI according to standard protocol, followed by incubation with 75µg/ml LDL and enzymatically modified LDL (eLDL) respectively. After 15 min, 6 and 12 hours (h) cells were harvested, and analysed for the expression of CD14, CD16, HLA-DR and toll-like receptors TLR2 & TLR4 by flowcytometry. Shape and viability of cells were analysed after 15 min, 6 and 12 hrs by uptake of trypan-blue, and transmission electronmicroscopy. Beside routine laboratory parameters patients with primary HCH were analysed before and during treatment with HMG-CoA reductase inhibitors.
eLDL, but not LDL, downregulated CD14 & CD16 expression of Mo (compared to controls), whereas HLA-DR increased after 12 h incubation with LDL and eLDL, which a higher increment by eLDL ($p<0.01$). In contrast to native LDL a maximum increase of TLR2 and TLR4 expression (x 120 fold) was induced by eLDL after 6 hours, restricted to selected male donors. Serial analyses of cells by transmission electronmicroscopy revealed a progressive accumulation of intracellular lipid droplets starting from 15 min up to a maximum between 6 and 12 h after incubation with eLDL only. Blood levels of CD14+CD16+ Mo subsets were higher in patients with HCO ($p<0.001$) compared to healthy controls (n=27), and rapidly dropped within 3-4 days after starting a treatment with statins ($p<0.01$), whereas CD14++ cells were not effected; in parallel serum-CRP levels decreased to a certain extent ($p<0.07$).
The data evidence a high biological in vitro activity of eLDL concerning the modulation of major Mo antigens as well as concerning its cellular uptake and accumulation. Antilipaemic therapy improves a proinflammatory cellular status in patients with primary hyperchole- sterolaemia.

E10

Macrophage-derived NGAL promotes tumor growth in breast cancer via EMT

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Tumor-associated macrophages (TAM) play a critical role in breast cancer and are indicative of poor prognosis and a low survival rate. After infiltrating tumor tissue, macrophages are re-programmed by cancer cells to shift their phenotype from an inflammatory to an immunosuppressive one in order to support tumor growth, metastasis, and angiogenesis. One recently described factor released by TAM is the neutrophil gelatinase-associated lipocalin (NGAL).
NGAL is highly expressed in a variety of cancer types and is associated with the oncogenic process of epithelial-to-mesenchymal transition (EMT), a hallmark of cancer metastasis. During EMT, epithelial cells lose their apical-basal polarity and change their phenotype to a prolonged, mesenchymal one, thereby allowing the escape of cancer cells from the primary tumor site.
We provide evidence regarding the potential of TAM-derived NGAL in breast cancer metastasis by inducing EMT. The interaction of apoptotic breast cancer cells with primary human macrophages provokes the release of NGAL. In a MCF-7 breast cancer spheroid model macrophage-conditioned media containing NGAL induces EMT. This is reflected by a decreased expression of epithelial markers, such as cytokeratines and increased levels of mesenchymal markers such as N-cadherin. Furthermore, macrophage-derived NGAL not only affects EMT-associated genes, but also promotes migration and invasion of MCF-7 cancer cells. Furthermore, the knockdown of NGAL in macrophages significantly inhibits EMT-induction in MCF-7 breast cancer spheroids.
Our data suggest an essential role of TAM-derived NGAL during cancer progression.

E11

Evaluation of CLEC5A and CD163L1 as markers of M1 and M2 polarization in human macrophages

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Macrophages (Mph) are innate immune cells with remarkable plasticity and heterogeneity in phenotype and function according to the local microenvironment. Mph can be polarized into distinct phenotypes under the influence of GM-CSF (M1-Mph) and M-CSF (M2-Mph). M1-Mph are pro-inflammatory and tumoricide, whereas M2-Mph have anti-inflammatory and pro-tumoral functions. We performed microarray assays between M1- and M2-Mph generated *in vitro* from human CD14+ monocytes. We found a selective expression of the lectin CLEC5A transcripts in M1-Mph, and of the scavenger receptor CD163L1 in M2-Mph. *In vitro*, CD163L1 was only expressed in M2-Mph, and its expression was induced by IL-10 and M-CSF. CLEC5A was preferentially expressed in M1-Mph, and was induced by inflammatory cytokines and by cell adherence. In lymph nodes, CLEC5A+ cells were restricted to the interfollicular regions, while CD163L1+ cells were also located at the follicles. A high percentage of both cell types co-expressed CD68. Localization of CLEC5A+CD68+ and CD163L1+CD68+ tumor-associated Mph (TAM) was assessed in primary melanomas and in lymph node metastases. CLEC5A+ TAM were virtually absent in primary tumors. The number of CD163L1+ TAM was higher, but they were mostly located at the peri-tumoral region. Both types of Mph increased their number in metastatic lymph nodes at the intra- and peri-tumoral zones. However, CLEC5A+ TAM were not associated with tumor cells in advanced metastases, whereas CD163L1+ TAM did, suggesting a positive correlation between the expression of CD163L1 and deeper tumor invasion. These results indicate a strong association of CLEC5A and CD163L1 with M1 and M2 polarization, respectively.

E12

Low and intermediate dose ionising radiation even up to 2Gy does not alter viability and phagocytic behaviour of activated macrophages but induces an anti-inflammatory cytokine milieu

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Macrophages act as professional phagocytes and influence their microenvironment by cytokine secretion. While evidence exists that distinct tumor associated macrophages exert pro- or anti-tumorial effects, little is known how these immune cells get modulated by ionizing radiation (X-ray) when exposed to a single dose of 2Gy during standard radiotherapy or by low doses of X-ray in general.
We set up an *ex-vivo* model in which Lipopolysaccharide pre-activated peritoneal macrophages (pMph) from BALB/c mice were exposed to X-rays up to 2Gy. 24h later, the release of cytokines, the viability and the phagocytic behavior of the macrophages was tested.
Exposure of pMph up to single doses of 2Gy did not significantly influence their viability or phagocytic capability. Cytokine analyzes revealed that levels of the pro-inflammatory cytokines IL-1ß and TNFα were reduced by single doses of 0.01Gy and from 0.5 up to 2Gy. The level of the inflammatory cytokine IL-4 was significantly reduced in a dose range of 0.1 – 0.7Gy and that of IL-6 especially at 0.01 and 0.5Gy. The level of the anti-inflammatory cytokine TGF-ß showed an opposing, increasing trend in the dose range of 0.1 – 0.5Gy.
Especially the intermediate dose of X-ray of 0.5Gy results in an anti-inflammatory pMph microenvironment. Further, exposure of activated macrophages up to 2Gy of X-ray does not impair their viability and function. The anti-inflammatory microenvironment induced by X-ray might be also beneficial for anti-tumorial activities counteracting inflammation-induced tumor progression.
This work is supported by the European Commissions (DoReMi, European Network of Excellence, contract number 249689).

E13

Development of a solid flow cytometry-based assay for immunophenotyping of patients with inflammatory diseases

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Flow cytometry has become a very important tool for the analyses of phenotype and function of cells over the years in clinic and science. The availability to measure multiple parameters at once on a single cell basis makes it to one of the most powerful technologies for screening of patient blood samples. It allows characterization of many different cell subsets, including rare ones, and activation status in a small sample size through detection of expression patterns of surface, intracellular and extracellular proteins.
Here we developed a flow cytometry-based assay to study in detail the changes of the immune status, caused by treatment with low-dose radiotherapy of X-ray or Radon, in patients with chronic inflammatory degenerative diseases. This assay covers the detection of all main immune cell types in peripheral blood such as T cells, B cells, monocytes, granulocytes, natural killer cells, dendritic cells, and stem cells. Besides the analyses of morphology, activation status and cell death, it further distinguishes the cells in 30 subsets with recognition of additional surface markers. This flow cytometry-based immunophenotyping assay was tested with fresh blood samples from different healthy donors and 100 patients with chronic inflammatory diseases and found to be solid.
The possibility to observe changes in dendritic cell and monocyte populations in patients during various therapies and the possibility to specifically upgrade this assay makes it valuable for researchers of the EMDS society.
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E14

Mild hyperthermia enhances human monocyte-derived dendritic cell functions and offers potential for applications in vaccination strategies

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Dendritic cell (DC) based immunotherapy has been shown to be a promising strategy for anti-cancer therapy. Nevertheless, only a low overall clinical response rate has been observed in vaccinated patients with advanced cancer and therefore methods to improve DC immune-stimulatory functions are currently under intense investigation. In this respect, we exposed human monocyte-derived DCs to a physiological temperature stress of 40°C for up to 24 hours followed by analysis for (i) expression of different heat shock proteins, (ii) survival, (iii) cell surface maturation markers, (iv) cytokine secretion, and (v) migratory capacity. Furthermore, we examined the ability of heat shocked DCs to prime naive $CD8^+$ T cells after loading with MelanA peptide, by transfection with MelanA RNA, or by transduction with MelanA by an adenovirus vector. The results clearly indicate that in comparison to control DCs, which remained at 37°C, heat treated cells revealed no differences concerning the survival rate or their migratory capacity. However, DCs exposed to thermal stress showed a time-dependent enhanced expression of the immune-chaperone heat shock protein 70A and both an up-regulation of co-stimulatory molecules such as CD80, CD83, and CD86 and of the inflammatory cytokine TNF-α. Moreover, these cells had a markedly improved capacity to prime autologous naive $CD8^+$ T cells *in vitro* in an antigen-specific manner, independent of the method of antigen-loading. Thus, our strategy of heat treatment of DCs offers a promising means to improve DC functions during immune activation which, as a physical method, facilitates straight-forward applications in clinical DC vaccination protocols.

E15

Comparative transcriptomics reveals gain of proliferative capacity, loss of phagocytic capacity and a distinct phenotype of tumor-associated macrophages in murine chronic lymphatic leukemia

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Tumor-associated macrophages (TAM) are a major component of the tumor microenvironment linked to reduced survival in most tumor entities. Using comparative transcriptomics in macrophages derived from spleen and bone marrow at pre-leukemic versus leukemic state in a mouse model for chronic lymphocytic leukemia (CLL), we revealed profound re-programming of macrophages during leukemogenesis. Interestingly, gene set enrichment analysis showed just little overlap with M1-like or M2-like macrophage polarization patterns. Instead, major transcriptional changes indicated a gain of proliferative capacity and loss of phagocytic mechanisms showed by gene ontology enrichment analysis. Indeed, increased proliferation of F4/80+ macrophages could clearly be shown in leukemic animals at local tumor sites. Moreover, reduced phagocytic capacity of macrophages was validated in vivo in leukemic mice and in vitro using macrophages of CLL patients. Comparative transcriptomics of macrophages within the same tissue at pre-malignant versus malignant state allowed to identify important macrophage-mediated suppressive mechanisms in leukemia.

E16

Inducing T cells specific for mutated tumor antigens

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Mutations of BRAF and of NRAS both lead to a constitutive activation of the RAS-RAF-MEK-ERK-MAP kinase pathway. Both mutually exclusive mutations are frequently found in melanoma cells. Based on the high frequency of these mutations and their exclusive occurrence in tumor cells, these mutated proteins could be suitable targets for T-cell based immunotherapy of melanoma. This strategy requires the activation of cytotoxic T cells expressing T-cell receptors which are specific for the mutated BRAF or NRAS antigens.
To find out whether BRAF and NRAS are possible therapeutic targets, we tested for the occurrence of $CD8^+$ T cells in the blood of healthy donors, which are able to react specifically to $BRAF^{V600E}$-, $NRAS^{Q61K}$-, or $NRAS^{Q61R}$-presenting dendritic cells (DC). Furthermore, we compared the wild-type and the mutated versions of these antigens regarding their stimulatory capacity. Therefore, we electroporated mature DC with RNA that encoded the wild-type or the mutated BRAF or NRAS antigens. To increase and prolong the DC's stimulatory capacity, they were co-transfected with constitutively active IKKα and IKKβ. Autologous $CD8^+$ T cells were stimulated with these DC. To analyze the expansion and functionality of antigen-specific $CD8^+$ T cells, we measured their antigen-specific cytokine production by Elispot assays. The cytokine release showed that antigen-specific T cells were induced in most of the donors after stimulation with BRAF and $BRAF^{V600E}$-presenting cells. Similar experiments with NRAS encoding RNA, led to divergent results. Recent experiments, however, point out that DCLamp or the Myc-Tag linked to the Ag might also cause immunogenic reactions.

E17

Targeting mutated and over-expressed tumor antigens is a challenge for cancer immunotherapy

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We investigated whether mutated tumor antigens (Ag) (GNAQ and GNA11), and Wilms' tumor protein WT1, over-expressed in several leukemia and solid tumors, could serve as targets for cancer therapy.
Differences in the immunogenicity of wild-type or mutated GNAQ and GNA11 were tested on $CD8^+$ T cells, which were stimulated three times for 7 days with autologous mature monocyte-derived dendritic cells (mDC) that had been loaded with wild-type or mutated Ag by RNA-electroporation. The Ag-specific activity of stimulated CTL was detected by Multi-Functional T-Cell (MFTC) and IFNγ-Elispot assays. An antigen-specific increase in CTL producing TNF and IFNγ in response to target cells transfected with Ag-encoding RNA, but not control RNA, was detected in MFTC. Elispots indicated Ag-specific IFNγ-production by CTL transfected with wild-type or mutated Ag. Cross-reactivities between distinct Ag versions were observed. Therefore, no definite conclusion about differences in the immunogenicity of wild-type and mutated Ag can be drawn.
The immunogenicity of wild-type WT1 was compared to a chimeric WT1-DCLamp construct in various HLA-backgrounds. CTL were stimulated with mDC, which had been transfected with WT1 or WT1-DCLamp mRNA, and Ag-specific activity was demonstrated. In MFTC, an antigen-specific increase of CTL producing TNF and IFNγ was detectable. Corresponding results came from Elispots. However, recent experiments indicate that either DCLamp or Myc-Tag linked to the Ag might also cause immunogenic reactions.
In summary, this approach in cancer immunotherapy seems promising, but it is still necessary to improve experimental procedure and identify new targets.

E18

Cathelicidin mediates anti lymphoma cytotoxicity by human macrophages

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Introduction: In haematopoietic lymphatic malignancies, such as Burkitt's lymphoma (BL), infiltration by macrophages is a characteristic morphological hallmark**.** Although macrophages can be directly cytotoxic against tumor cells, tumor associated macrophages (TAM) fail to kill tumor cells. However, the mechanism for this loss of function is unknown. Given the fact that the vitamin D axis is a key regulator in macrophage mediated immune response and therefore essential for tumoricidal macrophages we therefore hypothesized that M2 macrophages and TAM in BL display an alteration of the vitamin D metabolic pathway, thereby circumventing macrophage mediated antitumor activity.
Methods: We included 19 patients diagnosed with BL and, as a control group, 20 patients with benign reactive lymphadenopathy. Phenotypic characterizations of TAM were evaluated by immunohistochemistry and by qPCR in paraffin embedded tissues. To investigate the role of distinct macrophage subsets in tumorigenesis in BL, we generated macrophages from PBMC either by GM-CSF to create the M1 phenotype, or by M-CSF to obtain the M2 phenotype, coincubated them with several BL cell lines and analyzed tumoricidal effects by FACS, qPCR and immunofluorescence.
Results: The results presented here demonstrate that human M1 macrophages selectively kill proliferating lymphoma cells with the vitamin D dependent peptide cathelicidin, by targeting the Achilles heel of cancer cells, the mitochondria. In contrast, M2 macrophages and TAM in BL, which exhibit an M2-like phenotype, express low levels of cathelicidin, due to an altered vitamin D metabolic pathway, and fail to lyse lymphoma cells. We also showed that addition of exogenous $1,25D_3$ or a VDR agonist to M2 macrophages triggered the cathelicidin mediated antitumor activity against BL cells
Conclusion: These results suggest a mechanism in which vitamin D is required for innate immunity to overcome the ability of lymphoma cells to evade macrophage-mediated antitumoral responses. The present findings underscore the importance of adequate amounts of vitamin D for sustaining innate immunity and imply that the therapeutic activation of the vitamin D pathway may even result in triggering tumoricidal effector mechanisms of TAM.

E19

Characterization of TAMP1 expression and function in tumor-associated macrophages:

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Tumor-associated macrophages (TAM) are important regulatory immune cells in the tumor microenvironment whose involvement in smoldering inflammatory processes in cancer and cancer metastasis have been recognized.
By gene expression profiling of bone-marrow derived macrophages stimulated with tumor conditioned medium, we have identified the novel type one transmembrane protein TAMP1 selectively expressed by TAM in colorectal carcinoma and malignant melanoma. TAMP1 is a member of a protein family involved in the regulation of the adaptive immune system. To further investigate TAMP1 *in vivo* and *in vitro*, a monoclonal antibody was generated against a n-terminal peptide sequence of the protein in rats. In order to verify the specificity of the antibody, a transgenic TAMP1 expressing monocytes-like RAW264.7 cell line was generated. These transgenic cells were analyzed by western blot and immunohistochemistry stainings. In western blot analysis two specific bands were identified by the monoclonal antibody, which points to a posttranscriptional modification of the protein. Furthermore, immunohistochemical stainings revealed a strong TAMP1 expression in TAM of both subcutaneous B16F10 melanoma and of CT26 colon carcinoma transplant tumors. To study the function of TAMP1, proliferation and apoptosis were tested in the Raw264.7 transgenic cell line but no differences were observed. Additionally, the migration was analyzed by a wound healing assay: TAMP1 positive RAW264.7 cells displayed a delayed closure of the artificial scratch. Concluding, these results indicate a possible role of TAMP1 for macrophage migration or adhesion, but further studies are needed.

E20

Ms4a8a, a novel marker of M2-like macrophages

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Tumor-associated macrophages (TAM) represent M2 (or alternatively activated) macrophages that support tumor growth.
Gene profiling of a Stabilin-1$^+$, Lyve-1$^+$ M2 macrophage subset identified Ms4a8a as a novel TAM molecule which *in vitro* is induced in bone marrow-derived macrophages (BMDM) by combining M2 mediators (IL4, dexamethasone) and tumor-conditioned media (TCM).
In vivo, Ms4a8a protein was preferentially expressed by TAM in mammary carcinoma and malignant melanoma.
In addition, we identified Ms4a8a$^+$ macrophages in other M2-associated pathologies as late stage Trypanosomiasis and Cysticercosis. These infection models are characterized by a clear shift from M1 (early stage) to M2 (late stage) predominance.
Innate immunity in these infections is modulated by toll-like receptor (TLR) signalling. In line with this, TLR2/4/7 agonists strongly induced Ms4a8a expression in BMDM given simultaneous treatment with M2 mediators IL4 and dexamethasone (dexa). Co-induction of Ms4a8a by M2 mediators and TLR agonists activated the classical TLR signalling cascade via MyD88/TRIF and NFkB.
Upon forced over-expression in Raw264.7 cells, Ms4a8a activated a special M2-like gene expression program including *Tcfec*, *Spink5* and *Sla*. When the LPS response of Ms4a8a transfected cells was analysed, *Tcfec* and *Sla* were further up-regulated, while *Hdc* was expressed *de novo*. Induction of these Ms4a8a dependent genes were also found in Ms4a8a$^+$ LPS dexa/IL4 stimulated BMDM and PEMs from *T. crassiceps* infected mice.

E21

Characterization of the biological function of CD83 using tissue-specific knock-out mice

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CD83 is one of the best known cell surface markers for mature dendritic cells which can potently activate naïve T cells. In addition, CD83 is expressed on a variety of cell types including activated T and B cells, regulatory T cells and thymus epithelial cells. Up to date two isoforms of CD83 have been reported: a membrane bound (mCD83) and a soluble form (sCD83) which consists of the extracellular part of mCD83. Functionally it has been shown that mCD83 on mature DCs acts as a co-stimulatory molecule, can induce T cell proliferation and its expression on thymic epithelial cells plays an important role during T cell development in the thymus. While *in vitro* mCD83 has been shown to possess immune stimulatory capacities when expressed on DC, sCD83 displays inhibitory functions down-modulating immune responses. Hence CD83 is a highly interesting molecule for prospective clinical investigations. However, the elucidation of the specific functions on different cell types for mCD83 and sCD83 is a prerequisite for its clinical applications. Thus, one of our goals is the phenotypic and functional characterisation of CD83 on DCs and B cells using tissue-specific CD83 knockout mice. Therefore CD83-loxP floxed mice have been crossed with CD11c-Cre mice as well as with mb1-Cre mice to deplete CD83 specifically on DCs and B cells, respectively. In first experimental *in vitro* and *in vivo* settings the resulting offspring have been analysed showing phenotypical and functional differences between wildtyp, complete k.o. and the cell-type specific k.o. animals. These data will be presented during the meeting.

E22

Hypofractionated local radiotherapy induces a timely restricted infiltration of innate immune cells in CT26 colon cancer tumors of BALB/c mice

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It is well known that the major mode of action of radiotherapy (RT) is the induction of DNA-damage that finally leads to cell cycle arrest or cell death. Additionally, RT modifies the phenotype of tumor cells and the tumor-microenvironment which contributes to induction of specific and systemic anti-tumor immune responses. The latter can be boosted when additional immune therapy is applied at distinct time points during RT. We therefore aimed to examine whether and which immune cells are recruited into the tumor after RT and if their presence is timely restricted in a syngeneic CT26-BALB/c tumor model.
The tumor outgrowth was significantly retarded after RT. While CD4 T-cells migrated into non-irradiated and irradiated tumors, CD8 T-cells were only found in tumors that had been irradiated. Innate immune cells were found in irradiated and non-irradiated tumors. However, an increased amount of especially NK-cells and MHC-II positive antigen presenting cells was found in irradiated tumors with a peak between day 2 and 5 after the last irradiation. We also detected an increased infiltration of MDSC into the tumors after RT. However, the amount of such immune suppressive cells could be decreased by applying additional immune therapy with the immune stimulatory protein AnnexinA5.
Innate and adaptive immune cells infiltrate into the tumor after RT and their presence is timely restricted. This has to be taken into account when innovative multimodal treatment concepts consisting of RT and immune therapy are established.
Acknowledgement: This work is supported by the German Research Foundation (GA1507/1-1 and GK1660).

E23

IL-28 links Perforin expression in CTLs via Smurf2 and protects APC^{min} mice from tumorigenesis

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Type III Interferons like IL-28 were recently discovered and possess beside their antiviral properties also cancer fighting activities. The IL28 receptor has been demonstrated to be highly expressed on lung and gut epithelial cells but also on dendritic cells. The aim of this study was to determine the functional role of IL28 signaling on dendritic cells in the pathogenesis of sporadic coloncarcinogenesis. Tumors from APCmin mice expresses significant higher levels of IL-28 and Perforin compared to control tissue indicating a relevant role of IL-28 in sporadic carcinogenesis. Interestingly, we found an additional significant upregulation of a splice form of Smurf2 (ΔSmurf2) in tumors compared to its WTSmurf2 variant suggesting that Smurf2 plays a pivotal role in sporadic tumorigenesis. Furthermore, in-vivo administration of IL-28A into mice lead to a significant increase of Perforin and ΔSmurf2 in cytotoxic T lymphocytes (CTL) resulting in a dramatic hightened cytotoxicity. Tumors significant lower in number and size in the colon of transgenic mice as well as higher Perforin/GranzymB/Eomesodermin but not T-bet expression were found in mice overexpressing ΔSmurf2 in CD8 T cells indicating that Smurf2 targets these genes for proteasomal degradation. Therefore, overexpression of ΔSmurf2 in T cells resulted in a loss of WTSmurf2 activity and contributes to an improved tumor burden. We conclude that IL-28 directly affects dendritic cells, activating ΔSmurf2 in CTLs and promotes cytotoxicity in the context of carcinogenesis aiding in immune surveillance. Thus, treatment with type III interferons may serve as a potential new therapeutic method to protect against colitis independent coloncarcinogenesis.

E24

CCR2 expression by macrophages promotes resolution of inflammation and tumor progression through induction of IL-10 production

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Chemokines are well known to play a major role in tumor progression and metastasis. In particular CCL2 and its receptor CCR2 are over-expressed in several human cancers and their higher levels correlate with poor prognosis and shorter outcomes. Whereas CCR2 has been demonstrated to recruit monocytic cells such as tumor-associated macrophages (TAM), little is known about its role in macrophage polarization. In order to directly investigate this aspect wt and CCR2-/- macrophages were polarized with M1 and M2 stimuli and analyzed for gene expression and cytokines production. While no difference was found in M2 polarized macrophages, CCR2-/- M1 or LPS activated macrophages showed higher expression of inflammatory genes and reduced production of the anti-inflammatory cytokine IL-10 and of the pro-angiogenic cytokine VEGF when compared to wt macrophages. After LPS and CCL2 co-stimulation, CCR2-/- macrophages showed reduced activation of NF-kB and p38 MAPK when compared to wt macrophages indicating a cross talk between CCR2 and TLR4 signaling pathways. The contribution of CCR2 to cancer growth was evaluated with a trasplantable lung cancer model that grew slower when co-injected with CCR2-/- macrophages. Taken together these data indicate that CCR2 expression by macrophages not only induce their recruitment to tumor site but also affect their polarization and anti-tumoral potential.

E25

Modulation of the LPS Response of Human Monocytes by (Tumor-derived) Lactic Acid

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The aim of this work was to study the influence of lactic acid (LA) on the activation of human monocytes. LA is known to accumulate under inflammatory conditions in wounds or tumors. We had initially observed an inhibitory effect of LA on TNF secretion and mRNA expression in LPS-stimulated monocytes. To globally analyze LA effects on monocytes, we performed whole genome microarray expression analyses. Monocytes were treated for one or four hours with LPS and LA, corresponding sodium lactate or acidic pH to separate LA-specific effects from lactate- or pH-dependent effects. Gene expression analyses revealed that LA modulates the LPS-response primarily during the early stimulation phase. Up-regulation of the majority of LPS-induced genes was significantly delayed by LA. LA targets included genes encoding for classical monocyte effector proteins like interleukins (IL) (e.g. IL-7 and TNF) or chemokines (e.g. CCL2 and CCL7). Microarray results were confirmed by quantitative RT-PCR. Analysis of LPS-signaling pathways showed that LA inhibits the degradation of IκBα and diminishes the level of phospho-Akt. This might represent a possible mechanism underlying the modulation of the LPS-response by LA. In conclusion, we demonstrate a profound effect of LA on monocyte LPS-signaling and gene expression suggesting a role for LA in suppressing the inflammatory (anti-tumor) response of monocytes.

E26

Different subpopulations of tumor associated macrophages impact an epithelial- mesenchymal transition in pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) still ranking 4th in the order of fatal tumor diseases, which is characterized by a profound tumor stroma with high numbers of tumor associated macrophages (TAMs). It is described that in tumors an pro-inflammatory M1- and suppressive M2-macrophage subtype can be identified. The latter are regarded as being pro-tumorigenic, but a detailed analysis of the role of TAMs in PDAC development is still lacking. Therefore, this study freshly isolated TAMs derived from tumor`s tissue of PDAC patients were analysed for their phenotype and impact on epithelial-mesenchymal transition (EMT) in co-culture with benign (H6c7) and malignant (Colo357) pancreatic ductal epithelial cells. Those TAMs exhibited characteristics of both, M1- and M2-macrophages. In the presence of TAMs, H6c7 and Colo357 cells increased EMT-marker expression such as an elongated cell shape along with an increased expression of the mesenchymal marker vimentin and reduced expression of epithelial e-cadherin. In parallel to TAMs, M1- and M2-macrophages were in vitro generated from peripheral blood monocytes and also co-cultured with the pancreatic epithelial cell lines. Both macrophage populations similarly mediated EMT in H6c7 and Colo357 cells. Furthermore, we could demonstrate that M1-macrophages acquired M2-characteristics during co-culture that could be prevented by GM-CSF treatment, but then M1-macrophages still potently induced EMT in H6c7 and Colo357 cells although lacking M2-characteristics. Overall, these data demonstrate that TAMs exhibit anti- as well as pro-inflammatory properties that equally contribute to EMT induction in PDAC.

E27

Stress-induced Glucocorticoids Mediate Recruitment of IMC into Sencondary Lymphoid Organs

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Chronic psychosocial stress has long been recognized as a risk factor for various immunological disorders. In physiological stress responses glucocorticoids (GC) are released from the adrenals to prepare the body for a fight-or-flight response. In contrast to the activating acute effects of GC, chronically elevated levels of GC lead to immune suppression, particularly for T- and B-cell functions. There are no data available indicating any activating effect on myeloid cells. Chronic subordinate colony housing (CSC) is an established model for chronic psycho-social stress in male mice. In the present study we investigated the effect of stress on the recruitment of $CD11b^+$ cells into secondary lymphoid organs. After 19 days of CSC increased numbers of immature $CD11b^+$ $Ly6G^+$ $Ly6C^{int}$ polymorpho-nuclear cells (PMN) as well as $CD11b^+$ $Ly6G^-$ $Ly6C^{high}$ monocytic myeloid cells (MO), together known as inflammatory myeloid cells (IMC), were found in the spleen. Stress-induced IMC produced more pro-inflammatory cytokines upon LPS stimulation. Serum levels of G-CSF were highly elevated in the very early phase (after 10 hours) of CSC indicating increased myelopoiesis. Elevated expression of chemokines in the spleen after 19 days of CSC mediated prolonged recruitment of IMC to the spleen. In order to test whether GC can cause these stress-induced alterations, we injected dexamethasone into naïve mice and observed similar effects.
In conclusion, our data indicate that chronic stress induces increased myelopoiesis and recruitment of IMC with high production capacity for pro-inflammatory cytokines to the spleen.

E28

The inhibition of adhesion and migration of Myeloid Derived Suppressor Cells in pancreatic cancer immunotherapy

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Pancreatic Ductal Adenocarcinoma (PDAC) is an aggressive tumor. The global five-years survival is of 5% and surgery is the only potentially curative treatment. The immunotherapy might be a chance. Antibodies against α-enolase (ENO1), are detected in more than 60% of patients with PDA. ENO1 vaccination in GEM elicits humoral, cellular response, and reduces number of myeloid derived suppressor cells (MDSC) (Cappello P. *et al*., 2013). MDSC increase in cancer patients, with correlation between circulating MDSC, metastatic burden, and cancer stage, therefore offer a new target in cancer therapy. We propose to clarify whether blocking the ENO1 interferes with MDSC migration, infiltration to the tumor site and suppressive activity. BM cells from C57BL/6 mice, were cultured in presence of GM-CSF, stimulated or not with LPS for 48h, and stained with anti CD11b, GR1 and ENO1 antibodies for FACS analysis; the expression of ENO1 in GR1+CD11b+ cells increased about two fold after LPS stimulation vs control. Then MDSC were treated or not with LPS in presence of antibody antiENO1(αENO1) and tested *in vitro* for adhesion, migration, arginase activity and cytokine production. *A*dhesion and migration were inhibited by αENO1, the migration decreased of 50% in treated cells vs control and similarly arginase activity. The production of cytokine (TNFα, IL10, IL6), in cells treated with four different concentrations of αENO1 decreased, but not in a dose-dependent way. Thus the antibodies anti ENO1 in vaccinate GEM, could inhibit the MDSC infiltration and interaction with the tumor microenvironment, making the immunotherapy more effective against MDSC-induced immunosuppression.

E29

Antigen targeting of Fc-receptors induces strong T cell responses *in vivo*

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Dendritic cells are very important antigen presenting cells and responsible for the initial induction of effective immune responses but also for the maintenance of peripheral T cell tolerance. We recently found that different DC subpopulations are able to induce different T cell responses after targeting antigens to the endocytosis receptors DEC205 and DCIR2 (33D1) in vivo. Beside C-type lectin receptors, Fc receptors are highly efficient endocytotic receptors expressed on a variety of antigen presenting cells. We found that Fc gamma receptors were differentially expressed on the DC subpopulations. To explore if Fc gamma receptors are suitable for in vivo antigen targeting, we have cloned the variable regions of a variety of anti Fc gamma receptor antibodies (Ly17.2-FcγRIIB, 9E9-FcγRIV) in frame to a non Fc receptor binding murine IgG1 constant region. For the immunological readout we genetically engineered the ovalbumin model antigen into the C-terminal region of the cloned antibodies. Here, we show that targeting antigens via recombinant ovalbumin carrying Fc receptor antibodies in vivo induces different T cell responses. Although Fc-gamma receptors are also expressed on other lymphoid cell populations (monocytes, B cells, granulocytes) we provide evidence that only the expression of Fc gamma receptors on the DC subpopulations is needed for the induction of T cell responses. Therefore, we suggest antigen targeting to Fc gamma receptors as useful tool for future therapeutic applications.
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E30

The role of microRNA 155 in innate immunity and arthritis

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Background: MicroRNA 155 (miR155) has been demonstrated to be essential for the development of collagen induced arthritis by controlling the generation of autoreactive T and B cells. However, the contribution of miR155 in innate immune cells is not known.
Materials and Methods: We analyzed activation and cytokine production of macrophages and dendritic cells (DCs) *in vitro* and *in vivo*. We analyzed T-cells stimulatory capacity of DCs. We crossed miR155 deficient mice into hTNFtg mice and analyzed arthritis development clinically as well as histologically.
Results: MiR155 deficiency did not alter the differentiation of BMDC from bone marrow cells or the expression of costimulatory molecules or MHCII expression after stimulation of macrophages and DCs *in vitro* and *in vivo*. We also Facs-sorted DCs after stimulation with LPS in vivo and determined the production of proinflammatory cytokines such as IL-23, IL-6 as well as TNF. We did not detect differences between wt and miR155$^{-/-}$ mice. In addition, the T cell stimulatory capacity of wt and miR155$^{-/-}$ was identical *in vitro* and *in vivo*. When we analyzed miR155$^{-/-}$ mice compared to wt mice in the hTNFtg model of inflammatory arthritis, which is independent of the adaptive immune system, we did not detect differences in the clinical signs and symptoms of arthritis. Histologically, we even found slightly increased synovial inflammation in hTNFtg/ miR155$^{-/-}$ mice compared to wt mice.
Conclusion: In contrast to the pivotal role of miR155 in autoimmunity requiring the adaptive immune system, the role of miR155 in innate immunity seems to be limited. This is emphasized by the fact that miR155 hardly influences the course of TNF-driven arthritis, which is mainly dependent on components of the innate immune system.

F04

The use of the Nanobody Technology to Block the MIF-CD74 Interaction.

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Cytokines play a critical role in the immune system as they are responsible for initiating and coordinating the host inflammatory responses. Although essential for the control of an invader, deregulation of such cytokines can culminate into major problems for the host, leading to a wide variety of chronic inflammatory diseases. The involvement of the interaction between macrophage migration inhibitory factor (MIF) and its receptor (CD74) has been identified as a key culprit in prolonging the inflammatory status/disease pathology in a variety of disease processes including autoimmune diseases, metabolic disorders, systemic infections as well as sepsis and cancer. The upstream actions in the inflammatory cascade and the ability to sustain inflammatory responses make targeting the MIF/CD74 axis an attractive, steroid sparing, therapeutic strategy. However, recently a MIF homologue (i.e. D-DT) has been identified rendering current MIF-targeting strategies insufficient. CD74 is found primarily on antigen presenting cells, has a high turnover rate and its expression is up-regulated during infection, making it a prime target. Therefore, we developed single domain antigen-binding fragments of camelid heavy-chain antibodies (termed Nanobodies; Nbs) to specifically target CD74, thereby blocking both the MIF and D-DT mediated effects. Hereby, we demonstrated that the anti-CD74 Nbs could (i) specifically recognize CD11b+Ly6c+ monocytic cells, (ii) block binding of MIF/D-DT on intact cells and (iii) block MIF-mediated TNF induction following LPS stimulation of myeloid cells. Therefore, we hypothesize that the Nb-based targeting of CD74 may offer a novel and more efficient therapeutic strategy to block MIF/CD74-mediated pathological effects.

F05

GM-CSF stimulation of monocytes enhances migration over endothelial cells

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system. Infiltration of macrophages into the central nervous system is crucial for disease onset and progression. Granulocyte-macrophage colony-stimulating factor (GM-CSF) has been shown to be essential for the migration of monocytes acrossthe blood brain barrier, since blocking GM-CSF inhibits clinical signs of experimental autoimmune encephalomyelitis(EAE), an experimental model for MS. Although the role of GM-CSF in MS is less apparent, GM-CSF can activate monocytes and macrophages. Higher levels of GM-CSF in cerebrospinal fluid during MS relapses indicate that GM-CSF may play an important role in leukocyte recruitment**.**

To elucidate which cells produce GM-CSF and express the GM-CSF receptor we performed immunohistochemistry on MS lesions. GM-CSF receptor is expressed by neurons in the rim of the lesion, whereas GM-CSF is expressed by inflammatory cells in the perivascular space. To investigate the effect of GM-CSF on macrophages, the levels of expression ofCD40and mannose receptor (MR) were determined using flow cytometry to investigate the activation status. GM-CSF induces the expression of both CD40 and MR on macrophages as shown in active MS lesions.

Next we assessed the migratory capacity of monocytes towards CCL2 in the presence of GM-CSF in a transwell culture system using a monolayer of human endothelial cells.

Altogether our data indicate that GM-CSF is a potent stimulator of monocyte migration in human in vitro models, which is supporting the relevance of previous findings in EAE models for neuroinflammation in human disease.

F06

NR4A1 mediates anti-inflammatory effects of apoptotic cells

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The nuclear receptor NR4A1 has been implicated as negative feedback regulator of NFkappaB signalling and as key regulator during the differentiation of Ly6C-low resident monocytes. Apoptotic cells are known to exert anti-inflammatory effects on macrophages but the underlying mechanisms are still poorly understood. Here we studied a potential role of NR4A1 as mediator of the macrophage response to apoptotic cells.
We analysed the effect of apoptotic thymocytes on wild type and NR4A1-/- peritoneal resident macrophages, and determined the consequences on intracellular signalling, gene expression and cytokine profile. Moreover, we examined the consequences of the lack of NR4A1 during maintenance of self tolerance by using the pristane-induced model of murine sytemic lupus erythematosus.
The expression of NR4A1 was rapidly and highly induced in resident macrophages after incubation with apoptotic thymocytes. NR4A1-/- resident macrophages showed an exacerbated pro-inflammatory profile as well as an increased activity of NFkappaB. Moreover, the anti-inflammatory effects of apoptotic cells were reduced in NR4A1-/- macrophages. In the pristane model of murine lupus, NR4A1-/- mice displayed increased levels of autoantibodies such as ds-DNA antibodies.
Tacking together, this data show for the first time that NR4A1 is an important mediator of the anti-inflammatory effects of apoptotic cells in tissue resident macrophages and thereby contributes to the maintenance of self-tolerance.

F07

Topical application of soluble CD83 induces IDO-mediated immune modulation, increases Foxp3$^+$ T cells and prolongs allogeneic corneal graft survival

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Modulation of immune responses is one of the main research aims in transplant immunology. Here we investigate the local immune-modulatory properties of soluble CD83 (sCD83) at the graft-host interface using the high-risk corneal transplantation model. In this model, which mimics the inflammatory status and the preexisting vascularization of high-risk patients undergoing corneal transplantation, allogeneic donor corneas are transplanted onto sCD83-treated recipient animals. This model allows the direct and precise application of the immune modulator at the transplantation side. Interestingly, sCD83 was able to prolong graft survival not only after systemic, but therapeutically even more relevant, also after topical application. The therapeutic effect was accompanied by an increase in the frequency of regulatory T cells and was mediated by the immune-regulatory enzyme IDO as well as TGF-ß. *In vitro*, sCD83 induced long-term IDO expression in both conventional and plasmacytoid dendritic cells via autocrine/paracrine production of TGF-β, a cytokine previously shown to be an essential mediator of IDO-dependent, long-term tolerance. Thus these findings open new treatment avenues for local immune modulation after organ and tissue transplantation.

F08

Molecular Imaging with Macrophage VSIG4-targeting Nanobodies for Diagnosis and Prognosis in a mouse model of Rheumatoid Arthritis.

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An accurate and noninvasive tracer able to detect molecular events underlying the development of rheumatoid arthritis (RA) would be of great help during RA diagnosis and to assess drug efficacy. The V-set and Ig domain-containing 4 (VSIG4) receptor is expressed on synovial macrophages of RA patients and is an interesting target for molecular imaging of RA using radiotracers. Here we report the visualization of VSIG4 in a mouse model for RA using a radiolabeled single domain variable antibody VHH fragment (also called Nanobody). A highly selective Nanobody (Nb) with nanomolar affinity, NbV4m119, was selected after screening a VSIG4-specific VHH-phage library. Flow cytometric analysis confirmed binding of the Nb to VSIG4^{+} transfected cells as well as to liver macrophage suspensions. In naive mice, single-photon emission computed tomography (SPECT/CT) imaging revealed that ^{99m}Tc-NbV4m119 exclusively targeted VSIG4^{+} liver macrophages, whereas no ^{99m}Tc-NbV4m119 tissue accumulation was found in VSIG4$^{-/-}$ mice. In collagen-induced arthritis (CIA) mice, arthritic lesions in inflamed paws could be successfully imaged using the ^{99m}Tc-NbV4m119 Nb. Phosphorimaging and confocal microscopy further confirmed NbVm119 binding to CD68^{+} synovial cells. Interestingly, the ^{99m}Tc-NbV4m119 signal in arthritic lesions increased according to the severity of the inflammation. In knees of collagen challenged mice, ^{99m}Tc-NbV4m119 was found to accumulate even before the onset of macroscopic clinical symptoms. Thus, imaging of the knees with the Nb could predict which mice will develop inflammation during CIA. Consequently, imaging of joint inflammation with VSIG4-specific Nanobodies offers perspectives for clinical applications in RA patients.

F09

Macrophage-fibroblast communication in pulmonary fibrosis: the OPG/RANK/RANKL-axis

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Macrophages and myofibroblasts are the main cells regulating extracellular matrix (ECM) deposition in lung tissue and we postulated that these cells communicate via the Receptor Activator of Nuclear factor kappa b (RANK), its ligand (RANKL) and osteoprotegerin (OPG) for ECM regulation. RANKL can bind to RANK on lung macrophages, inducing a proteolytic phenotype. OPG, possibly produced by profibrotic myofibroblasts, is the decoy receptor for RANKL and can prevent induction of proteolytic macrophages. We hypothesized that the RANK/RANKL/OPG-axis is disturbed in pulmonary fibrosis (PF).
Presence of RANK, RANKL, and OPG was analyzed by western blot and ELISA in lung tissue of PF patients (n=11) and controls (n=7). Cytokines affecting OPG production were studied using cultures of 3T3 fibroblasts. Furthermore, C57BL/6 mice with established silica-induced PF were treated 3x/week intranasally for two weeks with 1, 2.5 or 5ug RANKL. Fibrosis was assessed by lung collagen content.
OPG levels were higher in lung tissue of PF patients compared to controls ($p<0.05$), while RANK and RANKL levels were similar. TGFβ and to a lesser extent IL-13 induced OPG production in myofibroblasts. In mice with PF, RANKL treatment led to dose-dependent lower levels of collagen I in lung compared to PBS treatment ($p<0.05$).
PF patients had higher levels of OPG, which can be induced in myofibroblasts by the profibrotic cytokines TGFβ and/or IL-13. OPG may be responsible for lower proteolytic activity of macrophages, because treatment with RANKL increased collagen breakdown in a PF model. These results support a role for a disturbed OPG/RANK/RANKL-axis in PF.

F10

Role of heme oxygenase-1 in mononuclear phagocyte system.

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Heme oxygenase 1 (HO-1) catalyzes degradation of heme to biliverdin, iron, and carbon monoxide. Expression of HO-1 is induced by Nrf2 and can be regulated by miR-155 which reduces translation of Bach1, an Nrf2 suppressor. HO-1 deficiency leads to decrease in population of spleen macrophages. It has also been demonstrated that Bach1/HO-1 pathway regulates the generation of APCs, as in HO-1-/- Bach1-/- double mutants the level of macrophages and dendritic cells was decreased. Yet, there are no data on monocytes subsets in HO-1 deficiency.
Our results displayed decreased macrophage level, but not altered level of dendritic cells in spleens of HO-1 knock-out mice. We also showed that level of non-classical and intermediate monocytes was statistically lower in HO-1-/-, yet level of classical monocytes was not changed. This suggests that HO-1 might be important in terminal differentiation of mononuclear phagocytes. We also tested the level of miR-146a and miR-155 in sorted monocyte subsets. Consistently with previous results, we found that miR-146a is strongly expressed in non-classical monocytes. However, no differences in miR-146a expression between HO-1-/- and WT mice in all monocyte subsets were detected. The level of miR-155 was also significantly increased in non-classical mononcytes in both genotypes, but non-classical monocytes from HO-1-/- mice displayed much higher expression of miR-155 than wild type cells. Interestingly, classical monocytes, revealed no difference in miR-155 expression between genotypes. High level of miR-155 in non-classical monocytes in HO-1-/- mice might suggests that miR-155 can be negatively regulated by HO-1 or products of heme degradation.

F11

Adoptive Transfer of Cytokine-Induced Immunomodulatory Microglia Attenuates Experimental Autoimmune Encephalomyelitis in DBA/1 Mice

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Microglia are resident antigen presenting cells in the central nervous system (CNS) that may either suppress or promote disease depending on their activation phenotype and microenvironment. Multiple Sclerosis (MS) is a chronic inflammatory disease causing demyelination and nerve loss in the CNS and Experimental Autoimmune Encephalomyelitis (EAE) is an animal model of MS that is widely used to investigate complex pathogenic mechanisms and therapeutic effects. We isolated and cultured microglia from adult DBA/1 mouse brains and exposed them to specific combinations of stimulatory molecules and cytokines, the combination of IL-4, IL-10 and TGF-β yielding the optimal regime for induction of an immunosuppressive microglial phenotype (M2). M2 microglia were characterized by decreased expression or production of CD86, F4/80, PDL1, nitric oxide, and IL-6, and having a potent deactivating effect on LPS/IFN-γ pre-stimulated microglia. Myelin oligodendrocyte glycoprotein (MOG)-induced EAE was induced in DBA/1 mice and at different time points (0, 5, 7, 12 or 15 days p.i) either 3 x 10^5 M2 microglia or vehicle were transferred intranasally. A single transfer of M2 microglia attenuated the severity of ongoing EAE, which was particularly obvious when the cells were injected at 15 days p.i. M2 microglia-treated mice had reduced inflammatory responses and less demyelination in the CNS. Our findings demonstrate that M2 microglia therapy represents a novel intervention that alleviated ongoing EAE and that may have relevance for treatment of MS patients.

F12

Effector molecules released by Th1 but not Th17 cells drive an M1 response in microglia

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Microglia, the resident macrophage-like population in the central nervous system (CNS), act as sensors of inflammation and respond to various stimuli by upregulating major histocompatibility (MHC) class-II and co-stimulatory molecules on their surface. Furthermore, activated microglia release reactive oxygen species (ROS) and secrete pro-inflammatory mediators, which are toxic to the neural tissue. In certain neuroinflammatory diseases, pathogenic $CD4^+$ T helper cell subsets that characteristically secrete IFN-γ (Th1) or IL-17 (Th17) infiltrate from the periphery into the CNS, interact with microglia and subsequently results in mutual activation. However, the potential of a distinct cytokine milieu generated by these effector T cell subsets to activate microglia is poorly understood. In this study, we tested the ability of factors secreted by *in vitro* generated Th1 and Th17 cells to induce microglial activation. Interestingly, we found that only Th1-associated factors had the potential to activate microglia while Th17-associated factors only had a minimal effect. Furthermore, Th1-associated factors triggered a proinflammatory M1-type gene expression profile in microglia. Recently GM-CSF has been described as an important effector molecule produced by pathogenic Th17 cells. GM-CSF was found lacking in our *in vitro* generated Th17 culture supernatants. Nevertheless this was not the reason for the inability of Th17-associated factors to activate microglia as exogenous addition of recombinant GM-CSF to Th17 culture supernatants did not alter the microglial phenotype. Together, these findings suggest that while Th1 cells can mediate its effects directly by acting on microglia, Th17 has no direct influence on microglial activation.

F13

Definition of the serotonin (5HT)-dependent gene expression profile in human macrophages and identification of specific 5HT receptors involved.

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Serotonin (5-hydroxytryptamine, 5HT) regulates inflammation and tissue repair via a set of receptors (5HT1–7) whose pattern of expression varies among cell lineages. Considering the importance of macrophage polarization plasticity for inflammatory responses and tissue repair, we evaluated whether 5HT modulates human macrophage polarization. 5HT inhibited the LPS-induced release of proinflammatory cytokines without affecting IL-10 production, upregulated the expression of M2 polarization–associated genes, and reduced the expression of M1-associated genes. 5HT7 receptor mediated the inhibitory action of 5HT on the release of proinflammatory cytokines, and both 5HT2B and 5HT7 receptors mediated the pro-M2 skewing effect of 5HT. Pharmacological blockade of both receptors during in vitro monocyte-to-macrophage differentiation preferentially modulated the acquisition of M2 polarization markers. Gene expression profiling allowed us to define the gene expression profiles of human macrophages specifically controlled by either 5HT or receptor-specific agonists, as well as to identify the intracellular signaling pathways that mediate the modulation of macrophage effector functions by 5HT. We will also present evidences that 5HT greatly influence the expression of several growth factors and functionally relevant cell surface molecules on human macrophages.

Impact of the dermal microenvironment on dendritic cell function

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Dendritic cells (DCs) are in close contact with the tissue microenvironment under steady state conditions as well as upon initiation of an immune response. For a long time the tissue microenvironment was respected to provide only the scaffold. However, stromal cells via a wide and variable biosynthetic repertoire have important functions in tissue development and homeostasis. In this way stromal cells act as important regulators of the function of immune cells.
In the skin we showed a close colocalisation of DC and fibroblasts. Consequences of these interactions are the control of the production of matrix-degrading enzymes in both the DCs and fibroblasts. Under inflammatory conditions TNFalpha and IL-1beta stimulate the secretion of IL-6 from fibroblasts which in turn enhances the migration of DCs through the extracellular matrix by upregulation of MMP-9 in DCs. In addition the interaction of DCs and fibroblasts stimulates the secretion of MMPs in fibroblasts which also supports the migration of DCs through the extracellular matrix. Moreover, the crosstalk of fibroblasts and DCs actively participate in the regulation of an immune response. Under inflammatory conditions DCs produce TNFalpha and IL-1beta, which in turn activate resident fibroblasts. Via the secretion of prostaglandin E2, these fibroblasts stimulate the IL-23 secretion from activated DCs, which results ultimately in a prominent expansion of Th17 cells.
In summary, we demonstrated an intensive crosstalk between DCs and fibroblasts as one component of the tissue microenvironment which results in the regulation of DC functions important for the initiation and maintenance of an immune response.

G03

Differential regulation of IDO by TLRs and CLRs in human DCs

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Indoleamine 2,3-dioxygenase (IDO), an enzyme which catabolizes tryptophan, has been shown to play a critical role in regulating immune responses. In the context of allergic diseases, IDO has been shown to have a protective effect in models of experimental asthma. However, there is not much information regarding the role of IDO in human allergy. Recently, data published by our group demonstrated that Der p 1 (a major allergen from house dust mite) could, through engaging mannose receptor (MR) on dendritic cells (DCs), down-regulate levels of IDO activity. This in turn could bias immune responses toward a Th2 phenotype.
Here we studied the IDO regulation by different pattern recognition receptors, such as MR, DC-SIGN, TLR4, and TLR9 in human monocyte-derived DCs in order to define the role of these molecules in controlling allergen induced Th2 immune responses. Our data indicates that IDO expression and activity is increased after LPS treatment. Conversely, we have shown for the first time that engagement of a number of C-type lectin receptors (CLRs) leads to a significant reduction in IDO activity, even in the presence of LPS. Furthermore, our data indicates that CpG, a TLR-9 ligand, can suppress IDO activity even under conditions leading to enhanced IDO activity such as TLR4 signalling. These data show an antagonistic relationship between TLR4 and TLR9/CLRs in the context of IDO activity regulation. Further experiments should aim at elucidating the intracellular pathways mediating such antagonistic effects on IDO activity and how this affects T cell activation and differentiation.

G04

Loss of HIF-1α in macrophages attenuates chemical-induced tumor formation

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Hypoxia-inducible factors (HIF) coordinate major transcriptional responses to hypoxia. Many solid tumors develop hypoxic areas that are highly infiltrated by macrophages. So far the role of HIF-1α and HIF-2α in these tumor-associated macrophages during tumor development remains unclear. Deletion of HIF-1α in macrophages in a 3-Methylcholanthrene (MCA)-induced tumor model largely reduced tumor outgrowth, whereas the conditional knockout of HIF-2α slightly enhanced tumor progression. Analysis of tumors arising in macrophage specific HIF-2α-knockout mice showed no significant differences in immune cell distribution, vascularization or macrophage polarization. As deletion of HIF-1α almost completely abolished tumor formation, the initiation phase was analyzed. Inflammation was not changed after the loss of HIF-1α but metabolism of MCA was impaired in these mice. We found decreased levels of the MCA-metabolizing enzyme cytochrome P450 1A1 (CYP 1A1) as well as attenuated DNA damage in mice lacking HIF-1α in macrophages. Therefore, we hypothesize that HIF-1α is crucial for tumor initiation in this tumor model and it attenuates the metabolic activation of a polycyclic arylhydrocarbon derivate by influencing
CYP expression. These data identify HIF-1α as a potential therapeutic target for chemical-induced tumors.
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G05

Macrophage growth by polyploidization via pathogen-mediated cell cycle regulation in mycobacterial disease

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Macrophage growth in infected tissues is critical for the formation of granulomas and control of mycobacterial disease. However the mechanisms by which macrophages grow at the single cell or population level in the context of mycobacterial infections are largely unknown. In infected tissues population growth is thought to occur mostly by recruitment of bone marrow monocytes. Recently macrophage growth by in situ proliferation was found to contribute to their population expansion in Th2 inflammation. Here we show that macrophage growth by polyploidization during Mycobacterium bovis infection in vivo represents a third pathway, beyond recruitment or in situ proliferation, for the expansion of the macrophage population in mycobacterial granulomas. In vitro, TLR2 ligand-mediated and MyD88-dependent macrophage polyploidization was induced by promoting DNA synthesis while suppressing mitosis, thus generating nuclei with increased DNA content. In this context, multinucleated giant cells were generated by cytokinesis failure due to supernumerary centrosomes and DNA trapped at the cleavage furrow. TLR2/Myd88 signals regulated cell cycle events by modulating the expression of cell cycle proteins and the macrophage transcription factor MafB. This pathway of macrophage polyploidization is distinct from the previously described cytokine-induced macrophage multinucleation by cell to cell fusion, which is not known to require cell cycle regulation. We propose that macrophage growth by pathogen-mediated cell cycle regulation and polyploidization may be an important mechanism for the maintenance of granulomas and control of mycobacterial infections.

G06

Tumor hypoxia does not drive differentiation of tumor-associated macrophages but rather fine-tunes the M2-like population.

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Tumor-associated macrophages (TAM) are exposed to multiple microenvironmental cues in tumors, which collaborate to endow these cells with protumoral activities. Hypoxia, caused by an imbalance in oxygen supply and demand due to a poorly organized vasculature, is often a prominent feature in solid tumors. However, to what extent tumor hypoxia regulates the TAM phenotype in vivo is unknown. Here, we show that the myeloid infiltrate in mouse lung carcinoma tumors encompasses two morphologically distinct $CD11b^{hi}F4/80^{hi}Ly6C^{lo}$ TAM subsets, designated as MHC-II^{lo} and MHC-II^{hi} TAM, both of which were derived from tumor-infiltrating $Ly6C^{hi}$ monocytes. MHC-II^{lo} TAM express higher levels of prototypical M2 markers and reside in more hypoxic regions. Consequently, MHC-II^{lo} TAM contain higher mRNA levels for hypoxia-regulated genes than their MHC-II^{hi} counterparts. To assess the in vivo role of hypoxia on these TAM features, cancer cells were inoculated in *PHD2*-haplodeficient mice, resulting in better oxygenated tumors. Interestingly, reduced tumor hypoxia did not alter the relative abundance of TAM subsets nor their M2 marker expression, but specifically lowers hypoxia-sensitive gene expression and angiogenic activity in the MHC-II^{lo} TAM subset. The same observation in $PHD2^{+/+}$ --> $PHD2^{+/-}$ bone marrow chimeras also suggests the implication of a better-oxygenized microenvironment. Together, our results show that hypoxia is not a major driver of TAM subset differentiation, but rather specifically fine-tunes the phenotype of M2-like MHC-II^{lo} TAM.

G07

Angiotensin-converting-enzyme-2 mRNA is highly expressed in classical monocytes

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Human monocytes can be subdivided into three main subpopulations: $CD14^{++}CD16$ (classical), $CD14^{++}CD16^{+}$ (intermediate) and $CD14^{+}CD16^{++}$ (non-classical). The latter two subsets, in comparison to the classical monocytes, produce proinflammatory cytokines and have been reported to increase in numbers during inflammatory disorders and to decrease with glucocorticoid therapy, which indicates their important role in inflammation. They are located in close vicinity of endothelium and could potentially affect vascular tone by the release of vasoactive factors. However, their potential role in regulation of blood pressure has never been investigated. The aim of this study was to determine the expression of two main enzymes involved in the regulation of blood pressure, angiotensin-converting enzyme 1 (ACE1) and 2 (ACE2) in sorted subpopulations of monocytes obtained from healthy blood donors. Relative level of both ACE1 and ACE2 mRNAs was significantly elevated in $CD14^{++}CD16^{-}$ subpopulation, with the preference of ACE2 expression. Our data indicate that classical monocytes may be the main subset involved in converting process of angiotensin I into angiotensin 1-7, which suggests their pivotal role in relaxation of arteries. Therefore, contribution of $CD14^{++}CD16^{+}$ and $CD14^{+}CD16^{++}$ monocytes to the vasorelaxation seems to be limited. Our data may be a further hint on the relative importance of particular monocyte subsets in the pathogenesis of cardiovascular disease.

G08

SWAP-70 Regulates Ceramide Increase in Dendritic Cells after LPS Stimulus

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In dendritic cells (DCs), signaling through different inflammatory stimuli (TNF-α, IL-1β or LPS) leads to increase levels of the sphingolipid ceramide. The increase of cellular ceramide regulates important DC functions: antigen uptake and presentation and apoptosis. Therefore, ceramide represents an important signaling molecule in DC biology. Ceramide originates from the hydrolysis of sphingomyelin due to the activity of neutral and/or acid sphingomyelinase. Alternatively, ceramide originates through *de novo* synthesis. Levels of ceramide are also regulated by the activity of acid and/or alkaline ceramidase that breaks down ceramide into sphingosine and fatty acids. The protein SWAP-70 regulates important DC functions: MHCII up-regulation after toll-like receptor (TLR) stimulation, migration and endocytosis in response to sphingosine 1-phosphate and spontaneous maturation. *Swap-70*$^{-/-}$ DCs fail to increase cellular ceramide after LPS activation when compared to their wild type (wt) counterparts. This failure was accompanied by altered endocytosis and apoptosis. Expression levels of sphingomyelinases and ceramidases show different kinetic between wt and *Swap-70*$^{-/-}$ DCs. We have also observed interaction of SWAP-70 and lysosomal acid ceramidase in lysates of DCs. These results demonstrate that SWAP-70 represents a novel and important element in the regulation of ceramide production after DC activation. Understanding the molecular mechanism involved in this regulation will be of interest to develop new strategies in immune therapy.

G09

The role of ATP in the release of nicotinamide phosphoribosyltransferase a protein with dual functions

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Nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme of a salvage pathway which serves the resynthesis of NAD after its degradation by NAD consuming enzymes. There is some evidence that NAMPT also has a role as an extracellular mediator, displaying cytokine-like effects.
Here we show that stimulating human monocytes with LPS leads to an upregulatinon of intracellular NAMPT expression and activity as well as an increase secretion of the protein.
The extracellular appearance of NAMPT which similar to IL-1β lacks a cytokine specific secretion gene sequence raises the question of how it can be transported out of the cell.
As ATP has been identified as a potent secretion stimulus we tested its role in NAMPT release by activated monocytes. We did not find any evidence for the involvement of endogeneous ATP in the secretion process. However, when added extracellularly to LPS-primed cells ATP greatly enhanced NAMPT release. Thus NAMPT like IL-1β requires a second signal for its efficient secretion. This mode of secretion might represent a general mechanism for the release of leaderless secretory proteins at locally restricted sites where ATP-concentrations are elevated.

G10

Hypoxia Modulates The Gene Expression Profile of Immunoregulatory Receptors in Human Monocytes and Dendritic Cells: Identification of TREM-1 and CD300a as New Hypoxia-Inducible Genes Endowed with Pro-Inflammatory Properties

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Mononuclear phagocytes are recruited as primary monocytes from the circulation to sites of infection, inflammation and tumor growth, where they undergo terminal differentiation into macrophages and dendritic cells. Mononuclear phagocytes integrate stimulatory and inhibitory signals present in the pathologic microenvironment through a defined repertoire of cell surface receptors, whose deregulated expression may result in amplification of inflammation or establishment of immune escape mechanisms. Characterization of the expression and function of these receptors is, thus, required for a better understanding of the regulation of mononuclear phagocyte activity at pathologic sites. Hypoxia is a common feature of pathological situations and an important regulator of mononuclear phagocyte differentiation and functions. We defined the hypoxic transcriptome of primary human monocytes and monocyte-derived dendritic cells. We demonstrated that hypoxia can finely tune the expression of a significant cluster of genes coding for immunoregulatory signaling (IRS) receptors and identified two of such receptors, TREM-1 and CD300a, as new hypoxia-inducible genes in primary monocytes, monocyte-derived macrophages and dendritic cells. TREM-1 and CD300a were expressed *in vivo* in cells infiltrating the inflamed hypoxic joints of children affected by Juvenile Idiopathic Arthritis. Engagement of these receptors by agonist Abs resulted in the production of proinflammatory, Th1/Th17-priming cytokines/chemokines and in T cell activation. These results suggest that TREM-1 and CD300a induction by the hypoxic environment represents a mechanism of amplification of mononuclear phagocyte proinflammatory responses at pathologic sites. The potential implications of these findings for the pathogenesis of chronic inflammatory diseases will be discussed.

G11

The Role of Heme Oxygenase 1 in Regulating Iron Homeostasis and Innate Immune Response to Salmonella Infection

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Macrophages play an essential role for the containment and elimination of microbes. Heme oxygenase-1 (HO-1, *hmox1*) the enzyme cleaving heme to ferric iron, billiverdin and carbon monoxide, is involved in the regulation of stress responses. However, underlying regulatory effects of *hmox* in host pathogen interaction involving macrophages and associated changes of iron homeostasis and innate immune function are incompletely understood.
Using the murine macrophage cell line RAW264.7 and lenti-virus based tetracycline inducible shRNA knock downs of stress response genes, we observed alterations in the expression of iron metabolism genes, most prominent with an up-regulation of the iron exporter ferroportin-1 upon *hmox* knock down. To study the relevance of these observations for host response we infected macrophages with *Salmonella enterica* serovar Thyphimurium. Interestingly, the knock down of *hmox* reduced the survival of Salmonella in macrophages whereas it had no effect on pathogen uptake. Further results suggest that the improved pathogen control could be traced back to reduced iron availability for intra-macrophage bacteria and partly improved innate immune function as a consequence of increased ferroportin expression and intracellular iron restriction. Taken together, our data highlight the central role of HO-1 in host response towards intracellular pathogens which can be traced back to its capacity to modulate anti-bacterial immune effector pathways and cellular iron homeostasis.

G12

The Role of JAK2 in Hepcidin-dependent Ferroportin Internalisation in Macrophages

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Macrophages play a key role in iron homeostasis. Especially with regard to their iron recycling function. Macrophages in the spleen and liver phagocytize damaged or senescent erythrocytes and by degrading heme, recycle iron. Iron can be stored intracellulary or released into the circulation by Ferroportin1 (Fpn1), a transmembrane protein and the only known iron exporter. Posttranslational regulation of Fpn1 is mediated by Hepcidin, the central iron regulator. Binding of hepcidin to Fpn1 results in Fpn1 internalisation, degradation and iron retention in macrophages. High levels of hepcidin are present in the anemia of chronic disease (ACD), a major form of anemia, developing in patients with chronic inflammation (cancer, HIV, rheumatoid arthritis), resulting in a much worser outcome. The exact mechanisms of hepcidin-dependent Fpn1 internalisation are incompletely understood and highly debated, especially with regard to the role of JAK2.
Therefore we established a mouse model of macrophage-specific JAK2 deficiency, by using a LysMCre-LoxP System, to study the role of JAK2 in hepcidin-dependent Fpn1 internalisation in macrophages, especially in states of inflammation and iron homeostatis disturbances. Our preliminary results show, that hepcidin is still able to induce the Fpn1 internalisation and degradation in case of macrophage specific JAK2-deficiency. Therefore it is likely, that JAK2 is dispensable for hepcidin mediated Fpn1 internalisation.

G13

Macrophage uptake and accumulation of folates is polarization-dependent *in vitro* and *in vivo*, and is regulated by activin A

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Vitamin B9, commonly known as folate, is an essential cofactor for one-carbon metabolism that enter cells through three major specialized transporter molecules (Reduced folate carrier, RFC; Folate receptors, FR; Proton-coupled folate transporter, PCFT) that differ in expression pattern, affinity for substrate and ligand-binding pH dependency. We now report that the expression of the folate transporters differs between macrophage subtypes, and explains the higher accumulation of 5-methyltetrahydrofolate, the major folate form found in serum, in M2 macrophages both in vitro and in vivo. M1 macrophages display a higher expression of RFC, whereas FRβ and PCFT are preferentially expressed by anti-inflammatory and homeostatic M2 macrophages. These differences are also seen in macrophages from normal tissues involved in folate transit (placenta, liver, colon) and inflamed tissues (ulcerative colitis, rheumatoid arthritis), as M2-like macrophages from normal tissues express FRβ and PCFT, whereas TNFα-expressing M1 macrophages from inflamed tissues are RFC+. Besides, we provide evidences that activin A is a critical factor controlling the set of folate transporters in macrophages, as it downregulates FRβ, upregulates RFC expression and modulates 5-methyltetrahydrofolate uptake. All these experiments support the notion that folate handling is dependent on the stage of macrophage polarization.

G14

CHOP-independent apoptosis of CD24+ conventional dendritic cells upon endoplasmic reticulum stress

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Endoplasmic reticulum (ER) stress, caused by an accumulation of unfolded/misfolded proteins, is involved in the pathogenesis of several diseases, including neurodegeneration, lung diseases, diabetes and cancer. Dendritic cells (DC) are potent antigen presenting cells that translate innate immune signals into T- and B-cell mediated adaptive immunity. *In vivo*, DCs have evolved in various subsets exerting different functions. Here, we investigated how different DC subsets react to ER stress using bone marrow-derived DC cultures supplemented with Flt3L as an *in vitro* model. A Flt3L DC culture gives rise to CD24+ and CD172+ conventional DCs (cDCs) and plasmacytoid DCs (pDCs). Exposing this culture to the ER stressor tunicamycin induces apoptosis of CD24+ cDCs but has no effect on CD172+ cDCs and pDCs. In literature, ER stress-induced death has been shown to rely on the pro-apoptotic protein CHOP. However, we observed that CD24+ cDCs derived from bone marrow cultures of CHOP-deficient mice are not rescued from tunicamycin-induced apoptosis, strongly suggesting that tunicamycin-induced apoptosis of CD24+ cDCs is CHOP-independent. Interestingly, TLR triggering by LPS or CpG of tunicamycin-treated Flt3L DCs inhibits apoptosis of CD24+ cDCs. Rescue induced by CpG depends on MyD88 while LPS-mediated survival of CD24+ cDCs mainly relies on TRIF. In conclusion, our findings show that DC subsets react differently to ER stress and that TLR stimulation promotes survival of ER stressed DCs, indicating that in ER stress conditions sensing innate immune signals preserves DC function. In addition, we showed that CHOP is not necessary for tunicamycin-induced apoptosis of CD24+ cDCs, suggesting a cell type-dependent requirement for CHOP in ER stress-induced cell death.

G15

Epoxomicin-treated Dendritic Cells Prevent Atherosclerotic Lesion Progression

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Dendritic Cells (DCs) are important in initiating and maintaining T cell responses in atherosclerosis. Targeted modulation of DCs is therefore a promising approach for disease therapy. Epoxomicin, a potent and selective proteasome inhibitor, blocks the degradation of cellular proteins. DCs treated with epoxomicin (epo-DCs) show decreased MHCI and MHCII expression and increased co-stimulatory molecules. Epoxomicin finally results in apoptosis. This prevents any further changes in the DCs, making a possible administration safer, but also enabling their final clearance by phagocytes.
In a co-culture with proliferating T cells, epo-DCs significantly increase regulatory T cells and decrease effector T cells. However, apoptotic epo-DCs can also be cleared by immature DCs. We found that this results in a significant reduction in pro-inflammatory IL-6 and TNF-α responses to 100 ng mL^{-1} LPS stimulation by imDCs. Clearance of epo-DC thus decreases inflammatory responses by imDCs.
When we injected epo-DCs into LDLrko mice that had been ten weeks on a Western-type diet and left these for another ten weeks on diet, we found a significant reduction of circulating and splenic inflammatory monocytes. Moreover, we saw a significant 40% reduction in the Th1 and a 52% reduction in the Th2 T cell subsets, indicating that mice treated with epo-DCs had less ongoing inflammation. The treated mice had a significant 21% reduction in lesion size and a significant 25% increase of collagen content.
We show that Epo-DC therapy significantly reduces progression of established lesions and could therefore serve as a potential therapy for patients presenting in the clinic.

Author Index (first or presenting and last authors of all abstracts)

Ghaemmaghami	Amir	G03
González	Erika	E11
Gonzalez Leal	Iris	D21
Griebenow	Evelin	E26
Guilliams	Martin	A01
Gurevich	Shakhar	B02
Gutiérrez	Laura	D32
Hahn	Christina	D11
Hallek	Michael	E15
Hannemann	Nicole	A11
Harris	Robert A.	A18, F11
Hartog	Anita	C05
Haschka	David	D22
Hauschildt	Sunna	A16, G09
Heger	Lukas	B09
Heidkamp	Gordon	B07
Heine	Gunnar H.	A17
Henke	Nina	G01, G04
Henneke	Philipp	G05
Hildner	Kai	D07, E08
Höfflin	Sandra	E17
Hogaboam	Cory M.	A13
Hoppstädter	Jessica	D23
Hübner	Sabine	D29
Ipseiz	Natacha	F06
Janssens	Sophie	G14
Jantsch	Jonathan	G02
Jessberger	Rolf	G08
Kalinke	Ulrich	D24, D25
Kaufmann	Stefan H. E.	D16
Kiemer	Alexandra	D23
Kittan	Nicolai	A13
Kitwoski	Vera	D07
Knippertz	Ilka	E14
Knoll	Dominique	D06
Kooij	Gijs	D09
Kool	Mirjam	B03
Kovarik	Pavel	D01
Kreutz	Marina	E25
Krönke	Gerhard	F06
Krzyzak	Lena	E21
Kubala	Lukas	C06
Kuebler	Joachim F.	B05
Kuiper	Johan	G15
Kungl	Jasmin	D35
Lambrecht	Bart N.	A01
Lang	Roland	D12, D28, D34
Lechner	Anja	E27
Lehmann	Christian	E29
Leitherer	Sabine	D20
Leppek	Kathrin	D31
Li	Yan	A02
Loeffler	J.	D35
Mahnke	Alexander	G02
Mattner	Jochen	C02, C04
Melgert	Barbro N.	F09
Meng	Wei	D27

Merad	Miriam	C01
Meyer	Ralf G.	A21
Michel	Julia	E19
Minderjahn	Julia	A15
Misharin	Alexander	B01
Mitterstiller	Anna-Maria	G11
Moll	Heidrun	D21
Mortha	Arthur	C01
Müller	Dominik N.	F03
Nairz	Manfred	C03
Nettelbeck	Dirk M.	E14
Neurath	Markus F.	E23
Niemann	Stefan	D14
Novelli	Francesco	E28
Nuebling	Kathrin	E08
Ocaña Morgner	Carlos	G08
Oeren	Bilge	E10
Ostrop	Jenny	D12
Paijo	Jennifer	D24
Pekarova	Michaela	C06
Perlman	Harris	B01
Peter	Katrin	E25
Pfeiffer	Isabell	A08
Prommersberger	Sabrina	E16
Puig Kröger	Amaya	G13
Raes	Geert	E01
Rehli	Michael	A14, A15
Reiling	Norbert	D14
Richardson	Vernon	A12
Riedel	Christian U.	D26
Rubner	Yvonne	E07
Rühle	Paul	E13
Saalbach	Anja	F14
Salazar	Fabian	G03
Samaniego	Rafael	G13
Sánchez-Torres	Carmen	A05, E11
Sancho	David	D02
Schaft	Niels	A08, A09, E16, E17
Scheenstra	Maaike	D32
Scherberich	Juergen	E09
Schey	Regina	C02
Schilling	Erik	G09
Schledzewski	Kai	E19
Schleicher	Ulrike	D06
Schmidl	Christian	A14
Schmidt	Susanne	D15
Schmidt	Dominic	E27
Schmieder	Astrid	E20
Schönhaar	Kathrin	E20
Schoonooghe	Steve	F08
Schott	Johanna	F02
Schroll	Andrea	D33
Schultze	Joachim L.	A07, D15
Sebens	Susanne	E26
Shaffer	Kelly	A18
Shakhar	Guy	B02
Siedlar	Maciej	G07

Simon	Jan C.	F14
Sommer	Maria	A21
Soulat	Didier	D20
Sparkes	Amanda	F04
Stamatiades	Efstathios	F01
Stammler	Dominik	D04
Stangel	Martin	F12
Steinkasserer	Alexander	E21
Stijlemans	Benoit	D08
Stoecklin	Georg	D31, F02
Stoeger	Tobias	B08
Tavernier	Simon	G14
Theurl	Igor	G12
Tonoli	Elisabetta	E28
Triantafyllopoulou	Antigoni	G05
Tuckermann	Jan	D29
Ulas	Thomas	E15
Van Ginderachter	Jo A.	G06
Van Lent	P.	A10
van Vliet	Sandra J.	E03
van Zandbergen	Ger	A19, A20, D03
Varesio	Luigi	G10
Vogel	Daphne	F05
Vogelpoel	Lisa	E04
Vos	Paul	C05
Vroman	Heleen	B03
Waisman	Ari	B10
Weigert	Andreas	E05
Weiß	Ronald	A16
Weiss	Günter	C03, D22, D33, G11
Wentker	Pia	D13
Wenzel	Jens	D28
Werthmöller	Nina	E06
Winterberg	Thomas	B05
Wunderlich	Roland	E12
Xue	Jia	A07
Yogev	Nir	B10
Zawada	Adam	A17
Zhang	Xing-Mei	F11
Zheng	Fang	F08
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