MONOCYTES, DENDRITIC CELLS
AND MACROPHAGES – BASIC RESEARCH
AND PATHOPHYSIOLOGICAL ASPECTS

29th Annual Conference of the EMDS
11-13 September 2015, Krakow, Poland

www.emds2015krakow.pl

Conference co-financed by
the National Scientific Leadership Center 2012 - 2017
Jagiellonian University Medical College
Faculty of Medicine
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Dear colleagues and friends,

On behalf of the Conference Organising Committee it is our great pleasure to welcome you to the 29th edition of the Annual Conference of the European Macrophage and Dendritic Cell Society (EMDS), which this year is being held in Kraków, Poland. We are pleased that you all decided to join this meeting.

This is the second time that the EMDS meeting has been organised by the Jagiellonian University Medical College here in Kraków. The Jagiellonian University is the oldest higher-education institution in Poland and one of the oldest in this part of Europe. Last year, the Jagiellonian University celebrated the round 650th anniversary of its founding. Kraków University was founded by the Polish King, Casimir the Great, on May 12th, 1364. The Studium Generale – as the University was called then – comprised three faculties, namely liberal arts, law and medicine. The most famous alumni include Nicolaus Copernicus and Pope John Paul II.

The 29th EMDS meeting focuses on the basic research and pathophysiological aspects of monocytes, dendritic cells and macrophages. These central themes will be covered in seven symposia by 16 invited distinguished scientists, and 18 short oral presentations that were selected from 102 submitted abstracts. Each oral presentation was carefully reviewed and rated by three independent international experts in the field. More than 150 participants from 17 countries (12 European countries, as well as from the USA, Australia, Israel, Singapore and Japan) decided to participate in the meeting. Following the traditional format of EMDS meetings, there is only one lecture auditorium and one area dedicated to poster presentations; coffee and lunch breaks and sponsor exhibitions are all to be held in the same area.

The banquet and the presentation of the EMDS Awards will be held on Saturday evening at the Krzysztofory Palace on the Main Market Square. Throughout its history, many prominent figures have been guests at the palace, including Polish Kings Jan Kazimierz Waza and Stanislaw August Poniatowski.

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

On behalf of the Local Organising Committee and all the people that have helped to make this meeting possible

Maciej Siedlar

Karolina Bukowska-Strakova
ORGANIZERS

The 29th Annual Meeting is held and organized by the *European Macrophage and Dendritic Cell Society (EMDS)* in cooperation with the Jagiellonian University Medical College and Targi w Krakowie Ltd.

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CONFERENCE ORGANIZING OFFICE

Targi w Krakowie Ltd. (PCO/DMC)
9 Galicyjska Str.
31-586 Kraków
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www.targi.krakow.pl
THE EUROPEAN MACROPHAGE AND DENDRITIC CELL SOCIETY (EMDS)

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 group 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 centers of Strasbourg, Freiburg and Basel, but rapidly grew to a European format. On April 28, 1999, the European Macrophage Society (EMS) was founded in Regensburg. At the end of 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 currently has 485 members from 34 countries. For more information and registration as a member, please see our website: www.macrophage.de

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GENERAL INFORMATION

Conference Venue
The Conference takes place in the Conference Centre Faculty of Medicine JUMC. This modern building situated in the heart of Cracow was fund by Jagiellonian University Medical College and School of Medicine in English to held congresses, conferences, seminars and other events. Good location and efficient internal infra-structure are undoubtedly its great advantages.

Address:
Conference Centre Faculty of Medicine JUMC
16 Św. Łazarza Str.
Wi-Fi Internet access

Wireless Internet is available at the Conference Venue.
Login: UJCM_Conference
Password: ujcm16cdk2015

Registration desk
Registration desk will be open at the ground floor of the Conference Venue.
Opening hours:
Friday, 11th of September: 12:00 pm – 6:00 pm
Saturday, 12th of September: 8:30 am – 6:00 pm
Sunday, 13th of September: 8:30 am – 2:00 pm

Oral presentations
Each oral presentation will take 15 minutes, including 5 minute discussion. Please kindly check exact time allocated for your presentation in the conference program. Speakers are kindly requested to prepare their oral presentations in .pptx file format (Microsoft Power Point version 2010), compatible with Windows 10. There is no possibility of using your own computer during the presentation. The final deadline of the presentation up-load is during registration at the conference venue.

Poster presentations
Poster session will take place at the ground floor of the Conference Venue (side entrance to the building). All posters should be on display throughout the duration of the conference. Posters must be set up before starts 1st Session on Friday and removed by 2:00 pm on Sunday.
Poster boards size is 95 x 240 cm. Please note that poster should be not wider than 95 cm. Poster boards will be numbered. Poster tape will be available at the registration desk.
The Poster Sessions are scheduled for Friday (6:45 pm-9:00 pm) and Saturday (1:00 pm-3:00 pm). Authors are kindly asked to be present by their posters and discuss presented content, according to the following schedule:
• Authors of posters numbered 1-31 from 6:45 pm to 7:45 pm on Friday
• Authors of posters numbered 32-62 from 1:00 pm to 2:00 pm on Saturday
During the poster session please refer to the poster numbers in the Conference Book.

Coffee breaks
Served at the first floor of the Conference Venue on the following days:
Friday, 11th of September: 5:00 pm – 5:30 pm
Saturday, 12th of September: 10:45 am – 11:15 am and 5:00 pm – 5:30 pm
Sunday, 13th of September: 8:30 am – 2:00 pm

Lunch
Served at the first floor of the Conference Venue only on 12th of September at 1:00 pm – 3:00 pm.

Gala Dinner
Saturday, 12th of September
Time: 8:30 pm
All conference participants are invited to join the Gala Dinner on the 12th of September, which takes place in the Krzysztofory Palace, one of the most beautiful palaces in Krakow, on the Main Market Square. Throughout its history, many prominent figures were guests of the palace.

**Address:**
The Krzysztofory Palace
35 Main Market Square
Entrance from the Main Market Square
Invited speakers

Christian Bogdan
Sven Brandau
Antonio Celada
Grant Drummond
Frederic Geissmann
Tomasz Guzik
David Hume
Alicja Józkowicz
Steffen Jung
Jerzy Kupiec-Weglinski
Pasquale Maffia
Cecilia Garlanda
Leo Otterbein
Michael Sieweke
Filip Swirski
Loems Ziegler-Heitbrock
MONOCYTES, DENDRITIC CELLS AND MACROPHAGES – BASIC RESEARCH AND PATHOPHYSIOLOGICAL ASPECTS

Friday, September 11th

12:00  Registration opens, poster set-up
12:30  EMDS Council Meeting (main Lecture Hall)
14:45-15:00  Opening remarks
15:00-17:00  Session I: Origin and development
  Chairmen: ... and Guenter Weiss
15:00-15:30  Blood monocytes and their subsets
  Loems Ziegler-Heitbrock
15:30-16:00  Development and functions of macrophages
  Frederic Geissmann
16:00-16:30  Gut and brain macrophages – development and tissue specialization
  Steffen Jung
16:30-16:45  M-CSF and GM-CSF receptor signaling differentially affect the maturation and polarization of monocytes and macrophage subsets in the tumor microenvironment
  Geert Raes
16:45-17:00  Macrophages from mice treated with antidepressant drugs express reduced antigen-presenting activity
  Katarzyna Nazimek
17:00-17:30  Coffee break, industrial exhibition and poster viewing
17:30-18:45  Session II: Malignancy: pro – and anticancer activities
  Chairmen: Sven Brandau and Jarek Baran
17:30-18:00  Role of neutrophils and MDSC in the tumor host
  Sven Brandau
18:00-18:15  Investigating the role of radiotherapy on macrophages and on macrophage-cancer cell communication
  Ana Pinto
18:15-18:30  Colorectal cancer-derived microvesicles affect differentiation of human monocytes to macrophages
  Monika Baj-Krzyworzeka
18:30-18:45  Macrophage polarization is distinctly modulated by normal and tumour decellularized matrices derived from human colorectal cancer patients
  Marta Pinto
18:45-21:00  Industrial exhibition and Poster Session I with dinner buffet and drinks
Saturday, September 12th

09:00-10:45 **Session III: Innate immunity aspects**

**Chairmen:** Geert Raes and Joanna Cichy

09:00-09:30 Molecular biology of macrophage differentiation
**David Hume**

09:30-10:00 The interplay between phagocytes and humoral innate immunity
**Cecilia Garlanda**

10:00-10:15 Comparison of two different strategies for human monocyte subsets gating within the large-scale prospective CARE FOR HOME study
**Adam Zawada**

10:15-10:30 A novel Tsc2-mTORC1-CDK4 axis controls tissue homeostasis by regulating polarization, proliferation, and metabolism in macrophages
**Thomas Weichhart**

10:30-10:45 Antigen Uptake and Presentation by Mononuclear Phagocytes in the Peyer’s Patch
**Guy Shakhar**

10:45-11:15 Coffee break, industrial exhibition and poster viewing

11:15-13:00 **Session IV: Infection and inflammation**

**Chairmen:** Christian Bogdan and Janusz Marcinkiewicz

11:15-11:45 Macrophages and dendritic cells in antileishmanial defense and relation to reactive chlorine intermediates
**Christian Bogdan**

11:45-12:15 Mechanisms of macrophage protection against DNA breaks produced during pro-inflammatory activation
**Antonio Celada**

12:15-12:30 Low oxygen tensions found in Salmonella-infected gut tissue boost Salmonella replication in macrophages by impairing antimicrobial activity and augmenting Salmonella virulence
**Jonathan Jantsch**

12:30-12:45 Lung-residing myeloid regulatory cells control susceptibility to disease in murine pulmonary tuberculosis
**Anca Dorhoi**

12:45-13:00 DNA damage and replication stress mediate macrophage precursor differentiation into giant anti-inflammatory foam cells
**Antigoni Triantafyllopoulou**

13:00-15:00 Lunch and Poster Session II

15:00-17:00 **Session V: Microenvironment/ Mononuclear cells associated with blood vessels**

**Chairmen:** Filip Swirski and Tomasz Guzik

15:00-15:30 Monocyte subsets in hypertension and obesity
**Tomasz Guzik**

15:30-16:00 Vascular macrophages in hypertension and atherosclerosis
**Grant Drummond**

16:00-16:30 Plasmacytoid dendritic cells in vascular microenvironment
**Pasquale Maffia**

16:30-16:45 Stabilin-1 is a functional biomarker for pro-fibrotic alternative macrophages predicting pathological heart remodelling in patients with heart failure during the left ventricular assist device therapy
**Julia Kłyszyńska**

16:45-17:00 Characterization of human CD14+ cells in peripheral blood that are responsible for hematopoietic stem and progenitor cell survival
**Esther Heideveld**

17:00-17:30 Coffee break, industrial exhibition and poster viewing

17:30-19:00 **EMDS Business Meeting (EMDS Members only)**

20:30 Gala Dinner and presentation of the EMDS Awards
### Sunday, September 13th

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<tr>
<th>Time</th>
<th>Session VI: Metabolic aspects /Heme oxygenase-1: from hematopoietic stem cells to macrophages</th>
<th>Chairman(s)</th>
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<tbody>
<tr>
<td>09:00-09:30</td>
<td>Role of heme oxygenase-1 in hematopoietic stem cells differentiation: does the niche matter?</td>
<td>Alicja Józkowicz</td>
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<tr>
<td>09:30-10:00</td>
<td>Carbon monoxide in inflammasome dependent bacteria elimination by macrophages</td>
<td>Leo Otterbein</td>
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<td>10:00-10:30</td>
<td>HO-1 in macrophage-mediated rescue after liver ischemia/reperfusion injury</td>
<td>Jerzy W. Kupiec-Weglinski</td>
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<td>10:30-10:45</td>
<td>Methotrexate selectively targets human pro-inflammatory macrophages through a thymidylate synthase/p53 axis</td>
<td>Amaya Puig Kröger</td>
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<tr>
<td>10:45-11:00</td>
<td>An emergency mechanism for erythrocyte disposal and iron recycling in the liver</td>
<td>Igor Theurl</td>
</tr>
<tr>
<td>11:00-11:30</td>
<td>Coffee break, industrial exhibition and poster viewing</td>
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</tr>
</tbody>
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### 11:30-13:15 Session VII: Macrophages and DC/basic aspects and specific pathologies

<table>
<thead>
<tr>
<th>Time</th>
<th>Session VII: Macrophages and DC/basic aspects and specific pathologies</th>
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<td>11:30-12:00</td>
<td>Growth factor influence on the macrophage lineage in infection and metabolism</td>
<td>Filip Swirski</td>
</tr>
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<td>12:00-12:30</td>
<td>Transcriptional control of macrophage self renewal and Activation status</td>
<td>Michael Sieweke</td>
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<td>12:30-13:00</td>
<td>Ontogeny And Homeostasis Of Resident Serous Cavity Macrophages and Dendritic Cells</td>
<td>Stephen Jenkins</td>
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<td>13:00-13:15</td>
<td>Formation of different lipid-laden human macrophages in response to terminally oxidized LDL (oxLDL) and enzymatically modified LDL (eLDL): a multi-omics approach</td>
<td>Gerd Schmitz</td>
</tr>
<tr>
<td>13:15-13:30</td>
<td>From tissue dissociation to flow cytometric analysis – product offer of MEDianus Ltd.</td>
<td>Anna Kępka</td>
</tr>
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<td>13:30-13:45</td>
<td>Closing remarks</td>
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</tr>
</tbody>
</table>
Session I: Origin and development

O1 Geert Raes

M-CSF and GM-CSF receptor signaling differentially affect the maturation and polarization of monocytes and macrophage subsets in the tumor microenvironment.

Eva Van Overmeire1,2, Benoît Stijlemans1,2, Felix Heymann3, Yannick Morias1,2, Yvon Elkrim1,2, Jiri Keirsse1,2, Chloé Abels1,2, Qods Lahmar1,2, Frank Tacke3, Patrick De Baetselier1,2, Geert Raes1,2, Jo A Van Ginderachter1,2, and Damya Laoui1,2

1Laboratory of Myeloid Cell Immunology, VIB, Brussels, Belgium
2Laboratory of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Brussels, Belgium
3Department of Medicine III, RWTH University-Hospital Aachen, Aachen, Germany

Tumors contain a heterogeneous myeloid fraction, encompassing discrete MHC-IIhi and MHC-IIlo tumor-associated macrophage (TAM) subpopulations, which originate from Ly6Chi monocytes. However, the mechanisms regulating the numbers and the phenotype of distinct TAM subsets remain unknown.

Here, we showed that treatment of tumor-bearing mice with a blocking anti-M-CSFR mAb resulted in a reduction of mature TAM in different tumor models, by impairing the recruitment, extravasation, proliferation and maturation of their Ly6Chi monocytic precursors. At the macrophage level, M-CSFR signaling blockade caused a shift in the MHC-IIlo/MHC-IIhi TAM balance in favor of the latter due to a preferential differentiation of Ly6C hi monocytes towards MHC-IIhi TAM. In addition, M-CSFR signaling appeared to be crucial in shaping the MHC-IIlo TAM phenotype, since genes, proteins and functions associated with MHC-IIlo TAM were downregulated upon M-CSFR blockade. Conversely, GM-CSFR had no role in the recruitment or intratumoral differentiation of Ly6C hi monocytes, since the mononuclear tumor infiltrate and relative abundance of TAM subsets was unaltered in GM-CSFR-deficient mice. However, GM-CSFR signaling played an important role in the fine-tuning of the MHC-IIhi phenotype.

Overall, our data uncover the multifaceted and opposing roles of M-CSFR and GM-CSFR signaling in determining the phenotype of macrophage subsets in tumors.

O2 Katarzyna Nazimek

Macrophages from mice treated with antidepressant drugs express reduced antigen-presenting activity.

Pawel Bryniarski1, Michał Myszka1, Agata Bryk1, Michał Santocki1, Spencer Strobel1, Anna Tyszka1, Piotr Gnady1, Pawel Wojcicki1, Katarzyna Nazimek2

1Students’ Scientific Society of Department of Immunology, Jagiellonian University Medical College, Krakow, Poland
2Department of Immunology, Jagiellonian University Medical College, Krakow, Poland

Introduction. Depression is associated with altered local and peripheral inflammatory response and antidepressant drugs were shown to alleviate local adverse inflammation. Our studies were aimed to determine the influence of antidepressant drugs on murine peripheral macrophages (Mf) acting as antigen-presenting cells.

Methodology. CBA/J mouse donors of peritoneal Mf were intraperitoneally treated for 7 days with imipramine (20mg/kg), fluoxetine (10mg/kg), venlafaxine (5mg/kg) or moclobemide (5mg/kg). Expression of selected Mf surface markers was analyzed cytometrically and zymosan-stimulated reactive oxygen species (ROS) generation was estimated in chemiluminescence assay. Cytokines concentration in culture supernatant of unstimulated or LPS-stimulated Mf was measured in ELISA and NO concentration was assessed colorimetrically. Contact sensitivity (CS) reaction in hapten-sensitized recip-
Ivants of hapten-labeled Mf was measured as ear swelling response, while humoral immunity in recipients of corpuscular antigen-pulsed Mf was assessed in plaque-forming and hemagglutination assays.

**Results and conclusion.** In vivo therapy with tested drugs (except imipramine) leads to significant impairment of Mf antigen-presenting ability associated with decreased MHC and co-stimulatory molecules expression, diminished release of IL-6, TNFα, IL-12p40, NO and ROS as well as increased secretion of IL-10 and TGFβ. This results in suppression of symptoms of CS reaction as well as in reduced specific antibody serum titres and decreased number of antibody-producing cells in recipient mice. To conclude, long-term therapy with antidepressant drugs may alleviate inflammatory response in pathogenesis of depression or other inflammatory disorders, like humoral and cellular allergies and autoimmunity (beneficial effect), but also may impair peripheral immune defense mechanisms (adverse effect).
O3 Ana Pinto

Investigating the role of radiotherapy on macrophages and on macrophage-cancer cell communication

AT Pinto\textsuperscript{1,2,3}, ML Pinto\textsuperscript{1,2,4}, AP Cardoso\textsuperscript{2,5}, C Monteiro\textsuperscript{1,2,6}, MT Pinto\textsuperscript{1,5}, AF Maia\textsuperscript{1,4}, P Castro\textsuperscript{1,2}, R Figueira\textsuperscript{7}, A Monteiro\textsuperscript{7}, M Marques\textsuperscript{7}, M Mareel\textsuperscript{8}, SG Santos\textsuperscript{1,2,4}, R Seruca\textsuperscript{1,5,9}, MA Barbosa\textsuperscript{1,2,4}, S Rocha\textsuperscript{10} and MJ Oliveira\textsuperscript{1,2,9}

\textsuperscript{1}I3S-Instituto de Investigação e Inovação em Saúde, Universidade do Porto; \textsuperscript{2}INEB-Institute of Biomedical Engineering, University of Porto; \textsuperscript{3}FEUP-Faculty of Engineering, University of Porto; \textsuperscript{4}ICBAS-Institute of Biomedical Sciences Abel Salazar, University of Porto; \textsuperscript{5}IPATIMUP-Institute of Molecular Pathology and Immunology, University of Porto; \textsuperscript{6}IBMC-Institute for Molecular and Cell Biology, University of Porto; \textsuperscript{7}Radiotherapy Service, Centro Hospitalar de São João, EPE, Porto; \textsuperscript{8}Department of Radiation Oncology and Experimental Cancer Research, Ghent University Hospital, Ghent, Belgium; \textsuperscript{9}Department of Pathology and Oncology, Faculty of Medicine, University of Porto, Porto, Portugal; \textsuperscript{10}Centre for Gene Regulation and Expression, College of Life Sciences, University of Dundee, Dundee, UK

Despite scientific/technological advances in the field of radiotherapy, therapy resistance is still a major challenge in cancer management. To improve conventional radiotherapy, fundamental research aiming to understand how radiation affects tumor microenvironment components, particularly macrophages, and also the crosstalk between these and cancer cells, is essential.

To address this, primary human monocyte-derived macrophages were subjected, during five days, to conventional doses of ionizing radiation (2Gy/fraction/day), as used for cancer patients’ treatment. Macrophage polarization profile and function, as well as the impact of irradiated macrophages on cancer cell invasion and cancer cell-induced angiogenesis, were investigated. To closer mimic macrophage-cancer cell communication, we established and irradiated a co-culture model.

Our results evidence that ionizing radiation induces macrophage DNA damage, without causing apoptosis, activates NF-\kappa B signalling pathway and increases Bcl-xL expression, contributing to pro-survival activity of irradiated macrophages. Additionally, ionizing radiation reduces macrophage anti-inflammatory phenotype, by decreasing CD163/MRC1/VCAN/IL-10 and increasing CD80/CD86/HLA-DR expression, maintaining their ability to promote cancer cell invasion and cancer cell-induced angiogenesis. Interestingly, CXCL12, a chemokine which autocrine production modulates differentiation towards an anti-inflammatory phenotype, is downregulated in macrophages from irradiated co-culture, when compared with those from non-irradiated one.

In summary, our data evidences that 10Gy-irradiated macrophages exhibit a reduced anti-inflammatory phenotype but still maintain their pro-invasive ability, suggesting that they could sustain the activity of residual radioresistant cancer cells. Additional work on macrophages from irradiated co-cultures will allow a better understanding of ionizing radiation effect on macrophage-cancer cell communication, which is fundamental to design new therapeutic strategies complementary to radiotherapy.

O4 Monika Baj-Krzyworzeka

Colorectal cancer-derived microvesicles affect differentiation of human monocytes to macrophages.

Monika Baj-Krzyworzeka\textsuperscript{1}, Bożenna Mytar\textsuperscript{1}, Rafal Szatanek\textsuperscript{1}, Marcin Surmiak\textsuperscript{2}, Jarek Baran\textsuperscript{1} and Maciej Siedlar\textsuperscript{1}

\textsuperscript{1}Department of Clinical Immunology and Transplantology, Jagiellonian University Medical College, Cracow, Poland \textsuperscript{2}Department of Internal Medicine, Jagiellonian University Medical College, Cracow, Poland

Tumour-derived microvesicles (TMVs) are small membrane fragments released by tumour cells during their life span. They have a substantial contribution in intercellular communication during tumour progression as they may change biological activity of immune cells e.g. monocytes and macrophages.
In the present study, the role of colon cancer cell-derived microvesicles in monocyte differentiation and activity profile (polarization) was investigated.

Monocyte-derived macrophages (MDM) were differentiated in vitro in the presence of TMVs obtained from colon cancer Caco-2, SW620, LoVo or SW480 cell lines and analysed according to their morphology and biological functions as defined by cytokine secretion, reactive oxygen intermediate (ROI) production and cytotoxic activity against respective colon cancer cells.

Monocytes differentiated with TMVs exhibited morphological and phenotypical characteristics of macrophages. An early contact of monocytes with TMVs resulted in increased IL-10 secretion, low ROI production and low cytotoxicity against tumour cells. On the other hand, late contact of MDM with TMVs, stimulated MDM to significant TNF and IL-12 secretion, ROI production and enhanced cytotoxicity against tumour cells in vitro. Biological activity and microRNA profiling of MDMs indicated differences in their polarization/activation status which may suggest mixed polarization type M1/M2 with the predominance of M2-like after early contact with TMVs and M1-like cells after late contact with TMVs.

In summary, macrophages activity (polarization status) may be influence by contact with not only tumour cells but also with TMVs, however final polarization status depends on the contact time, and vesicle “cargo”.

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**O5 Marta L Pinto**

**Macrophage polarization is distinctly modulated by normal and tumour decellularized matrices derived from human colorectal cancer patients**

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Tumours, besides cancer cells, are composed of various other cell types together with a highly dynamic network – the extracellular matrix (ECM). This complexity is being perceived as a great opportunity for cancer prevention and treatment. In this context, macrophages emerged as modulators of cancer progression, regulating breast cancer cell migration, invasion and metastasis. We are particularly interested in elucidating how the ECM modulates macrophage polarization.

To address this question, and to mimic more closely the natural tumour microenvironment, we created an innovative 3D-organotypic culture model using decellularized human colorectal cancer tissue repopulated with monocytes.

DNA quantification confirmed the efficiency of the decellularization protocol used while Scanning Electron Microscopy(SEM) analysis revealed an ECM fiber meshwork architecturally similar to native tissues. Decellularization reduced glycosaminoglycans(GAGS) content but not other ECM components, such as laminin and fibronectin. Repopulation experiments clearly evidenced that monocytes colonize these matrices differentiating into macrophages within the fiber network. Interestingly, normal and tumour-derived matrices distinctly modulated macrophage polarization. Our results revealed that macrophages repopulating normal decellularized matrices secrete higher levels of IL-6 and MMP-9. Real-time PCR analyses demonstrate that monocytes differentiated on tumour decellularized matrices express less CCL18, TNF and MMP1 but significantly more CCL18.

Our results clearly emphasize the critical impact of normal/tumour ECM on macrophage polarization and reinforce the relevance of using in vitro models that resemble native microenvironments. Elucidating the role of ECM components, derived from the tumour microecosystem, on macrophage differentiation and polarization opens new perspectives in the design of novel therapeutic strategies targeting macrophages.
**Session III: Innate immunity aspects**

**O6 Adam M. Zawada**

**Comparison of two different strategies for human monocyte subsets gating within the large-scale prospective CARE FOR HOMe study**

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Monocytes are heterogeneous cells consisting of three subsets: classical, intermediate and nonclassical monocytes. Correct enumeration of cell counts necessitates well-defined gating strategies. For delineation of intermediate from nonclassical monocytes, a “rectangular gating (RG) strategy” and a “trapezoid gating (TG) strategy” have been proposed. We compared the two gating strategies in a well-defined cohort of patients with chronic kidney disease (CKD).

Within the CARE FOR HOMe study, monocyte subsets were reanalyzed in 416 CKD patients, who were followed 3.6±1.6 years for the occurrence of a cardiovascular event. Gating was performed by either RG or TG. We analyzed the expression of surface markers, and compared the predictive role monocyte subsets, as defined by RG and TG, respectively.

With both gating strategies, higher intermediate monocytes counts predicted the cardiovascular endpoint in Kaplan-Meier analyses (p<0.001 with RG; p<0.001 with TG). After correction for confounders, intermediate monocyte counts remained independent predictors (HR=1.013 [95% CI: 1.006-1.020; p<0.001] with RG; HR=1.015 [95% CI: 1.006-1.024; p=0.001] with TG). NRI was 3.9% when reclassifying patients from quartiles of intermediate monocyte counts with RG towards quartiles of intermediate monocytes counts with TG. In expression analyses, those monocytes which are defined as intermediate monocytes by RG and as nonclassical monocytes by TG share characteristics of both subsets.

In conclusion, intermediate monocytes were independent predictors of cardiovascular outcome irrespective of the applied gating strategy. Future studies should aim to identify markers that allow for an unequivocal definition of intermediate monocytes, which may further improve their power to predict cardiovascular events.

**O7 Thomas Weichhart**

**A novel Tsc2-mTORC1-CDK4 axis controls tissue homeostasis by regulating polarization, proliferation, and metabolism in macrophages.**


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Maintenance of tissue homeostasis requires a tight control of the in situ-proliferative capacity versus quiescence of tissue-resident M2-like macrophages. Here we investigated a potential role of Tsc2, the key negative regulator of mTORC1,
for this process. Deletion of Tsc2 in myeloid cells broke quiescence and strongly induced macrophage proliferation and granuloma formation \textit{in vivo} in an mTORC1-dependent manner. Intriguingly, Tsc2-mTORC1 directly controlled M-CSF-stimulated cell cycle progression by inducing the expression of CDK4 but repressing inflammatory NF-κB signaling. Tsc2-deficient macrophages showed constitutive CDK4 expression and enhanced M2-like polarization that was accompanied by a reconfiguration of the cellular metabolism towards increased aerobic glycolysis and mitochondrial metabolism. Inhibition of mTORC1, CDK4, and hexokinase abrogated cell cycle progression indicating an intrinsic need of glucose flux and CDK4 for M-CSF-dependent M2-like macrophage proliferation. Strikingly, we found that CDK4 and glucose flux directly contributed to the M2-like polarization potential of Tsc2-deficient macrophages. In conclusion, cell cycle regulation in macrophages is exerted on the level of CDK4 expression through Tsc2-mTORC1 that couples macrophage proliferation, M2 polarization and metabolic reconfiguration to prevent granulomatous disease \textit{in vivo}.

O8 Guy Shakhar

Antigen Uptake and Presentation by Mononuclear Phagocytes in the Peyer’s Patch
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The gut-associated-lymphatic-tissue (GALT) contains mononuclear phagocytes (MPs) including dendritic cells (DCs) and macrophages. An important inductive GALT site is the Peyer’s patch (PP). At least two different DC populations populate the PPs, one resides underneath the follicle-associated epithelium (FAE) covering the PP while the second inhabits T cell zones.

MPs can sample luminal antigens which traverse microfold (M) cells located within the FAE. These antigens are then presented in the T cell zone. However, it is unclear how M cells and MPs cooperate as they sample antigen. It is also unclear whether the antigen is presented to T cells by the MPs that initially sampled it, or transferred to DCs found in the T cell zone.

Using two-photon microscopy in live transgenic mice, we investigated the interactions between M cells, MPs and T cells in the PP. We captured previously-unobserved behavior of MPs as they migrate within the epithelium, enter into microfolds and interact with M cells. Antigen uptake and T cell activation by MPs was assessed following administration of fluorescent antigens. MPs accumulated and presented particulate antigen \textit{in vitro}, resulting in T cell proliferation. Particles injected to the intestines accumulated in MPs in the PP. Administration of soluble antigen activated T cells, which formed dynamic clusters in the T cell zones and upregulated CD69. These results present our abilities to follow MPs as they interact with M cells and T cells as they are activated within the PP.
Low oxygen tensions found in Salmonella-infected gut tissue boost Salmonella replication in macrophages by impairing antimicrobial activity and augmenting Salmonella virulence

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In Salmonella infection, the Salmonella Pathogenicity Island-2 (SPI-2)-encoded type three secretion system (T3SS2) is of key importance for systemic disease and survival in host cells. For instance, in the streptomycin-pretreated mouse model SPI-2-dependent Salmonella replication in lamina propria CD11c CXCR1– monocytic phagocytes/ macrophages (MΦ) is required for the development of colitis. In addition, containment of intracellular Salmonella in the gut critically depends on the antimicrobial effects of the phagocyte NADPH oxidase (PHOX), and possibly type 2 NO synthase (NOS2). For both antimicrobial enzyme complexes oxygen is an essential substrate. However, the amount of available oxygen upon Salmonella infection in the gut tissue and its impact on Salmonella-MΦ interactions was unknown.

Therefore, we measured the gut tissue oxygen levels in a model of Salmonella enterocolitis using luminescence-2D-in vivo oxygen imaging. We found that gut tissue oxygen levels dropped from ~78 Torr (~11% O2) to values of ~16 Torr (~2% O2) during infection. Since in vivo virulence of Salmonella depends on the Salmonella survival in MΦ, Salmonella-MΦ interaction was analysed under such low oxygen values. These experiments revealed an increased intracellular survival and replication of wild type and t3ss2 non-expressing Salmonella. These findings were paralleled by blunted NO and ROS production and reduced Salmonella ROS perception. In addition, hypoxia enhanced SPI-2 transcription and translocation of a SPI-2-encoded virulence protein. Neither blockade of PHOX and NOS2 nor impairment of T3SS2 virulence function alone mimicked the effect of hypoxia on Salmonella replication under normoxic conditions. However, if t3ss2 non-expressing Salmonella were used, hypoxia did not further enhance Salmonella recovery in a PHOX and NOS2-deficient situation. Hence, these data suggest that hypoxia-induced impairment of antimicrobial activity and Salmonella virulence cooperate to allow for enhanced Salmonella replication in MΦ.
O10 Anca Dorhoi

**Lung-residing myeloid regulatory cells control susceptibility to disease in murine pulmonary tuberculosis**

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Innate myeloid cells exert multiple roles during *Mycobacterium tuberculosis* (*Mtb*) infection. A subset of innate cells, namely myeloid regulatory cells (MRCs)/myeloid-derived suppressor cells (MDSCs) have been described in cancer and shown to facilitate disease progression by suppressing T-cell responses. The roles of MDSCs in infectious diseases are incompletely understood. Here, we studied the interaction of MDSCs and *Mtb* and elucidated their roles in tuberculosis (TB). Cell-based approaches and experimental mouse models for pulmonary TB were employed to identify and characterize MDSCs during TB. *In vitro* generated MDSCs phagocytosed *Mtb*, retained their suppressive capacity and released selected pro- (IL-6, IL-1α) and anti-inflammatory (IL-10) mediators following infection. MDSCs were identified in lungs of both disease-resistant and susceptible mice during TB. MDSC kinetics at the site of pathology indicated that an accelerated accumulation into the lungs paralleled TB lethality and that a heightened surface expression of IL-4Rα on MDSCs correlated with disease severity. Drug-mediated maturation/ablation of MDSCs by systemic delivery of all-trans retinoic acid reduced bacterial burdens and ameliorated lung pathology. MDSCs represent a reservoir for *Mtb* and fine-tune immunity in TB. Future investigations should clarify the feasibility of drug-mediated ablation of MDSCs as a host-directed therapy against TB and perhaps other chronic bacterial infections.

O11 Antigoni Triantafyllopoulou

**DNA damage and replication stress mediate macrophage precursor differentiation into giant anti-inflammatory foam cells**

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The pathways that guide macrophage differentiation in granulomas are unclear. Foamy macrophages and multinucleated giant cells (MGCs) are hallmarks of mature granulomas. Here, using high content image cytometry and long term live cell imaging, we show that purified bacterial lipoproteins *in vitro* and *Mycobacterium bovis* Bacillus Calmette Guérin (BCG) *in vivo* promote macrophage precursor differentiation into giant anti-inflammatory foam cells by inducing replication stress and subsequent cytokinesis failure. Gene set enrichment analysis revealed that bacterial lipoproteins induced global reprogramming of macrophage cell cycle regulators and activated the DNA damage response, while concomitantly suppressing ApoE and NF-κB dependent genes. DNA damage, necrosis and differentiation of the surviving cells into polyploid giant foam cells were regulated by c-Myc and ATR signaling. Foamy macrophages and multinucleated giant cells in BCG and *M. tuberculosis*-induced granulomas *in vivo* showed evidence of DNA damage and replication stress. Our data support a model where cell cycle and DNA damage response pathways are integrated downstream of inflammatory cues to instruct granulomatous tissue-specific macrophage differentiation. Interfering with this pathway may be a valid way to re-program macrophage function in granulomatous diseases and potentially atherosclerosis.
Session V: Microenvironment/ Mononuclear cells associated with blood vessels

O12 Julia Kzhyshkowska

Stabilin-1 is a functional biomarker for pro-fibrotic alternative macrophages predicting pathological heart remodelling in patients with heart failure during the left ventricular assist device therapy.

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Alternatively activated macrophages (M2) control immunological tolerance and healing processes. Chronic inflammation in myocardium results in development of cardiomyopathy and heart failure (HF). Advanced HF is a life-threatening disorder affecting 6-10% of people above 65 y.o. Less than 50% of HF patients survive 5 year after the first symptoms are identified. Advanced HF is treated with mechanical circulatory support LVAD (left ventricular assist device). The outcome of LVAD therapy differs significantly between patients with over 50% mortality after LVAD explantation and successful myocardial recovery in others. Identifying the immunopathological mechanisms and biomarkers of patients’ reaction to LVAD are urgently needed. Heart sections were obtained directly before LVAD installation and out the explained hearts at the moment of donor heart transplantation. Analysis of macrophages phenotypes in paraffin sections of 21 patients with HF was performed using IHC, IF/confocal microscopy. Three types of macrophage subtypes have been identified: CD68+stabilin-1; CD68+Stabilin-1+; CD68-stabilin-1+. In 10 patients the percentage of CD68-Stabilin1+ was decreased or not changed during LVAD therapy. In 8 out of these 10 cases ejection fraction (EF) of the heart was improved after LVAD therapy. In 11 cases the percentage of CD68-Stab1+ was increased after LVAD therapy. In 10 out of these 11 cases EF was not improved. Pro-fibrotic cytokine TGFbeta strongly activated functionally active stabilin-1 in human macrophages in vitro. We concluded that CD68-Stabilin-1+ macrophages represent new subtype of pro-fibrotic M2 in healing heart, and dynamic accumulation of CD68-Stabilin-1+ macrophages is predictive for the pathological heart remodelling during LVAD therapy.

O13 Esther Heideveld

Characterization of human CD14+ cells in PERIPHERAL BLOOD that are responsible for Hematopoietic stem and progenitor cell survival

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Hematopoietic stem (and progenitor) cells (HS(P)C) reside in a complex microenvironment in the bone marrow. In mice, CD15-CD163+CD169+VCAM1+ macrophages regulate HSC retention and depletion of these macrophages mobilizes HSPC. We previously observed a 4-15 fold increase in erythroid yield from total peripheral blood mononuclear cell (PBMC) cultures compared to CD34+ cells purified from PBMC, suggesting a role for feeder/stromal cells in PBMC that increase hematopoietic output.
Immunodepleting PBMC for CD14 showed a twofold reduction in erythroblasts, indicating that monocytes/macrophages regulate CD34+ differentiation. Co-culturing CD34+ with CD14+ cells for eight days yielded 5-10 fold more erythroblasts compared to CD34+ cells alone. CD14+ co-culture with erythroblasts did not affect the cellular yield, in contrast short term cultures of CD34+ with CD14+ cells showed fivefold increase in CD34+ cell numbers and colony forming units within two days. CD34+CD36– HSPC co-cultured with CD14+ cells significantly increased both numbers of CD34+CD36– HSPC and CD34+CD36+ megakaryocyte-erythroid progenitors. The presence of CD14+ cells did not affect CD34+ proliferation, but increased CD34+ cell survival.

Cell contact plays a minor role as conditioned medium of CD14+ cells and transwell experiments reconstituted 70-80% of the co-culture effect. In particular CD14++CD16+ intermediate monocytes/macrophages are responsible for increased erythroid yield. During culture, CD14+ cells differentiate and gain expression of CD163, CD169, and CD206, suggesting differentiation towards tissue resident-like macrophages.

These data indicate that HSPC survival is regulated by CD14+ cells present in PBMC, which share characteristics with bone marrow resident macrophages and adds to the retention signals elicited by these cells.
Abstracts of Oral Presentations

Session VI: Metabolic aspects /Heme oxygenase-1: from hematopoietic stem cells to macrophages

014 Leo E. Otterbein

Carbon Monoxide in Inflammasome Dependent Bacteria Elimination by Macrophages
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Microbial clearance by eukaryotes relies on complex and coordinated processes that remain poorly understood. The gasotransmitter carbon monoxide (CO) is generated by the stress-responsive enzyme heme oxygenase-1 (HO-1, encoded by Hmox1), which is highly induced in macrophages (mφ) in response to bacterial infection. HO-1 deficiency results in inadequate pathogen clearance, exaggerated tissue damage, and increased mortality. Here, we determined that macrophage-generated CO promotes ATP production and release by bacteria, which then activates the mφ inflammasome, intensifying bacterial killing. Bacterial killing defects in HO-1-deficient murine mφ were restored by administration of CO. Moreover, increased CO levels enhanced the bacterial clearance capacity of human mφ and WT murine mφ. CO-dependent bacterial clearance required the NALP-3 inflammasome, as CO did not increase bacterial killing in mφ isolated from NALP-3-deficient or caspase-1-deficient mice. IL-1β cleavage and secretion was impaired in HO-1-deficient mφ, and CO-dependent processing of IL-1β required the presence of exogenous ATP. We found that bacteria remain viable to generate and release ATP in response to CO. The ATP then binds to mφ nucleotide P2 receptors resulting in activation of the NALP3/IL-1β inflammasome to amplify bacterial phagocytosis by mφ. Taken together, our results indicate that mφ-derived CO permits efficient and coordinated regulation of the host innate response to invading microbes.

015 Amaya Puig-Kröger

Methotrexate selectively targets human pro-inflammatory macrophages through a thymidylate synthase/p53 axis
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Methotrexate (MTX) functions as an anti-proliferative agent in cancer and an anti-inflammatory drug in rheumatoid arthritis (RA). Although macrophages critically contribute to RA pathology, their response to MTX remains unknown. As a mean to identify MTX-response markers, we have explored its transcriptional effect on macrophages polarized by GM-CSF (GM-MØ) or M-CSF (M-MØ), which resemble pro-inflammatory and anti-inflammatory macrophages found in RA and normal joints, respectively. MTX exhibited a minor transcriptional effect on M-MØ but modulated the expression of 2024 genes in pro-inflammatory GM-MØ, where it induced CCL20 and LIF at the mRNA and protein level. Pharmacological and siRNA-mediated approaches indicated that the macrophage subset-specific MTX responsiveness correlates with thymidylate synthase (TS) expression, as pro-inflammatory TS+ GM-MØ are susceptible to MTX, whereas anti-inflammatory TSlow/- M-MØ and monocytes are refractory to MTX. Furthermore, p53 activity was found to mediate the TS-dependent MTX-responsiveness of pro-inflammatory TS+ GM-MØ. Importantly, TS and p53 were found to be expressed by CD163+/TNFα+ GM-CSF-polarized macrophages from RA joints but not from normal synovium. Altogether, our results demonstrate that macrophage response to MTX is polarization-dependent and determined by the TS-p53 axis, thus providing the molecular explanation for
the selectivity of MTX for pathology-driving pro-inflammatory macrophages. Moreover, our results show that CCL20 and LIF constitute novel macrophage markers for MTX responsiveness.

O16 Igor Theurl

An emergency mechanism for erythrocyte disposal and iron recycling in the liver

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Iron is an essential component of the erythrocyte protein hemoglobin and is crucial to oxygen transport in vertebrates. In the steady state, erythrocyte production is in equilibrium with erythrocyte removal. In various pathophysiological conditions, erythrocyte life span is severely compromised, which threatens the organism with anemia and iron toxicity. Here we identify an essential emergency on-demand mechanism specific to the liver that clears erythrocytes and recycles iron. We show that Ly-6Chigh monocytes ingest damaged and senescent erythrocytes, accumulate in the liver, and differentiate to ferroportin 1 (FPN1)-expressing macrophages that can deliver iron to hepatocytes. Monocyte-derived FPN1+ macrophages are transient and their appearance in the liver requires Csf1 and Nrf2. The spleen likewise recruits iron-loaded Ly-6C<sup>high</sup> monocytes, but they do not differentiate into iron-recycling macrophages due to the suppressive action of Csf2, and are instead shuttled to the liver via coordinated chemotactic cues. Inhibiting this mechanism by preventing monocyte recruitment to the liver leads to kidney failure and liver damage. These observations identify the liver as the primary organ supporting emergency erythrocyte removal and iron recycling, and uncover a mechanism by which the body adapts to fluctuations in erythrocyte integrity.
Session VII: Macrophages and DC/basic aspects and specific pathologies

O17 Allan McI Mowat

Tissue Specific Processes and TGFβ Drive the Differentiation of Monocytes into Homeostatic Intestinal Macrophages
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Resident macrophages (mφs) from different tissues are highly heterogeneous in terms of their functions and origins. Intestinal mφs are one of the largest populations in the body and have several characteristic features, including expression of high levels of MHCII and CX3CR1, constitutive production of IL10 and TNFα and avid phagocytic activity. However they are refractory to stimuli such as TLR ligands. We have shown that adult intestinal mφs are derived from circulating Ly6Chi monocytes that acquire the properties of the resident cells in the mucosa. However the mechanisms responsible for this local differentiation process are unknown. By comparing their transcriptome with monocyte – and embryonically derived mφs from other tissues, here we identify a genetic signature of resident intestinal mφs and validated it by Q-PCR and FACS. Soon after entry into the intestine, maturing mφs become genetically distinct, even from mφs in other tissues that are replenished by monocytes, such as the dermis. Many of the selectively expressed molecules are involved in tissue remodeling and in uptake of apoptotic cells, including αvβ5 integrin, metalloproteases 2, 9 & 14, C1Q and CD36. Several are TGFβ inducible genes and although intestinal mφ numbers are normal in CD11c-cre-TGFβR1fl/fl mice, their gene signature is altered and they show reduced expression of IL10 and CX3CR1. Thus the fate and functions of resident mφs are determined by the local environment and TGFβR signaling is at least partly involved in this process in the intestine, driving the development of mφs with homeostatic functions in tissue remodeling.

O18 Stephen J Jenkins

Ontogeny And Homeostasis Of Resident Serous Cavity Macrophages and Dendritic Cells
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Re-examination of the ontogeny of serous cavity mononuclear phagocytes (MPs) is prompted by discrepancies in previously published work and advances in macrophage and dendritic cell (DC) theory, such as identification of a dedicated circulating precursor of conventional DC (cDC) and a lineage of autonomous tissue resident macrophages arising from the yolk sac and/or fetal liver progenitors. We have used surface marker expression, growth factor dependence and population kinetics, together with a novel method to fate map monocytes to delineate the composition of the MP compartment within the serous cavities. We show that in addition to the numerically dominant F4/80+ macrophages, the cavity contains fit3L-dependent conventional DC (CD11c–CSF1R+), CSF1R-dependent monocyte-derived small peritoneal macrophages (CD11c- CSF1R+), but also cells expressing both CD11c and CSF1R that comprise a mixture of both cDC and monocyte-derived cells. Importantly, despite a degree of phenotypic convergence, monocyte-derived MP and cDC retain molecular differences that suggest discrete functional programmes exist between these lineages. In addition, we show that classical Ly-6C+ monocytes constitutively enter the cavities in a CCR2-dependent manner and rapidly replenish the MHCII+ CSF1R+ small
peritoneal macrophages. These then act, at least in part, as intermediaries between monocytes and F4/80^highGATA6^+ macrophages via a differentiation programme that includes up-regulation of RALDH2, the rate limiting enzyme in retinoic acid synthesis. Thus, similar to the macrophage compartments in the gut wall and dermis, macrophages in the serous cavities require replenishment by BM-derived monocytes.
ABSTRACTS OF POSTER PRESENTATIONS
Session II Malignancy: pro – and anticancer activities

P1 Iveta Gazova

Roles of chromatin-modifying enzymes in macrophage differentiation

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During normal cell development genes are activated and repressed, usually through epigenetic mechanisms. Gene repression is usually imposed by repressive marks created by polycomb repressive complexes 1 and 2 (PRC1 and PRC2). Epigenetic modifiers, deubiquitinases for PRC1 and demethylases for PRC2, remove the epigenetic mark and thus pave the way for activation of gene expression. In this project the role of a set of epigenetic modifiers (USP12, USP16, BAP1, KDM6A, KDM6B and UTY) in THP-1 and U937 acute monocytic leukaemia cell lines is being investigated. These cell lines can be stimulated to halt proliferation and differentiate into macrophages with phorbol esters. We have shown that inhibition of KDM6B reduces the rate of differentiation to macrophages by THP-1 cells. We are currently further investigating the role of the epigenetic modifiers in cellular growth and differentiation by perturbing the levels of the chromatin-modifying enzymes using CRISPR-Cas9 knockouts, siRNA and a range of inhibitors during differentiation of THP-1 and U937. In addition, the transcriptome of THP-1 during the differentiation time course is being analysed, to find gene expression changes correlated with changes in the chromatin modifying enzymes and hence gain better understanding of regulatory factors in macrophage differentiation.

P2 Genard Géraldine

Interactions between co-cultured cancer cells and macrophages during proton beam irradiation

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Introduction The immune system is essential to protect the organism against diseases. Everyone needs it to detect, neutralize and eliminate host agents. But sometimes, immune system can take part of pathologies as autoimmune diseases, inflammatory diseases, and more importantly, tumors. The tumor microenvironment is composed of several cell types including cancer cells, immune cells, endothelial cells and other stromal cells. Among immune cells, the macrophages are the major component of the tumor. The tumor associated macrophages (TAMs) promote key processes in tumor progression, such as angiogenesis, immunosuppression, invasion and metastasis. Two phenotypes of macrophages are distinguished: classical (M1) and alternative (M2). It is proposed that M1 macrophages are anti-tumoral whereas M2 ones favor tumor growth.

Nowadays the available treatments for tumors are mainly surgery, chemotherapy, radiotherapy or a combination of those. More than half of the patients receive radiotherapy. But one of the major problems with the use of the conventional radiotherapy is the irradiation of healthy tissues upstream and downstream of the tumor. During the past decade, new treatment modalities have emerged: protontherapy and hadrontherapy. These therapies are less invasive since the dose delivery is much more precise.

In addition to the role in the tumor initiation and progression, the immune system influences the response to the therapy. Several studies showed that TAMs can drive reparative mechanisms in tumors after radiotherapy. It was also shown that low dose irradiation can modify the macrophage polarization toward a M1 phenotype hence enhancing the efficacy of immunotherapy. However, the response of macrophages to protontherapy is not known.
Objective We aimed to study the molecular interactions that could lead to enhanced survival of cancer cells co-cultured with M0, M1 or M2 macrophages after exposure to a broad beam of protons. To this purpose, two cell lines were used: pulmonary cancer cells (A549) and differentiated THP-1 macrophages.

Results The irradiation of cancer cells with 1.5 Gy proton beam induced a decrease in cell survival of 60%, as measured by clonogenic assays, when A549 cells were cultured separately. In parallel, a cell cycle arrest in G2/M phase was observed which was maximal 8 hours after the irradiation. On the other hand, proton irradiation induced a direct cytotoxic effect on macrophages, whose intensity depends on the macrophage phenotype. M1 and to a lesser extent M2 are more sensitive to proton irradiation than M0 macrophages. Finally, proton irradiation does not affect the macrophage polarization but modify the inflammatory process in the three phenotypes as evidenced by changes in the mRNA level of several inflammatory markers.

Conclusion These experiments revealed effects of proton beam on macrophages and cancer cells separately. In the future, co-cultures will be irradiated to investigate whether a dialog between the two cell types may influence cancer cell sensitivity to proton irradiation.

P3 Iwona Homa

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Introduction. Dendritic cells (DCs) are the major antigen-presenting cells and nowadays play an important role in immunotherapy of cancers. DCs can be stimulated by tumor antigens and therefore could be useful for the construction of anti-cancer vaccines.

Aim. Identify the differences in phenotype of DCs generated ex vivo from patients with lung adenocarcinoma, depending on the type of tumor antigen used for stimulation.

Material and methods. Monocytes were isolated from peripheral blood from 20 patients. DCs were generated in CellGro medium in the presence of IL-4, GM-CSF, TNFα. DCs were stimulated with synthetic epitopes of tumor antigens: EGFR, MUC-1,2 and MAGE-A3. The phenotype and biological activity of DCs were evaluated by flow cytometry using monoclonal antibody.

Results. Generation of myeloid lineage DCs (CD45+CD11+) was effective. DCs were mature (CD1a+/CD83+, B7H1+/B7DC+, CD80+/CD86+) and able to activate and stimulate naive T cells. DCs were able to phagocytose (CD209+) and to migrate (CCR7+). A significant differences concerning the percentages of DCs as well as the expression of specific molecules depending on the tumor epitopes stimulation was observed.

Conclusions. There is possibility to generate mature and functional DCs using synthetic epitopes of tumor antigens. There are differences in phenotype of DCs depending on the type of tumor antigens. Sensitized DCs by synthetic epitopes of tumor antigens: EGFR, MUC-1,2 or MAGE-A3 can be useful as vaccine for immunotherapy of lung cancer.

P4 Mario Kuttke

PTEN-deficiency in myeloid cells alters tumor immune surveillance in a murine model of inflammation driven colon cancer

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Cells of the innate immune system are the first line of defense against invading microbes, as they produce cytokines, activate other leukocytes and are implicated in the resolution of inflammatory processes and tissue remodeling. They have been found to contribute also to tumor progression as they undergo phenotypic conversion from an inflammatory state into
an alternatively activated, tumor tolerating state. We could show that the PI3K/PTEN signaling pathway plays a role in this fate decision process. Deletion of PTEN in myeloid cells leading to sustained PI3K activation results in an M2-like phenotype characterized by an increase in M2-markers and release of anti-inflammatory factors.

In acute models of inflammation this anti-inflammatory state in myeloid cells is beneficial, but a diminished innate immune response could be detrimental during tumor development. We addressed this question by applying a model of inflammation-driven colon cancer in myeloid-PTEN deficient mice.

PTENfl/fl LysM cre conditional knock-out mice showed an increased tumor incidence, progression and mortality during CAC development. Isolated myeloid cells exhibited an up-regulation of M2-marker genes and a down-regulation of pro-inflammatory cytokines. Moreover, we found an increase in immune-regulatory innate cells in the secondary lymphoid organs. T-cells isolated from myeloid-PTEN deficient mice had decreased cytokine production as well as a reduced proliferative potential ex vivo. Therefore we suggest that myeloid PTEN deficiency leads to a hypo-responsiveness in T-cells allowing for unimpeded intestinal tumor growth.

P5 Marzena Lenart

The mechanism of IL-10 induction in monocytes by tumor-derived microvesicles

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Interleukin 10 (IL-10) production is elevated in various types of cancer, where is produced by both tumor cells and/or tumor-infiltrating monocytes/macrophages, and is associated with tumor-mediated immunosuppression. Tumor-derived microvesicles (TMV) can mimic effects of tumor cells leading to an increased anti-inflammatory cytokine production, such as IL-10, by monocytes and macrophages. Yet, the mechanism of IL-10 induction by TMV in monocytes is largely unknown.

The co-incubation of TMV derived from the human pancreas carcinoma cell line (HPC-4) with human monocytes resulted in a nearly 30-fold increase in IL-10 protein production, while monocytes transduced with an adenovirus containing IL-10-promoter luciferase reporter gene showed a 5-fold induction of luciferase activity after treatment with TMVHPC. Since tumor cells can express hyaluronan (HA), which participates in tumor invasion and metastases, we have tested its effect on IL-10 expression. HA induces IL-10 protein expression and the IL-10 promoter activation in monocytes. Moreover, hyaluronidase treatment of TMV reduced IL-10 protein production and promoter activity by half. Inhibitors of the PI3K/Akt/mTOR pathway reduced both, TMV-induced IL-10 promoter activity and protein production, and the same was observed in monocytes when stimulated by HPC-4 cells or HA. Inhibition of PI3K activity down-regulated phosphorylation of the Akt and mTOR proteins in monocytes following TMVHPC or HA stimulation. When comparing monocyte subsets, TMVHPC induced IL-10 protein and mRNA synthesis only in classical CD14++CD16− but not in CD16-positive monocytes.
Our data show that TMV induce IL-10 synthesis in human classical monocytes via HA, which, in turn, activates the PI3K/Akt/mTOR pathway.

**P6 Mie Nieda**

**Effective induction of melanoma-antigen-specific CD8+ T cells via Vγ9γδ T cell-expansion by CD56high+Interferon-α-induced dendritic cells**

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Dendritic cells (DCs) can be differentiated from CD14+ monocytes in the presence of interferon-α (IFNα) and granulocyte/macrophage colony stimulating factor (GM-CSF) in vitro, and are known as IFN-DCs. Circulating blood CD56+ cells expressing high levels of CD14, HLA-DR, and CD86 have been shown to spontaneously differentiate into DC-like cells in vitro after their isolation from blood. We show here that IFN-DCs expressing high levels of CD56 (hereafter, CD56high+IFN-DCs) can be differentiated in vitro from monocytes obtained as adherent cells from healthy donors and patients with metastatic melanoma. These cells expressed high levels of CD14, HLA-DR, and CD86, and possessed many pseudopodia. These CD56high+IFN-DCs may be an in vitro counterpart of the circulating CD56+CD14+CD86+HLA-DR+ cells in blood. Conventional mature DCs differentiated from monocytes as adherent cells in the presence of GM-CSF, IL-4, and TNF-α (hereafter, mIL-4DCs) did not express CD56 or CD14. In contrast to mIL-4DCs, the CD56high+IFN-DCs exhibited a stronger capacity to stimulate autologous CD56+Vγ9γδT cells highly producing IFNα in the presence of zoledronate and IL-2. The CD56high+IFN-DCs possessing HLA-A*0201 effectively induced Mart-1-modified melanoma peptide (A27L)-specific CD8+ T cells through preferential expansion of CD56+Vγ9γδT cells in the presence of A27L, zoledronate, and IL-2. Vaccination with CD56high+IFN-DCs copulsed with tumour antigens and zoledronate may orchestrate the induction of various CD56+ immune cells possessing high effector functions, resulting in strong immunological responses against tumour cells. This study may be relevant to the design of future clinical trials of CD56high+IFN-DCs-based immunotherapies for patients with melanoma.

**P7 Maria J Oliveira**

**An interferon-γ-delivery system based on chitosan/poly(γ-glutamic acid) polyelectrolyte complexes modulates macrophage-derived stimulation of cancer cell invasion in vitro**

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The functional plasticity of macrophages upon stimulation from the environment makes them susceptible to the influence of cancer cells and also renders them as promising therapeutic targets.

In this work, we describe a drug delivery system to modulate the phenotype of macrophages, converting them from the pro-tumour M2-like phenotype to the anti-tumour M1-like phenotype, based on the incorporation of a pro-inflammatory cytokine (interferon-γ) in chitosan (Ch)/poly(γ-glutamic acid) (γ-PGA) complexes. Ch is a biocompatible cationic polysaccharide extensively studied and γ-PGA is a biodegradable, hydrophilic and negatively charged poly-amino acid. These components interact electrostatically, due to opposite charges, resulting in self-assembled structures that can be designed to deliver active molecules such as drugs and proteins.
Ch and γ-PGA were self-assembled into polyelectrolyte multilayer films (PEMs) of 371 nm thickness, using the layer-by-layer method. Interferon-γ (IFN-γ) was incorporated within the Ch layers at 100 and 500 ng/mL. Ch/γ-PGA PEMs with IFN-γ were able to modulate the phenotype of IL-10-treated macrophages at the cell cytoskeleton and cytokine profile levels, inducing an increase of IL-6 and a decrease of IL-10 production. More interestingly, the pro-invasive role of IL-10-treated macrophages was hindered, as their stimulation of gastric cancer cell invasion in vitro decreased from 4 to 2-fold, upon modulation by Ch/γ-PGA PEMs with IFN-γ.

This is the first report proposing Ch/γ-PGA PEMs as a suitable strategy to incorporate and release bioactive IFN-γ with the aim of modulating macrophage phenotype, counteracting their stimulating role on gastric cancer cell invasion.

P8 Katarzyna Sawa-Wejksza

Influence of colon cancer cells soluble factors on THP-1 cells differentiation.

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Macrophages play an important role in the immune response and in the maintenance of tissue homeostasis. It is well known that many tumors recruit monocytes from circulation and influence their differentiation. Tumor associated macrophages exhibit mainly M2-like phenotype. However, tumor can give rise to a heterogeneous population of tumor infiltrating cells depending on the local microenvironment. As there are contradictory data considering importance of macrophages for colon cancer progression, we examined four colon cancer cell lines that represent different stages of tumor development (HT29, LS180, SW948, SW620). As a control cells, the primary human skin fibroblast were used. An acute monocytic leukemia cell line, THP-1 was examined as a human model of monocytes. Our work revealed that the tumor condition media (CM) induced activation of THP-1 cells. The changes of morphology and increased expression of surface activation markers CD11b were observed. Moreover, our work showed that tumor CM induce production of reactive oxide species in THP-1 cells in dose dependant manner. We also showed that CM of colon cancer cells increase the expression of CD163, CD206, B7H1 markers, typical for M2 macrophages. The tumor CM media at highest examined concentration (50%) did not influence the viability of THP-1 cells, however it strongly and dose-dependently decreased their proliferation and influence the cell cycle. These preliminary studies suggest that colon tumor cells produce soluble factors that influence the monocytes differentiation, most probably into the suppressive subsets. The obtained date provide better understanding of colon cancer influence on macrophages polarization.

P9 Piotr Tymoszuk

Role of Macrophage-Expressed Ferritin H in Progression of Mammary Carcinoma

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Tumor-associated macrophages (TAMs) constitute a major population of tumor-infiltrating immune cells in animal models and human mammary carcinoma. Those alternatively activated macrophages support tumor growth by plethora of various mechanisms such as stimulation of tumor vascularization, tumor cell motility, provision of growth factors and dampening anti-tumor immunity. Accordingly, their presence and quantity is tightly associated with unfavorable patient’s prognosis.

Here, we show that TAMs present in murine mammary neoplasms ingest endogenous (e.g. aged erythrocytes) and exogenous iron (e.g. clinically applicable iron nanoparticles) and export it further to rapidly proliferating malignant cells. Furthermore, a myeloid-specific knockout of the crucial iron storage protein ferritin H led to a faster tumor growth and increased rate of metastasis. We demonstrate, that this effect can be attributed to a more efficient export of iron from TAMs and better iron absorption in the tumor epithelium.
Our results let us propose a hypothesis whereby TAMs play a central role in the local iron metabolism in the mammary tumors and their iron-recycling properties contribute substantially to their tumorigenic potential. The lack of TAM-expressed ferritin H elevates the turnover rate even further and leads to an accelerated tumor progression. Importantly, those two observations possess some clinical relevance as numerous patients experiencing malignancy-related anemia are prescribed an intravenous iron and/or red blood transfusions. We postulate that parameters like TAM presence in the neoplasm and TAM ferritin status should become a decisive factor in the risk evaluation of anemia-correcting medication.
Session III Innate immunity aspects

P10 Peter Delputte

Characterization of antibody-induced internalization of the macrophage-specific receptor sialoadhesin
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Sialoadhesin (Sn) is a member of the family of sialic acid-binding immunoglobulin-like lectins (Siglec) and exclusively expressed on a subset of resident tissue macrophages, but also on inflammatory monocytes and macrophages. The last decade, researchers investigated the interaction of Sn and pathogens, which may result in cellular uptake, suggesting that Sn is an internalization receptor. For most Siglecs, internalization mechanisms and pathways are characterized, for Sn however, the cytoplasmic tail does not contain a known internalization motif. Detailed research was done for porcine Sn (pSn), which showed that antibody-induced internalization of pSn occurred through clathrin-mediated endocytosis. However, for human Sn (hSn) and murine Sn (mSn) there is less evidence that internalization also occurs. Furthermore, since the amino acid sequence of the cytoplasmic tail of Sn is highly variable between species, another internalization mechanism might be possible.

With this work, we aimed to investigate hSn and mSn internalization and identify the mechanism by which antibody-induced internalization occurs. Therefore, CHO cells expressing either hSn or mSn were incubated with Sn-specific mAb at 37 °C for different times until 120 min. Cells were fixed, permeabilized and stained with secondary labeled antibodies. Internalization was analyzed by confocal fluorescence microscopy and flow cytometry. Internalization of hSn and mSn was comparable to that of pSn and reaches a maximum of internalization at 60 min. To identify the internalization mechanism, different inhibitors and dominant-negative forms of cellular components involved in endocytic mechanisms were used. These results indicate that hSn and mSn is internalized via a clathrin-mediated process.

P11 Karl Katholnig

mTORC2 regulates macrophage polarization and the cellular energy metabolism
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Macrophages are innate immune cells and pivotal for the regulation of immune and metabolic responses. A dysregulation of immune or metabolic processes contributes to many chronic diseases such as obesity, cancer, or autoimmunity. The mammalian target of rapamycin complex 2 (mTORC2) is an important kinase that regulates many basic cellular and metabolic processes; however, its function in macrophages is largely ill-defined. The aim of our project is to understand the role of mTORC2 for macrophage polarization and the associated implications on cellular and whole body metabolism. In vitro, we show that deletion of the mTORC2 component Rictor (rapamycin-insensitive companion of mTOR) in macrophages leads to a stronger inflammatory M1 phenotype and reduced M2 polarization potential. However, this inflammatory phenotype has no effect on whole body glucose metabolism, as glucose – and insulin-tolerance tests do not yield significant differences between control and macrophage-specific Rictor-KO mice. Interestingly, less macrophages accumulate in adipose tissue of macrophage-specific Rictor-KO mice on high-fat diet compared to control mice. In vitro, we find that the knockout of Rictor diminishes cell proliferation of macrophages in the presence of M-CSF, but does not influence apoptosis. Moreover, the migratory capacity of macrophages is higher if mTORC2 is intact. Molecularly, glucose influx and mitochondrial potential are reduced in Rictor-deficient macrophages. In conclusion, our results point to a role of mTORC2 in the regulation of macrophage polarization, cell cycle progression and migration that might be important in the onset of severe inflammatory diseases and suggest inhibition of mTORC2 as a possible therapeutic approach.
P12 Katarzyna Kwiatkowska

CD14-enriched rafts of the plasma membrane as sites of PI(4,5)P2 generation in LPS-stimulated cells
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Lipopolysaccharide (LPS) is the main constituent of the outer membrane of Gram-negative bacteria which activates pro-inflammatory reactions of immune cells after a sequential binding to CD14 and TLR4/MD-2 proteins. Stimulation of cells by LPS requires integrity of lipid rafts, the plasma membrane nanodomains enriched with distinct lipids, GPI-anchored proteins, including CD14, and palmitoylated proteins. The aim of these studies was to decipher the role of lipid rafts in generation of PI(4,5)P2, a plasma membrane lipid vital for TLR4 signaling. We found that 100 ng/ml LPS triggered a bi-phasic generation of PI(4,5)P2 that was dependent on the engagement of CD14. Immunoelectron microscopy analysis revealed that LPS induced rapid and transient clustering of CD14 in the plasma membrane and that aggregates of CD14 co-localized with PI(4,5)P2. The accumulation of PI(4,5)P2 at CD14 was inhibited by antibodies blocking LPS-CD14 interactions, while clustering of CD14 without LPS participation led to the increase of PI(4,5)P2 level in cells. CD14, the newly generated PI(4,5)P2 and PI(4,5)P2-kinase Iα and Iγ, which catalyze PI(4,5)P2 formation, were abundant in the detergent-resistant membrane fraction of cells enriched with rafts. The kinases co-localized with CD14 clusters in the plasma membrane and their gene silencing abolished LPS-induced PI(4,5)P2 generation and the downstream pro-inflammatory signaling. The similar effect was exerted by 2-bromopalmitic acid that, on the other hand, inhibited also the protein palmitoylation, as revealing by “click chemistry”. The studies indicate that CD14-enriched rafts can serve as platforms for PI(4,5)P2 generation in LPS-stimulated cells which is required for pro-inflammatory signaling pathways of TLR4.

P13 Julia Kzhyshkowska

High glucose differentially affects expression of TLRs in human macrophages: potential mechanism of chronic inflammation in diabetes
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Diabetics suffer from chronic inflammation. Toll-like receptor (TLR)-mediated response to exogenous and endogenous factors results in pro-inflammatory activation of macrophages. Increased TLR expression is associated with diabetic inflammation and complications. Our aim was to examine how elevated glucose levels affect expression of TLRs in M1 and M2 macrophages. CD14+ monocytes were isolated from healthy donors and cultured in serum-free medium in the presence of 5mM or 25mM glucose for 6 days. M1 and M2 differentiation was driven by IFN-γ and IL-4. Quantification of TLR mRNA was performed by RT-PCR, showing that TLR1, 2, 4, 6 and 8 are expressed in human macrophages, while TLR5 and 9 were not detectable. All TLRs were expressed in M1 on higher levels compared to M2. Increased glucose had most pronounced stimulatory effect on TLR2 expression and less on TLR6. In individual M1 cultures high glucose stimulated up to 8-fold increase of TLR2 expression and up to 28% increase of TLR6. In M2, TLR2 expression was up to 6-fold higher in high glucose conditions. High glucose had suppressive effect on TLR4 in M1 and M2. Analysis of fat tissue and blood parameters from metabolic syndrome patients revealed a negative correlation between TLR2 and ALAT and between TLR6 and HDL. TLR2 correlates positively with TNF-α. We conclude that glucose modifies expression of TLR2, TLR4 and TLR6 in a donor-specific way an can sensitise macrophages to respond to endogenous unwanted-self products. Such individual responses suggest new glucose-mediated mechanism for development of patient-specific inflammation-mediated complications in diabetes.
P14 Monika Linke

A novel Tsc2-mTORC1-CDK4 axis controls tissue homeostasis by regulating polarization, proliferation, and metabolism in macrophages.

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Maintenance of tissue homeostasis requires tight control of the in situ-proliferative capacity of tissue-resident M2-like macrophages. However, the signaling pathways that regulate quiescence versus proliferation in tissue macrophages are unknown. Here we investigated a potential role of tuberous sclerosis 2 (Tsc2), the key negative regulator of mammalian target of rapamycin complex 1 (mTORC1), for this process.

Deletion of Tsc2 in myeloid cells broke quiescence and strongly induced macrophage proliferation and granuloma formation in vivo in an mTORC1-dependent manner. Intriguingly, Tsc2-mTORC1 directly controlled cell cycle progression by activation of cyclin-dependent kinase 4 (CDK4), while inflammatory NF-κB signaling was repressed. Tsc2-deficient macrophages showed enhanced M2-like polarization that was accompanied by a reconfiguration of the cellular metabolism towards increased aerobic glycolysis and mitochondrial metabolism. Inhibition of mTORC1, CDK4, and hexokinase abrogated cell cycle progression, indicating an intrinsic need of glucose flux and CDK4 for M-CSF-dependent M2-like macrophage proliferation. Strikingly, we found that CDK4 and glucose flux directly contributed to the M2-like polarization potential of Tsc2-deficient macrophages.

In conclusion, we demonstrate that activation of an mTORC1-CDK4 axis stimulates macrophage proliferation, M2 polarization and metabolic reconfiguration, which promotes granulomatous disease in vivo. The precise molecular elucidation of how macrophages dynamically regulate proliferation versus quiescence will have fundamental implications for the understanding of tissue homeostasis and immunity.

P15 Daniel B. McKim

Repeated Social Stress Caused Long Lasting Sensitization of Splenic-Monocytes that Contributed to Recurring Anxiety

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Priming and redistribution of monocytes may contribute to the pathophysiology of recurring anxiety disorders. Previous work showed that repeated social defeat (RSD) in mice promoted sensitized behavioral and immune responses to acute stress 24 days later. For instance, acute stress 24 days after RSD was sufficient to promote the recurrence of anxiety-like behavior, and this was associated with enhanced monocyte redistribution from the spleen to the brain. We hypothesized that the spleen of RSD-exposed mice became a reservoir of primed monocytes that were released following neuroendocrine activation by acute stress. Acute stress 24 days after RSD re-established anxiety-like behavior that was associated with egress of Ly6Chi monocytes from the spleen that accumulated in blood and the brain. Moreover, splenectomy prior to RSD blocked monocyte redistribution, and this prevented the recurrence of anxiety-like behavior following acute stress 24 days after RSD. In addition, splenocytes cultured 24 days after RSD exhibited a primed or inflammatory phenotype following ex vivo stimulation. Next, treatment with a peripheral sympathetic inhibitor (guanethidine) prior to acute stress blocked monocyte redistribution and prevented the recurrence of anxiety. Additionally, BrdU-pulse chase data indicated that increased availability of releasable monocytes 24 days after RSD may be related to chronic proliferation of myeloid progenitors within the spleen. Collectively, these data show that the spleen is capable of producing and storing primed monocytes that pro-
mote exaggerated behavioral responses to acute stress, even 24 days later. Collectively, these data show that the spleen is capable of promoting persistent.

**P16 Jelena Popović**

**Control of Dendritic Cell Differentiation by SWAP-70 and DEF6 in GM-CSF Cultures**

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Expression of maturation markers like CD86 and MHCII in GM-CSF-derived dendritic cells (BMDC) is due to the heterogeneity of these cultures that consist of CD11c+ macrophages (MO) and dendritic cells (DC) and not to spontaneous maturation. We recently found that the absence of SWAP-70 increases the expression of maturation markers of BMDCs. Preliminary data show that besides SWAP-70, the only closely related protein DEF6 also controls the expression of maturation markers of BMDC. We performed a deep sequencing transcriptome analysis of single “knockout” (SKO) (Swap-70/−, Def6/−) and double “knockout” (DKO) Swap-70/− − Def6/− − BMDCs. Variation analysis of transcript levels between SKOs, DKO and wild type (wt) BMDC shows that DC and MO signature genes are up – and down-regulated, respectively, in the absence of SWAP-70 and DEF6. Accordingly, BMDCs from SKOs and DKO had a higher DC/MO population ratio (CD11c+CD11bintCD86high/CD11c+CD11bhighCD86low) than wt cultures. Our data support the notion that difference in expression of maturation markers of BMDC is due to variation in the frequency of DC and MO populations in these cultures and suggest a mechanism of control by SWAP-70 and DEF6. Progenitor cells analysis of SKOs and DKO animals shows differences in frequency and number of macrophage-DC progenitor (MDP) and MCSFR-CD135+CD115 – myeloid progenitor cells when compared to wt, while common DC progenitor (CDP) remained the same. This study highlights the importance of the role played by SWAP-70 and DEF6 in the mechanism of DC differentiation in GM-CSF cultures and both proteins represent possible targets for future therapies requiring cell-based vaccines.

**P17 Kathleen Renault**

**Microglial transcriptome diversity in the healthy adult brain reveals regional heterogeneity in immunoregulatory and metabolic function and selective sensitivity to ageing**

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An expanding array of homeostatic functions for microglia during brain development and the adult steady-state is emerging in addition to their recognized roles as key immune effectors of the CNS. Heterogeneity in structure, cytoarchitecture and function across the CNS would be expected to place heterogenous demands on microglia. A better knowledge of microglial heterogeneity is needed to understand how microglia support normal brain function and may reveal region-specific sensitivities predisposing to age-related neurodegeneration. Here we show microglial regional heterogeneity on a genome-wide scale and the underlying functional pathways of the adult steady-state microglial diversity.

Microglia were purified from distinct regions of the adult mouse brain and whole-genome expression assessed by microarray. Principal component analysis revealed region-dependent heterogeneity in microglial transcriptomes and transcriptional network analysis identified three major patterns of gene co-expression underpinning this heterogeneity. Transcriptional networks controlling bioenergetic and immunoregulatory function were the major processes driving microglial diversity. Differences in immunophenotype indicated a more immune vigilant state of cerebellar and hippocampal microglia but this phenotype was distinct from conventional states of activation. Functional differences in the ability of microglia from different brain regions to sequester bacteria and control replication correlated with the regional pattern of immune vigilance. Comparison with systemic macrophage transcriptomes showed that microglial regional diversity is superimposed upon a core profile distinguishing microglia from non-CNS macrophages. Regional differences in microglial heterogeneity are enhanced during ageing, particularly cerebellar microglia, and suggest region-dependent kinetics of microglial ageing.
P18 Bartosz Wylot

Interleukin-33 drives immune response after injury of the central nervous system

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Interleukin-33 is a novel protein enhancing Th2 cytokine production, initially described as an alarmin which is released from dying, necrotic cells to affect the immune response. However, more recent studies suggest active production of IL-33 and its release in the injury activated tissues. Nevertheless, synthesis and secretion of IL-33 as a cytokine in the injured central nervous system (CNS) has not been comprehensively elucidated so far. Therefore, the aim of the present study was to examine the potential role of IL-33 as a cytokine released in response to CNS injury. Using qPCR, Western blotting and ELISA assay we showed significant up-regulation of IL-33 mRNA and protein in two pathological models: in a response to demyelination of the spinal cord white matter and stab wound injury of the brain cortex. Using in vitro cultures of activated glial cells we examined their potential to secrete IL-33. Moreover, we demonstrated that injury leads to increase in the number of IL-33 receptor (Il1rl1)-expressing cells, both in the CNS parenchyma and in the peripheral blood. Our results suggest that interleukin-33 functions not only as an alarmin but it is actively synthesized and potentially released to directly affect response of microglia and macrophages.

This study was co-financed by the European Regional Development Fund under the Operational Programme Innovative Economy, grant POIG.01.01.01-00-109/09-01 and National Science Centre grant 2011/03/B/NZ4/02988
P19 Nicole Armbruster

Staphylococcus aureus PSM peptides modulate dendritic cell functions and increase priming of regulatory T cells

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The major human pathogen Staphylococcus aureus has very efficient strategies to subvert the human immune system. Virulence of the emerging community-associated methicillin-resistant S. aureus (CA-MRSA) depends on phenol-soluble modulin (PSM) peptide toxins, which are known to attract neutrophils by binding to the formyl peptide receptor (FPR) 2. However, their influence on other immune cells is poorly understood. Here, we analyzed the impact of PSMs on mouse dendritic cells (DCs) playing an essential role in linking innate and adaptive immunity. PSMs generally inhibit TLR-induced secretion of the proinflammatory cytokines TNF, IL-12 and IL-6 while inducing IL-10 secretion by DCs. Interestingly, the induction of tolerogenic DCs by PSMs appeared to be independent of mFPRs as shown by experiments with mice lacking mFPR2 (mFPR2−/−). TLR2 stimulation in combination with PSMα3 induces ERK, p38 and CREB phosphorylation of DCs, which is most likely responsible for IL-10 secretion. As a consequence, in vitro treatment with PSMs impaired the capacity of DCs to induce activation and proliferation of CD4+ T cells, characterized by reduced Th1 but increased frequency of IL-10 secreting FOXP3+ regulatory T cells (Tregs). Furthermore, infection of mice with the PSM-expressing S. aureus wild type strain (USA300) revealed an increase in the frequency of FOXP3+ Tregs compared to the PSM deletion mutant Δαβδ. Thus, PSMs from S. aureus affect DC functions thereby modulating the adaptive immune response and probably increase the tolerance towards the pathogen.

P20 Kristin Bieber

THE INNATE IMMUNE SYSTEM FAVORS EMERGENCY MONOPOIESIS AT THE EXPENSE OF DC DIFFERENTIATION TO CONTROL BACTERIAL INFECTIONS

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Dendritic cells (DCs) are professional antigen-presenting cells playing a crucial role in the initiation of T-cell responses to combat infection. However, systemic bacterial infection with various pathogens leads to DC depletion in humans and mice. The mechanisms of pathogen-induced DC depletion remain poorly understood. Previously, we showed that infection of mice with *Yersinia enterocolitica* impaired the *de novo* DC development, one reason for DC depletion. Here, we extended these studies to gain insights into the molecular mechanisms of DC depletion and the impact of different bacteria on DC development. We found that the number of BM hematopoietic progenitors committed to the DC lineage was reduced following systemic infection with different Gram-positive and Gram-negative bacteria. This was associated with a TLR4 – and IFN-γ signaling dependent increase of committed monocyte progenitors in the BM and mature monocytes in the spleen. Adoptive transfer experiments revealed that the infection-induced monopoiesis occurred at the expense of DC development.

Our data provide evidence for a general response of hematopoietic progenitors upon systemic bacterial infections to enhance monocyte production, thereby increasing the availability of innate immune cells to promote pathogen control, whereas DC development is impaired leading to DC depletion probably responsible for transient immunosuppression in bacterial sepsis.

**P21 Anna Ciesielska**

*Lysophosphatidic acid inhibits LPS-dependent inflammatory responses of macrophages by regulation of TLR4 signaling pathways.*

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Macrophages contribute substantially to dissemination of bacterial infections by the activation of an array of innate immune responses. Lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, triggers pro-inflammatory responses of macrophages. The compound activates Toll like receptor 4 (TLR4) and induces signaling cascades leading to the production of pro-inflammatory cytokines, like TNF-α, and type I interferons. LPS also regulates an expression of GPCR receptors which recognize lysophosphatidic (LPA), and autotaxin, an enzyme involved in the phospholipid production in blood. LPA belongs to important signaling molecules and modulates cell processes including pro-inflammatory reactions upon recognition by nine specific receptors. Recent studies indicate a cross-talk between signaling cascades of LPS and LPA. We found that LPA regulates expression and release of pro-inflammatory cytokines induced by LPS in J774 and RAW264.7 cells which have a similar LPA receptor profile, as found by RT-qPCR. In J774 cells co-stimulated with LPS and LPA the production of TNF-α was reduced by 50 % while production of RANTES was inhibited to lower extent. The inhibitory activity of LPA was connected with attenuation of LPS-dependent phosphorylation of ERK1/2 and Akt in J774 cells. Using a subtype of RAW264.7 cells with changed LPA receptor profile we show that the LPA activity toward the cells depends on the expression of distinct LPA receptors. Our data indicate that the pro-inflammatory activity of LPS is negatively regulated by LPA.

**P22 Agnieszka Ciomber**

*CD274 expression on HSCs is increased after chemo – and radiotherapy*

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Hematopoietic stem cells (HSCs) are able to regulate expression of CD47 and CD274 proteins, which are believed to have immunosuppressive function. Cell-surface CD47 interacts with SIRPα receptor on macrophages to inhibit phagocytosis. CD274 inhibits T-cell immunity by induction of apoptosis in T lymphocytes. A necessary procedure preceding allo-HSC transplantation is chemo – and radiotherapy which destroys HSCs and their microenvironment.
The aim of our study was to evaluate the impact of chemo – and radiotherapy on the phenotype of HSCs and the level of selected cytokines in bone marrow.

The study was performed using bone marrow samples obtained from 6 patients treated with allogeneic HSC transplantation. These samples were collected before chemotherapy and at the day of transplantation. The frequency of CD47+ and CD274+ HSCs was evaluated by flow cytometry. Concentrations of IFN-gamma, IL-2, IL-10, IL-6, IL-17A and LAP-1 (latent form of TGF-beta) were measured by ELISA test. The statistical significance of differences between the groups was evaluated by Wilcoxon test.

Our results indicate increased percentage of CD274+ HSCs (from 0.4% to 9.6%, p=0.04) and the level of IFN-gamma (from 34 pg/ml to 1023 pg/ml, p=0.02) at the day of transplantation vs before chemotherapy. The changes in the percentage of CD47+ HSCs and in the concentration of IL-2, IL-10, IL-6, IL-17A and LAP-1 were not statistically significant.

We assume that high level of IFN-gamma proves proinflammatory properties of bone marrow microenvironment after chemotherapy. It results in the increased expression of CD274 on HSCs, what may protect them against destruction.

The study was supported by Grant no 2011/03/B/NZ6/04917

P23 Anna Dembek

Role of Kupffer cells in hepatic lipid accumulation during endotoxin tolerance

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Steatosis is associated with excessive accumulation of lipids in the liver and may progress to cirrhosis and hepatocellular carcinoma. Gut-derived bacterial endotoxins have been previously suggested to contribute to the pathogenesis of steatosis by activating Kupffer cells (KC), the resident macrophages of the liver. Exposure of macrophages to low doses of endotoxin causes hyporesponsiveness upon subsequent endotoxin challenge, a phenomenon termed endotoxin tolerance (ET).

In the present study we aimed to examine whether LPS-induced lipid accumulation is influenced by ET.

C57/BL6 mice were treated with repeated i.p. injections of low-dose LPS followed by treatment with high-dose LPS. LPS pretreatment resulted in reduced expression of proinflammatory mediators, such as TNF-α and IL-1β upon high dose LPS treatment in liver tissues. In contrast, total lipid analysis indicated that LPS-induced lipid accumulation was not affected by ET. Accordingly, the LPS-induced hepatic lipid content was comparable in a genetic mouse model, in which ET is suppressed.

IL-6 has been reported to induce lipid accumulation in the liver. Interestingly, a tolerance response could neither be observed in liver tissues of LPS-tolerized mice nor in primary human macrophages in terms of IL-6 expression. Both LPS-mediated induction of IL-6 in murine liver tissue and increased hepatic lipid levels were dependent on the presence of KC, as indicated by a macrophage depletion model employing clodronate liposomes.

In summary, our data suggest that KC-derived IL-6 drives hepatic lipid accumulation without being affected by ET.

P24 Stefanie Dichtl

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

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

Methods: We studied the effects of the catecholamine dopamine on the regulation of iron in bone marrow-derived macrophages obtained from C57Bl/6 mice and littermates knocked out for lipocalin-2, a mammalian siderophore binding pep-
tide. The *in vivo* effects of dopamine were studied in wild-type mice infected with the Gram-negative bacteria *Salmonella typhimurium* (S.tm.).

**Results:** Administration of dopamine to macrophages resulted in a dose dependent increase of hemeoxygenase-1 and ferroportin expression, the latter being the major cellular iron exporter, which subsequently resulted in reduced intramacrophage iron concentrations. This effect could also be reproduced upon infection of macrophages with S.tm and was independent from the presence/absence of the siderophore binding peptide lipocalin-2. The *in vivo* administration of dopamine to mice infected with S.tm resulted in an increased bacterial burden in liver and spleen as compared to mice receiving solvent. This is linked to an increased delivery of iron to bacteria in the presence of dopamine along with an impaired pro-inflammatory immune response of macrophages.

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

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**P25 Michael Fichter**

**Polymeric nanocapsules composed of hepatitis C virus non-structural protein 5A induce intrahepatic antigen-specific immune responses**

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Nanocarrier-based antigen delivery is a promising vaccination approach against pathogens lacking effective immunization strategies, e.g. the Hepatitis C Virus (HCV). Formulating polymeric nanocapsules (NCs) out of viral antigens in combination with vaccine adjuvants enables efficient targeting and maturation of dendritic cells (DCs), essential prerequisites for the induction of vigorous cellular immune responses. Aim of the present study was the synthesis of polymeric protein nanocapsules composed exclusively of HCV non-structural protein 5A (NS5A), and the evaluation of their potential to induce intrahepatic cellular immune responses.

NS5A-based nanocapsules were synthesized by the inverse miniemulsion technique and further functionalized by adsorption of monophosphoryl lipid A (MPLA) onto the capsule surface. Uptake of NCs by DCs was investigated by flow cytometry and confocal microscopy, cytokine response by ELISA. The potential of NS5A-NCs to induce intrahepatic cellular immune responses was determined by immunization of mice via intravenous injections followed by in vitro stimulation of splenic and hepatic lymphocytes with NS5A.

The inverse miniemulsion approach led to polymeric nanocapsules with a spherical morphology that were efficiently ingested by DCs. Adjuvantation with MPLA induced massive pro-inflammatory cytokine responses. Immunization with the latter NS5A nanocapsule formulation generated robust intrahepatic antigen-specific immune responses.

In conclusion, antigen-based polymeric nanocapsules are a promising delivery system for targeting antigens and vaccine adjuvants to dendritic cells promoting Th1-type immune responses. This novel vaccination strategy avoids the use of structural compounds, increases the antigen load of DCs and bears the potential to overcome tolerance and to induce vigorous antigen-specific cellular immunity.
P26 Nina Hachenthal

The natural compound curcumin induces the glucocorticoid induced leucine zipper (GILZ) via the mRNA binding protein HuR in macrophages

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GILZ is inducible by glucocorticoids and plays a key role in their mode of action. GILZ attenuates inflammation mainly by inhibition of nuclear factor κB (NF-κB) and MAP kinase activation. Interestingly, GILZ seems to be not involved in the severe side effects observed after glucocorticoid treatment. Therefore, GILZ might be a promising target for new therapeutic approaches. The present work focuses on the natural product curcumin, which has previously been reported to inhibit NF-κB. Our data employing siRNA in LPS-activated RAW264.7 cells show that curcumin facilitates this anti-inflammatory action by induction of GILZ in macrophages. Experiments in LPS-activated bone marrow-derived macrophages (BMDM) from wildtype and GILZ knockout mice demonstrate that curcumin inhibits the activity of inflammatory regulators like extracellular-signal-regulated kinases (ERK) and subsequent tumor necrosis factor alpha (TNF-α) production via GILZ. The upregulation of GILZ protein by curcumin was neither associated with glucocorticoid receptor activation nor transcriptional induction, mRNA – or protein-stabilization. Since GILZ 3'-UTR contains AU-rich elements (AREs), we analyzed the role of the mRNA binding protein (mRNA-BP) HuR, which has been shown to promote the translation of ARE-containing mRNAs. Our results suggest that curcumin treatment initiates shuttling of HuR from the nucleus to the cytoplasm. An RNA-immunoprecipitation assay confirmed that HuR can bind GILZ mRNA. Moreover, luciferase reporter assays indicated that HuR interacts with the GILZ 3' UTR. Taken together, our data indicate that HuR-dependent GILZ induction contributes to the anti-inflammatory properties of curcumin.

P27 David Haschka

LAMTOR2 as a critical regulator of macrophage host resistance to Salmonella infection

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Background: LAMTOR2/p14 is part of a multi protein complex localized on late endosomes, where it integrates ERK and mTOR complex 1 signaling. ERK activation takes place in response to bacterial infections and is involved in the induction of oxidative and nitrosative stress as major antimicrobial immune defense mechanisms. mTOR1 activity was linked to the expression of transferrin receptor 1 (TfR1) which is central for iron acquisition by cells. As both, iron availability and ERK signaling play decisive roles for bacterial infection we studied the role LAMTOR2 for host resistance of macrophages infected with the intracellular bacterium Salmonella Typhimurium (S.tm.).

Methods: We used LysM-Cre and LAMTOR2-flox mice on a C57Bl/6 background to generate mice with a myeloid cell line specific deletion of LAMTOR2. Primary bone marrow-derived macrophages (BMDM) were used for in vitro infection with S.tm.

Results: We found that BMDM of ControlLysCre(+) animals control infection with S.tm. significantly better than LAMTOR2LysCre(+) BMDM. LAMTOR2 deletion results in impaired endosomal transport of bacteria as well as in diminished activation of early and late antimicrobial effector systems. When investigating activation pathways for LAMTOR2 induced host resistance we found TfR1 protein levels significantly decreased in LAMTOR2LysCre(+) BMDM, which translated into an impaired control of intracellular iron trafficking in Salmonella infected LAMTOR2LysCre(+) BMDM.

Conclusion: LAMTOR2 confers resistance of macrophages against infection with S.tm. by promoting the transport of pathogens through the endolysosomal system, by ameliorating anti-microbial effector pathways and by limiting the availability of the essential nutrient iron to bacteria.
A comparative study of respiratory syncytial virus (RSV) infection of different murine macrophage cell lines reveals differences in susceptibility.

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RSV is responsible for severe bronchiolitis in children and elderly and is linked with chronic pulmonary problems. Macrophages are prominent cells of the lung immune system and appear to be permissive for RSV, but published results are often contradictory. A viable explanation is that diverse types of macrophages cell lines were used. The aim of this study is to evaluate the susceptibility of different macrophage cell lines for RSV.

The cell lines MH-S, RAW 264.7 and J774 were infected with the RSV strain A2 and fixed at 2 and 24h p.i. Cells were permeabilized and RSV antigens were visualized with a polyclonal anti-RSV serum and an AF488-labelled conjugate. Both MH-S (2%) and RAW 264.7 (0.4%) showed staining of RSV-antigens in the cytoplasm 24h p.i. This staining was more intense compared to the staining at 2h p.i., indicating that new RSV-antigens were synthesized. J774 cells showed no positive signal of RSV-antigens.

In contrast to MH-S cells, RAW 264.7 cells expressed no RSV-antigens on the surface. This suggests an abortive RSV-infection in RAW 264.7 cells. This notion was explored by inoculating HEp-2 cells with supernatants of infected cells, collected 24 and 72h p.i. The percentage of infected HEp-2 cells increased from 1.5 to 5% when inoculated with supernatants of MH-S from 24 or 72h p.i. This in contrast to HEp-2 cells inoculated with supernatants of RAW 264.7 cells, where the percentage of infected cells varied between 1.4 and 1.2%. In conclusion, the RSV-infection with the A2 strain varies among macrophage cell lines.

TLR2 induction in human alveolar macrophages: an anti-inflammatory principle shared by lipopolysaccharide and glucocorticoids?

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Alveolar macrophages (AMs) play a key role in pulmonary innate immunity and recognize microbial ligands via Toll-like receptors (TLRs). We aimed to examine the influence of prolonged lipopolysaccharide (LPS) exposure on TLR expression in primary human AMs.

Among all TLRs tested, only TLR2 was highly upregulated by LPS treatment for 24 hours on both mRNA and protein level. Microarray and qPCR analysis suggested that TLR2 upregulation is facilitated by mRNA stabilization due to downregulation of the TLR2-targeting microRNA miR-19.

TLR2 induction was paralleled by LPS tolerance and upregulation of genes associated with resolving inflammation, such as IL-10 or FPR2. Analysis of publicly available data sets (GSE4607, GSE8121) indicated that TLR2 was also induced in the immunosuppressive phase of sepsis or SIRS in human whole blood samples.

Both endogenous and exogenous glucocorticoids might influence TLR2 levels in sepsis and SIRS since the TLR2 promoter contains glucocorticoid-responsive elements. Thus, we examined TLR2 expression in AMs after dexamethasone treatment. Dexamethasone induced TLR2 in a glucocorticoid receptor (GR)-dependent manner and synergistically enhanced GR-independent LPS-mediated TLR2 upregulation.

TLR2 was not functional after its induction by LPS and/or dexamethasone, since the recognition of TLR2 ligands was highly impaired. Therefore, we speculated that upregulated membrane-bound TLR2 might serve as a precursor for soluble TLR2 (sTLR2). Supernatants of LPS – or dexamethasone-primed AMs indeed contained sTLR2, as indicated by Western Blot analysis.
In summary, our data suggest that AMs induce TLR2 under anti-inflammatory conditions. TLR2 might be further processed to the decoy receptor sTLR2, thereby contributing to immunosuppression.

P30 Katarzyna Nazimek

**Macrophages releasing secondary exosomes are required for suppression of contact sensitivity T effector cells by T CD8+ cell-derived exosomes.**

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**INTRODUCTION.** Murine contact sensitivity (CS) reaction could be suppressed by T CD8+ cell-derived exosomes acting through antigen-presenting macrophages (Mf). Present studies were aimed to determine the exact role of Mf in exosome-mediated immune suppression.

**METHODOLOGY.** Peritoneal Mf pulsed with T suppressor (Ts) cell exosomes were cultured in standard conditions for 48 hours. Culture supernatant was collected, filtered (down to 0.22 micrometer), ultracentrifuged at 100,000g for 70 minutes, and resulting pellets were tested in adoptive transfer of isolated CS effector T cells. In some instances, putative pelleted exosomes were pre-incubated with monoclonal antibodies anti-trinitrophenyl (TNP) hapten and anti-MHC class II. Further, CS effector cells depleted of Mf by clodronate liposomes (then in some cases supplemented with Mf) were incubated with Ts cell exosomes prior to adoptive transfer. CS reaction was measured as ear swelling after TNP-chloride challenge following the transfer.

**RESULTS AND CONCLUSION.** The results showed that secondary exosomes released by Ts cell-exosome-pulsed Mf were able to suppress adoptively transferred T effector lymphocytes and that this inhibitory effect was blocked by pre-incubation of pelleted vesicles with antibodies against TNP and MHC class II. Further, CS effector cells depleted of Mf by clodronate liposomes (then in some cases supplemented with Mf) were incubated with Ts cell exosomes prior to adoptive transfer. CS reaction was measured as ear swelling after TNP-chloride challenge following the transfer.

Our observations confirmed the requirement of Mf presence for effective suppression of CS allergic immune response by Ts cell exosomes, suggesting that this effect is due to Mf release of inhibitory secondary exosomes expressing hapten complexed with MHC class II. Studies supported by NCN Grant No. 2013/09/N/NZ6/00753.

P31 Stephanie Obermeyer

**Antimicrobial function and intracellular localization of inducible nitric oxide synthase in Leishmania infections**

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*Leishmania* are protozoan parasites whose iron-dependent survival within phagosomes is directly or indirectly counteracted by inducible nitric oxide (NO) synthase (NOS2). NOS2, which converts L-arginine into L-citrulline and NO, is expressed in phagocytes upon stimulation with cytokines (e.g. IFN-γ) or microbial ligands (e.g. LPS). Previously, NOS2 was detected in the cytosol, attached to the submembranous cytoskeleton and in vesicles provisionally termed “nitroxosomes”.

In the present study we analyzed whether NOS2-derived NO modulates the availability of intracellular iron by regulating the iron-exporter ferroportin-1 (Fpn-1) and where in the infected host cell NOS2 is located. As expected, stimulation of *Leishmania*-infected bone marrow-derived macrophages (BMM) by IFN-γ plus LPS or TNF induced high NO levels which killed most of the intracellular parasites within 72h. Addition of FeCl₃ or FeSO₄ 10h after stimulation was able to reverse the...
parasite killing. The rescuing effect of Fe$^{2+}$/Fe$^{3+}$ was also observed when killing was elicited by an exogenous NO donor added to NOS2-deficient BMM. However, in both uninfected and infected BMM NOS2-derived NO failed to upregulate Fpn-1 mRNA and protein. Multicolour confocal laser scanning fluorescence microscopy revealed that NOS2 co-localized with the early endosomal antigen EEA1, but not with typical phagosomal markers.

Together, our data indicate that iron withdrawal could be one mechanism of NOS2-dependent *Leishmania* control. Further experiments are required to elucidate the role of iron in the NOS2-dependent killing of *Leishmania* and the exact localization of NOS2.

P32 Katrin Paduch

**Control of *Leishmania* major infections by TNF is mediated by restriction of arginase 1 expression in myeloid cells**

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Effective control of the intracellular parasite *Leishmania major* is mediated by a Th1 immune response and the expression of inducible nitric oxide (NO) synthase (NOS2). NOS2, which metabolizes L-arginine into citrulline and antimicrobial NO, can be antagonized by host cell-derived arginase 1 (Arg1), which converts L-arginine into urea and ornithine, a precursor for polyamine synthesis. Tumor necrosis factor (TNF)-deficient mice succumb to *L. major* infections despite a sustained Th1 response and NOS2 protein expression. Here, we investigated whether expression of Arg1 contributes to the non-healing course of infection in TNF-deficient mice.

In vitro, TNF suppressed the IL-4-induced expression of Arg1 in both bone marrow-derived macrophages (BMM) and dendritic cells. *L. major*-infected C57BL/6 TNF-deficient mice expressed significantly more Arg1 in skin lesions and draining lymph nodes compared to wild-type controls. Arg1 and NOS2 were found to be coexpressed in the same cell indicating that both enzymes can directly compete for each other. In situ analysis revealed a significant reduction of NOS2 activity in TNF$^{-/-}$ mice. Hyperexpression of Arg1 and impairment of NO-production was also observed in *L. major*-susceptible BALB/c mice. The functional relevance of Arg1 hyperexpression was investigated in BALB/c mice lacking Arg1 in hematopoetic and endothelial cells (Tie2Cre Arg1$^{1/2}$). Tie2Cre Arg1$^{1/2}$ BALB/c mice showed an unaltered T cell response, but an increased NO-production in infected tissues leading to successful parasite control. Thus, TNF is an important negative regulator of Arg1, an enzyme which otherwise contributes to non-healing *L. major* infections by counteracting the activity of NOS2.

P33 Anette Pietrzak-Nguyen

**Evaluation of an optimized HBV nanocapsule vaccine formulation for neonates**

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Introduction: 350 million people are chronically infected with the Hepatitis B Virus. One common route of HBV infection is the mother-to-child transmission. 90% of infected neonates develop chronic hepatitis. The lack of efficient treatment underlines the need of novel approaches. Aim of the present study was the synthesis of polymeric HBsAg-nanocapsules, the
evaluation of the overall uptake by human monocyte derived dendritic cells (moDCs) and their potential to induce T cell responses in a human co-culture model.

Methods: HBsAg-nanocapsules (NCs) were synthesized by the inverse miniemulsion technique and were further functionalized by the adsorption of monophosphoryl lipid A (MPLA) onto the capsule surface as an adjuvant. In vitro generated human moDCs from cord and adult blood were stimulated with NCs and subsequently co-cultured with autologous T cells. Up-take of NCs by moDCs was investigated by flow cytometry, cytokine secretion by CBA and ELISA, and T cell proliferation was determined by 3H-thymidine incorporation assay.

Results: HBsAg-nanocapsules were efficiently ingested by human moDCs. NCs pulsed moDCs increased T cell proliferation and induced massive pro-inflammatory responses with respect to cytokine secretion levels in a dose depended manner. The response was more prominent after additional MPLA-coating of HBsAg-nanocapsules and IFNg stimulation.

Conclusion: DCs play an important role in antiviral immunity and have the capability to activate naïve T cells. The simultaneous delivery of MPLA and IFNg with HBsAg-nanocapsules induces a vigorous cellular immunity and inherits the potential to optimize the preventive and therapeutic properties of current vaccines.

Funding: Deutsche Forschungsgesellschaft (DFG) grant DFG GE1193-2/1

P34 Valentin Schatz

Cutaneous Na+ Storage Strengthens the Antimicrobial Barrier Function of the Skin and Boosts Macrophage-Driven Host Defense

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Immune cells regulate a hypertonic microenvironment in the skin; however, the biological advantage of increased skin Na+ concentrations is unknown. We found that Na+ accumulated at the site of bacterial skin infections in humans and in mice. We used the protozoan parasite Leishmania major as a model of skin-prone macrophage infection to test the hypothesis that skin-Na+ storage facilitates antimicrobial host defense. Activation of macrophages in the presence of high NaCl concentrations modified epigenetic markers and enhanced p38 mitogen-activated protein kinase (p38/MAPK)-dependent nuclear factor of activated T cells 5 (NFAT5) activation. This high-salt response resulted in elevated type-2 nitric oxide synthase (Nos2)-dependent NO production and improved Leishmania major control. Finally, we found that increasing Na+ content in the skin by a high-salt diet boosted activation of macrophages in a Nfat5-dependent manner and promoted cutaneous antimicrobial defense. We suggest that the hypertonic microenvironment could serve as a barrier to infection.
P35 Balachandar Selvakumar

Functional role of bone marrow derived M2-polarized macrophages in influenza virus-induced acute lung injury

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Resident and bone marrow derived exudate macrophages (BM-MØ) play an important role in host defense and tissue homeostasis. Even though macrophage polarization has been extensively studied in different disease models, their lineage relation and functional profiles during pathogen induced acute lung injury (ALI) and resolution has not been convincingly elucidated. During influenza virus (IV) induced ALI, alveolar and interstitial BM-MØ showed an M1 phenotype in the acute phase and shifted to an M2 phenotype in the late phase of infection. Bone marrow transplantation experiments using CD45.2/1 (donor/recipient) mice showed that the M2 polarized BM-MØ later restore the resident alveolar macrophage (rAM) pool, suggesting high functional plasticity after infection. Additionally, adoptive transfer of M2 BM-MØ from IV-challenged wild type mice into the lungs of IV-infected CCR2-/- mice (lacking BM-MØ recruitment), showed that transferred M1 BM-MØ increased alveolar barrier dysfunction whereas M2 BM-MØ preserved the rAM pool and induced alveolar epithelial cell proliferation and barrier repair. Further, our genome-wide transcriptome analysis on flow-sorted M1/M2 BM-MØ showed up-regulation of several growth factors, repair mediators and pro-survival genes in M2 BM-MØ when compared with M1 BM-MØ. In addition, differentiation and proliferation pathways are found to be highly induced in M2 BM-MØ. These data support that mediators produced by M2 BM-MØ contribute to preserving the rAM pool and improving lung barrier function. In summary, our data demonstrate high functional plasticity of BM-MØ during IV pneumonia and highlight these cells as targets for therapeutic (re-) programming.

P36 Amanda N. Sparkes

Molecular Imaging with Liver Macrophage-specific Nanobodies for Non-invasive Monitoring of Liver Pathogenesis in Mouse Models of Autoimmune Hepatitis and NASH

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The liver is an organ playing a critical role in metabolic homeostasis, toxin clearance and immunogenic/tolerogenic immune responses. These functions however predispose the liver to a variety of diseases ranging from toxin induced damage to metabolic disorders and autoimmunecom in aspiration. In this context, Kupffer cells (KCs), the liver resident macrophages, are important mediators of tissue homeostasis and pathogen clearance but interestingly have been involved in divergent hepatoprotective or – destructive immune responses. The plasticity and versatility of KCs in response to environmental triggers, in combination with the biomarkers they express, make these macrophages attractive in vivo targets for non-invasive monitoring of liver inflammation/pathogenesis. Herein, we investigated whether KCs can be monitored non-invasively using single-photon-emission computed tomography (SPECT) with 99mTc-labeled Nanobodies® (Nbs). Nbs targeting V-set and immunoglobulin domain-containing 4 (Vsig4) and C-type lectin 4F (Clec4F) were found to accumulate only in the liver of homeostatic mice. Ex-vivo flow cytometry analysis confirmed that both Nbs specifically targeted KCs but no other cell types in or outside the liver. Upon induction of acute hepatitis using concanavalin A (ConA), down-regulation of the in vivo imaging signal using the Nbs was observed, reflecting reduction in KC numbers in FACS. In contrast, induction of non-alcoholic steatohepatitis (NASH) using the methionine choline deficient diet, resulted in higher signals...
in the liver corresponding to higher density of KCs. Interestingly, the Nb signals returned to normal levels after recovery, i.e. switching to normal diet. In conclusion, Nbs targeting KCs as molecular imaging biomarker could allow non-invasive monitoring of liver pathogenesis.

P37 Juan Tur

The mitochondrial protein MFN2 is critical for macrophage activation

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Mitochondria are known for their role as bioenergetic and biosynthetic organelles. Recently, they also have emerged as one of the main regulators of innate immune responses, mostly for its ability to modulate several signaling pathways through mechanisms such ROS production. Mitofusin-2 (MFN2) is a GTPase located in the external mitochondrial and ER membranes. It's responsible for the fusion between mitochondria and the ER-mitochondria contacts, both necessary for the correct functioning of both organelles. Even that, the role of MFN2 in the signaling of innate immune responses is still unknown.

We report that MFN2 is crucial to macrophages for proper inflammatory and immune responses. Murine MFN2−/− macrophages show a dramatic decrease in the production of ROS and in triggering ER stress responses during activation. We found that both processes severely compromise the ability of these MFN2−/− cells to produce pro-inflammatory cytokines and nitric oxide, through decreasing the activation of ERK and p38. The ability of these macrophages to phagocytose, either bacteria or apoptotic cells, was also impaired, as well as its ability to process phagocytosed proteins and present them to T-CD4+ cells. Surprisingly, other mitochondrial functions such ATP production were not affected, demonstrating a specific role for MFN2 in immune responses. Finally we show the biological relevance of MFN2 by demonstrating how MFN2−/− mice have an increased susceptibility to tuberculosis infection.

These findings suggest that MFN2 plays an essential role in modulating macrophage activation, phagocytosis and antigen presentation, being crucial for the proper development of immune responses.

P38 Annabel F. Valledor

Deciphering new roles of Liver X receptors (LXRs) in pathogen-host macrophage interaction

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Macrophages exert potent effector functions against invading microorganisms but constitute, paradoxically, a preferential niche for many bacterial strains to replicate. Several pathogenic microorganisms have, indeed, developed strategies to actively invade host cells and evade microbial digestion within the host cell endosomal system. Nuclear receptors constitute a superfamily of transcription factors with diverse functions in the regulation of development and physiology. Liver X receptors (LXRs) are members of the nuclear receptor superfamily that act as sterol sensors to subsequently regulate the expression of genes involved in lipid homeostasis. In the last few years, increasing evidence indicates that LXRs also play important roles in the regulation of immune responses. In a model of infection by Salmonella Typhimurium, activators of the nuclear receptors LXRs interfered with pathogen-induced changes in macrophage morphology and in distribution of the F-actin cytoskeleton, and reduced the capability of non-opsonized Salmonella to infect macrophages. In line with these effects, LXR-deficient mice displayed more accelerated mortality during infection by Salmonella Typhimurium than their wild-type counterparts. Mechanisms transcriptionally activated by the LXR pathway that contribute to these effects will be discussed.
SESSION V Microenvironment/ Mononuclear cells associated with blood vessels

P39 Larissa Dyugovskaya

Differential Effects of Intermittent and Sustained Hypoxia on Human Blood Monocyte Differentiation

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Blood monocytes are versatile cells expressing distinct morpho-functional programs in response to environmental signals. Two dominant functionally heterogeneous morphotypes (round – and spindle-shaped) were spontaneously differentiated and co-exist in monocytes maintained in culture for 7-10 days. Herein we studied the effects of intermittent and sustained hypoxia (IH; SH) on monocyte differentiation. Each IH cycle lasted 25 min. The lowest \(O_2\) in the medium (5%) was achieved after 15 min and then reached 20% after the next 10 min of incubation. Monocytes were exposed to chronic IH (20 cycles/day for 9 consecutive days) or to acute IH during the last 24 hr of culture (56 cycles). Cells were studied by light and confocal microscopy. The changes in gene expression upon hypoxia treatments were evaluated by PCR. Both chronic and acute IH induced spindle cells formation, whereas SH for the same durations triggered predominantly round larger macrophages. Most subjects developed few spindle-shaped cells in normoxia, and intensive formation was evident under IH. Importantly, IH-induced monocyte transformation into spindle-like cells was strongly associated with 7.8-fold increased VEGF gene expression over normoxia. However, two small sub-groups of subjects exhibited high – or low – spindle development which was correlated with VEGF gene expression regardless of the oxygen conditions applied. Our findings may support a role for IH in angiogenesis and might be considered as new modalities for its regulation. Yet, the development of spindle-shaped cells which was an individual trait may provide vascular protection, by promoting neovascularization at ischemic sites, or might have unfavorable prognosis in oncogenesis.

P40 Alexandru Gudima

Primary macrophages demonstrate donor specific inflammatory reactions to polylactic acid-based implant surface coatings

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Macrophages are the cells responsible for the initiation of the foreign body response and therefore regulate the immune reaction to implant materials. A promising biomaterial is Polylactic acid (PLA) which provides high biocompatibility, processibility and good mechanical properties. PLA is a synthetic biodegradable polymer used in manufacturing of implants and coatings such as resorbable sutures, clips, plates and screws and in drug delivery devices. However, degradation of PLA in the body into lactic acid can cause chronic inflammation and implant intolerance. To address this issue, surface modifications of PLA-based biomaterials may be used. Macrophages regulate the inflammatory reaction to implants, therefore their response to unmodified and modified PLA-based materials was analysed. We found that human primary monocyte-derived macrophages react in a donor-specific way to PLA samples modified with Brilliant Green dye (BG1, BG2, BG3) and that
these modifications had a stimulatory effect on the production of TNFα and CCL18 released by both M1 and M2. Additionally we studied the expression of macrophage mannose receptor CD206 and stabilin-1 to analyse whether modified or unmodified PLA samples can induce an M2 tolerogenic phenotype in monocytes. We found that unmodified PLA samples increase the expression of both markers, while the modified PLA modulate their expression in a donor specific way. Our results show that the inflammatory responses of macrophages can be changed by modification of PLA material surface. A monocyte-based in-vitro system for testing individual responses to the implanted material was established for selecting personalized implant variant.

**Funding:** IMMODGEL project, Grant No (602694)
SESSION VI Metabolic aspects /Heme oxygenase-1: from hematopoietic stem cells to macrophages

P41 Urszula Florczyk

Nrf2 transcription factor and heme oxygenase-1 affects osteoclasts differentiation

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Acting as strong cytoprotectants and antioxidants Nrf2 transcription factor and heme oxygenase-1 (HO-1), its down-stream target, may influence osteoclasts (OCL) formation but it needs to be verified using genetic models.

To induce macrophage and osteoclasts differentiation, bone marrow (BM) cells from Nrf2–/– and HO-1–/– mice were cultured with, respectively, M-CSF (day 0 – > day 6) and RANKL (day 3-> day 6). Macrophage phenotype at day 3 was confirmed irrespective of the genotype. Nrf2 deficiency resulted in higher number of OCLs assessed by TRAP enzyme activity at day 6. Reversely, after Nrf2 activation by sulforaphane in Nrf2+/+ cells no TRAP-positive signal was observed. In addition, osteoclasts-specific genes, such as cathepsin K and integrin β3, were upregulated in Nrf2–/– BM cells (vs. Nrf2+/+). In contrast, the lack of HO-1 diminished the number of TRAP+ cells and expression of OCL markers. Confirming that observed effects are not related to the effect of Nrf2/HO-1 on macrophage abundance, stimulation of Nrf2-deficient BM macrophages (BMMs) with RANKL led to a high increase in osteoclastogenesis, while BMMs HO-1–/– formed less TRAP+ cells.

In RAW264.7 cells, both pharmacological induction of HO-1 by CoPPIX or hemin and treatment with siRNA against HO-1 inhibited NFATc-1 level. However, CoPP IX prestimulation and additional siRNA HO-1 transfection tended to reverse this effect – enhanced the expression of NFATc-1.

In summary, Nrf2 deficiency exerts stimulatory effect on osteoclastogenesis, while HO-1 effect seems to be more complex and presumably its specific basal level is required for proper process of osteoclastogenesis.

Supported by Iuventus Plus grant from the Ministry of Science and Higher Education (0244/IP1/2013/72)

P42 Laura Matuschik

CD163 expression in human differentially activated macrophages is affected by diabetic conditions

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Scavenging function of monocytes and macrophages is essential for the control of inflammatory reactions. The monocyte – and macrophage-specific scavenger receptor CD163 internalizes and degrades haemoglobin-haptoglobin complexes built due to intravascular haemolysis. This circumstance particularly occurs in inflammation, supporting the development of microvascular diabetic complications. The shedding of CD163 from the cell surface in inflammation leads to an impaired scavenging function and consequently increases the risk of vascular oxidative damage. We examined how high glucose conditions affect the CD163 expression in human primary differentially activated macrophages. CD14+ monocytes were isolated from the peripheral blood of healthy donors by density gradient centrifugation and magnetic separation. M0 (non-stimulated), M1 (IFNγ-stimulated) and M2 (IL-4-stimulated) macrophages were differentiated within 6 days in low (5mM) and high (25mM) glucose conditions. CD163-mRNA expression was quantified by qRT-PCR. CD163 surface
expression was analysed by flow cytometry. Increased glucose had a suppressing effect on CD163 mRNA expression in 8 out of 10 donors in M1; and in 7 out of 10 donors in M2. The suppressive effect of glucose was more pronounced in M2 compared to M1. Regarding surface expression, the effect of high glucose was donor-dependent in M0 and M2, whereas the expression of CD163 uniformly decreased in M1. Our data suggest that elevated glucose levels affect both transcriptional and posttranscriptional mechanisms of CD163 production in a donor-specific manner. We concluded that the suppression of CD163 expression in macrophages by high glucose can contribute to the development of inflammation-mediated complications in diabetic patients.

P43 Kondaiah Moganti

Primary human macrophages cytokine responses in diabetic conditions

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Hyperglycaemia is a factor for the induction and progression of micro- and microvascular diabetic complications. Both classically (M1) and alternatively (M2) activated macrophages implicated in progression of diabetic complications. However, the effect of hyperglycaemia on differentiation and functional programming of macrophages is poorly understood. We established unique model system based on primary human monocyte-derived macrophages to examine the effects of hyperglycaemia on M0, M1 and M2. CD14+ monocytes, isolated from buffy coats, were cultivated in the presence of 5mM and 25mM glucose for 6 days under stimulation with IFNgamma (M1), IL-4(M2) and without cytokine stimulation (M0). In order to identify the effect high glucose on macrophages cytokines were selected as key inflammatory regulators. Using RT-PCR and ELISA the expression and release of TNF-alpha and IL-1beta (M1 cytokines) and IL1ra and CCL18 (M2 cytokine) were quantified. RT-PCR analysis revealed that high glucose induced mRNA of TNF-alpha, IL-1beta, and IL1Ra only in part of donors. However, in all analysed donors the increased secretion of all three cytokine release was demonstrated by ELISA. RT-PCR showed that high glucose suppressed the M2 marker CCL18 mRNA levels, and this corresponded to the ELISA-detected suppression of CCL18 release. Our data indicated that increase glucose stimulates release of TNF-alpha, IL-1beta and IL1Ra and suppresses CCL18 in a donor-specific way. High glucose can stimulate release of TNF-alpha, IL-1beta and IL1Ra independently on transcriptional activation. Our data suggest that individual profile of vascular complications can be caused by patient-specific cytokine profile induced by elevated glucose levels.
P44 Calum C Bain

Exploring the Regenerative Capacity of Tissue Macrophages
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Until recently, tissue macrophages were thought to require constant replenishment from blood monocytes. Although this is still the case for macrophages in the gut wall, dermis and cardiac tissue, most other tissue macrophages appear to derive from embryonic precursors that seed tissue during development and subsequently maintain themselves through in situ self-renewal. In addition, multiple tissue macrophages have been shown to proliferate during and following an inflammatory insult to sustain their numbers. However, whether macrophages can self-renew indefinitely, or if they eventually become exhausted is poorly understood. Similarly, whether all tissue macrophages possess identical ability to proliferate, or if a regenerative hierarchy exists within the macrophage compartment remains enigmatic. To begin to address these alternatives, we have used a mouse strain that allows ubiquitous, doxycycline-inducible expression of a histone 2B (H2B)-GFP fusion protein to track the proliferative history of macrophages. Our preliminary data suggest that macrophage proliferation wanes with age and that there may be heterogeneity within the macrophage compartment in terms proliferative ability, with fast and slow proliferating cells amongst the peritoneal macrophage compartment. Future studies will investigate the mechanisms underlying the differential ability of these cells to proliferate and test the regenerative capacity of each of these cells.

P45 Annalisa Del Prete

CCRL2 regulates M1/M2 polarization during EAE recovery phase
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CCRL2 belongs to the family of atypical chemotactic receptors, seven-transmembrane domain proteins devoid of chemotactic activity and involved in the control of inflammation. Experimental Autoimmune Encephalitis (EAE) is an autoimmune disorder which recapitulates the inflammatory aspects of multiple sclerosis. CCRL2-deficient mice developed exacerbated, non-resolving disease with protracted inflammatory response and increased demyelination. The increased severity was associated with higher levels of microglia/macrophages activation markers and imbalanced M1/M2 polarization. Thus CCRL2 is involved in the downregulation of CNS-associated EAE inflammation in the recovery phase of EAE. Therefore CCRL2 should be considered as a new potential molecule involved in multiple sclerosis.
P46 Izabela Glowczyk

Role of virulence factors from Porphyromonas gingivalis in IL-6 signaling regulation in monocyte derived dendritic cells.

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Chronic periodontitis (CP), is mainly caused by pathogens, such as, P. gingivalis, however high level of IL-6 is postulated in the disease progression. The causation of P. gingivalis in CP depends on multiple virulence factors like fimbriae, LPS and cysteine proteinases gingipains (RgpA and RgpB), which utilize different signaling receptors to exert their influences. The main aim of our study was to determine the role of these factors in IL-6 production by monocyte derived dendritic cells (moDCs) and gingival keratinocytes (TIGKs). To this end it has been demonstrated that co-culture moDC with live P. gingivalis results in maturation of dendritic cells and production of inflammatory cytokines mainly IL-6. By evaluating the effect of individual virulence factors, our research has demonstrated that LPS is a more potent inducer of IL-6 compared to FimA, and only HRgpA induced the expression of IL-6 among the proteases produced by P. gingivalis. Stimulation with combination of antigens resulted in the cross talk between HRgpA and FimA with LPS. Additionally, we observed increase level of sIL-6R, gp130 and activation of STAT3 after incubation with antigens, indicating the broad roles of P. gingivalis in IL-6 signaling regulation. Therefore, the precise verification of P. gingivalis antigen’s mechanism of actions is important in the understanding of the inflammation process in periodontitis.

P47 Melker Göransson

Validating SIK as a novel COPD target in four different human systems

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Recent findings identify salt inducible kinases (SIKs) as a key molecular switch, whose inhibition reprograms murine macrophages to an anti-inflammatory phenotype. The underlying chronic inflammation, triggered by the inhalation of noxious substances such as tobacco smoke, leads to structural changes and narrowing of the small airways in COPD. This inflammatory state is believed to be driven primarily by macrophages, T-lymphocytes and neutrophils. Polarization of macrophages into a regulatory population, that produces high levels of IL-10 and low levels of pro-inflammatory cytokines like TNFα, may play an important role in the resolution of inflammation and is hypothesized to reduce frequency of COPD exacerbations.

We have set up assays in 4 different human systems ranging from monocyte-derived macrophages, alveolar macrophages and IPS-derived macrophages to lung explant cultures for human validation of this target.

Our data from both “normal” primary cells/tissue from cancer resections as well as COPD derived primary cells/tissue show that the concept of changing the TNFα/IL10 ratio, and thus the inflammatory state of the lung, by inhibiting SIK is conserved from mice to man.

The SIK family consists of 3 members and it is not yet fully clear which of the SIK1-3 proteins is important for polarization of macrophages, but recent evidence suggests SIK2 as being the major target in the mouse system. In parallel with the standard target validation strategies, the effect of mutating strategic phosphorylation sites on SIK1-3 in a human IPS cell line will be assessed using CRISPR technology.
P48 Cathy A Hawley

Development of a Model to Evaluate the Proliferative Capacity of Liver Kupffer Cells

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Until recently it was widely believed that most tissue resident macrophage populations (resMφ) were continually replenished by circulating monocytes. Current evidence, however, demonstrates that resMφ derive from cells seeded during embryogenesis, and in many tissues survive into adulthood by local proliferation or longevity.

The extent to which adult resMφ remain autonomous from the hematopoietic system varies between tissues but limited data suggest that F4/80+ liver Kupffer Cells (KC) require little contribution from monocytes in the steady state for at least one year. Consistent with such self-maintenance, KC are known to proliferate at a low basal rate, which can be up-regulated upon tissue perturbation. However, whether KC have a limit to their regenerative capacity in homeostasis and disease remains to be elucidated.

Our analysis of KC in mice aged 10 to 30 weeks showed that proliferative capacity may decline with age, which raises the possibility that proliferation could eventually exhaust. To test this, we have developed an in vivo system using exogenous growth factors to enforce proliferation leading to expansion and subsequent contraction of the local resident KC pool. We will use this method in combination with fate mapping of monocytes to determine if replenishment of KC occurs from the haematopoietic system should the resident population become exhausted.

P49 Kerstin Kiefer

Involvement of the disease-associated ORMDL family in ceramide synthesis in macrophages

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Orosomucoid-like 3 gene (ORMDL3) has been identified as a candidate gene for the susceptibility to develop proinflammatory diseases such as asthma and inflammatory bowel disease by genome-wide association studies (GWAS). ORMDLs are a family of endoplasmic reticulum proteins that participate in two major intracellular signalling pathways: Ca2+ movements and de novo ceramide synthesis. These proteins are inhibitors of the serine palmitoyl transferase (SPT), the rate-limiting enzyme of this de novo pathway and overexpression of ORMLD3 has been shown to influence the cellular ceramide content in RAW 264.7 macrophages. Moreover, ceramide synthesis is increased in activated macrophages and modulates processes like autophagy, phagocytosis and interleukin production. Therefore we want to explore the role of ORMDLs in ceramide production of macrophages. We have performed expression analysis and ceramide quantification in the RAW 264.7 cell line and Bone Marrow Derived Macrophages (BMDM) from WT and ORMDL3-KI mice. Our results demonstrated a coordinated regulation of the ORMDL family members that alter de novo ceramide synthesis during the activation process of macrophages triggered by LPS. The influence of increased ORMLD3 expression in macrophages was analyzed comparing the ceramide content in BMDM of WT and ORMDL3-KI mice. We observed slightly reduced ceramide levels with increased ORMLD3 expression with C16-ceramide being the most affected species. However, there were no major differences in the ceramide profile. Further experiments are needed to elucidate the role of ORMDLs in macrophage physiology and the involvement of this cell type in ORMLD3 associated pathologies.
P50 Yannick Morias

Understanding the role of epigenetics in regulating human alveolar macrophage polarisation and function in COPD.

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Macrophages display remarkable plasticity and undergo physiological reprogramming in response to their micro-environment. These changes can give rise to a pro-inflammatory M1 and tissue-remodelling M2 macrophage phenotypes. Human alveolar macrophages (AM) are thought to play a central role in pathogenesis of chronic obstructive pulmonary disease (COPD). However, it is unknown whether AM from late-stage COPD patients can adapt to environmental changes and what impact epigenetic regulation has on their polarisation status.

We demonstrated that AM show phenotypic plasticity independent of the disease status, with increased expression of CD54 for the M1 and CD163 for the M2 AM phenotypes. In addition, expression of epigenetic regulators (e.g. BRPF1 & BRD9) appears to be modulated by their polarisation states. The impact of these histone modifiers on AM function was further investigated using tool compounds.

This study demonstrated that AM from COPD patients are plastic, with epigenetic regulators impacting their polarisation and function.

P51 Allan Mowat

TGFβR Signalling Promotes CD103+ CD11b+ Dendritic Cell Development in the Intestine

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The intestine contains a unique subset of dendritic cells (DCs) that expresses both CD103 and CD11b. These are the dominant population of DCs in the small intestine, but their exact function remains obscure, as they have been associated with the generation of both regulatory and effector T[17] cells in vivo. Furthermore, although their development is dependent on several different factors such as IRF4, Notch2, CSF-2 and SIRPα, it is unknown what local processes might drive the differentiation of such unusual DCs in the gut. Here we show that mice with deletion of the TGFβRI on CD11c+ cells have a selective defect in CD103+CD11b+ DCs in the small and large intestinal mucosa, with preservation of the related subset of CD103 CD11b+ DCs. This is also no effect on intestinal macrophage numbers, despite the fact that these also express high levels of CD11c and TGFβRI. The defect in CD103+CD11b+ DCs was cell intrinsic and was not due to altered migration of intestinal DCs, as identical reductions occurred in mucosa and draining lymph nodes. Isolated downregulation of the CD103 molecule does not explain the apparent defect, as microarray and phenotypic analyses showed reduced expression of other markers normally specific to this population, including Siglec F and TREM-1. Preliminary studies indicate that CD11b+ DCs from CD11c-cre-TGFβRI mice have a defective ability to induce the generation of FoxP3+ Treg. Together our data indicate that TGFβ plays a crucial role in the terminal differentiation of potentially tolerogenic CD103+CD11b+ DCs from a CD103 CD11b+ precursor in the intestine.
P52 Thomas Naessens

Dominate presence of CD141+ monocyte-derived dendritic cells in the lungs of COPD patients

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Introduction: Dendritic cells (DCs) are critical regulators of immune responses. Several DC subsets have been identified in human lung tissue in non-inflammatory conditions. However, little is known about DCs in the pulmonary compartment during inflammation.

Aims: To compare the DC repertoire present in the lungs of COPD patients, non-smokers (NS), current smokers (S) and ex-smokers without COPD (Ex).

Methods: Lung tissue was digested and the phenotype and proportion of the different DC subpopulations were characterized via multicolor flow cytometry. The conventional (c)DC subsets were identified within the Lin-HLA-DR+CD14-CD1a- cells as CD11c⁺CD141⁺ and CD11c⁺CD1c⁺ respectively. Monocyte-derived (Mo)DCs were Lin-HLA-DR⁺CD14⁺CD1a⁺CD11c⁺ and based on the archetypal human DC markers, this population could also be further divided into CD141⁺ MoDCs and CD1c⁺ MoDCs.

Results: Additional phenotypic analysis revealed that both MoDC subsets were CD64⁺CD206⁺CD11bhiCX3CR1hiCD115⁺CD172a⁺. In addition, FceRI was common for CD1c expressing DCs while CLEC9a was present on both CD141⁺ DC subsets. Moreover, the CD141⁺ MoDCs uniquely expressed CD209 and the macrophage/monocyte marker CD163. Compared to NS, S and Ex, COPD lungs showed significantly (p=0.002) increased percentages of total MoDCs (3-fold). In contrast, percentages of total cDCs were only increased compared to NS and S controls. Strikingly, the increase in total MoDCs observed in COPD lungs was mainly due to an increased pulmonary infiltration of CD141⁺ MoDCs rendering this subpopulation the dominant DC subset in the COPD lung.

Conclusion: Given their dominant presence in COPD lungs and because little is known about their function, CD141⁺ MoDCs are an interesting target for future (functional) investigation.

P53 Valérie Pireaux

Myeloperoxidase-oxidized LDLs enhance an anti-inflammatory M2 phenotype in murine macrophages

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Macrophages and oxidized low-density lipoproteins (LDLs) play a key role in atheroma initiation and evolution. It has been shown that subsets of macrophages, such as M1, M2 or “Mox” cells, are present in those atherosclerotic lesions. LDLs can be oxidized chemically by copper (Ox-LDLs), but also enzymatically by myeloperoxidase resulting in oxidized LDLs poor in lipid peroxides. The effects of physiologically relevant myeloperoxidase-oxidized LDLs (MpOx-LDLs) on macrophage polarization or on polarized macrophages remain unknown.

After establishing a new model of in vitro polarization of macrophages, we studied the impact of LDLs on macrophage polarization, their interference with polarized macrophages and the ability of polarized macrophages to become foam cells. We also studied the impact of UDP on macrophage polarization, a potential modulator of atherosclerosis.

Our data shows that RAW 264.7 murine macrophages can be polarized towards M1, M2 and MOX macrophages. Moreover, stimulation with LDLs showed that MpOx-LDLs efficiently accumulated within cells and enhanced the anti-inflammatoriy M2 phenotype in M0, M1, M2 and MOX macrophages.

These results have been validated in murine bone marrow-derived macrophages (BMDMs) and are currently being analysed regarding the effect of UDP.
P54 Clare Pridans

**Lentiviral vectors containing mouse Csf1r control elements direct macrophage-restricted expression in multiple species**

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The development of macrophages requires signaling through the lineage-restricted receptor Csf1r. Macrophage-restricted expression of transgenic reporters based upon Csf1r requires the highly conserved Fms-intronic regulatory element (FIRE). We have created a lentiviral construct containing mouse FIRE and promoter which is capable of directing macrophage-restricted reporter gene expression in mouse, rat, human, pig, cow, sheep, and even chicken.

The lentivirus was used to create Csf1r-EGFP transgenic sheep. Rat bone marrow cells transduced with the lentivirus were capable of differentiating into macrophages expressing the reporter gene in vitro. Macrophage-restricted expression may be desirable for immunization or immune response modulation, and for gene therapy for lysosomal storage diseases and some immunodeficiencies. The small size of the Csf1r transcription control elements will allow the insertion of large "cargo" for applications in gene therapy and vaccine delivery.

P55 Federica Raggi

**Hypoxia reprograms human macrophages towards a proinflammatory direction: role of TREM-1**

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Mononuclear phagocytes are recruited as primary monocytes from the circulation to sites of infection, inflammation, and tumor growth, were they undergo terminal differentiation into macrophages. Macrophages can be polarized by microenvironment factors into classically activated (M1) or alternatively activated macrophages (M2) which are characterized by a proinflammatory or an anti-inflammatory phenotype, respectively. A common feature of pathological situations is represented by hypoxia, an important regulator of cell differentiation and functions. Little is known about the impact of hypoxia on M1/M2 development. To address this issue, monocyte-derived macrophages were cultured with LPS or IL4 for 24h to generate M1 (CD80+) or M2 (CD206+) macrophages, respectively, under normoxic (20%O2) or hypoxic (1%O2) conditions. We present data showing that hypoxia amplifies M1 macrophage proinflammatory state and reprograms M2 macrophages towards a proinflammatory direction by increasing the production of inflammatory and proangiogenic M1 type cytokines/chemokines. The hypoxic pathologic microenvironment can finely tune the expression of immunoregulatory receptors, whose deregulated expression may result in amplification of inflammation or establishment of immune escape situations. We demonstrate that hypoxia strongly upregulates the expression of one of such receptors, TREM – 1, in both M1 and M2 macrophages. Engagement of TREM-1 by agonist Ab triggers further production of M1-type cytokines/chemokines in both macrophage populations. These results suggest the role of the hypoxic environment present at pathological sites in macrophage reprogramming towards a proinflammatory phenotype by inducing TREM-1, highlighting the potential of targeting TREM-1 as a strategy to counteract inflammation in inflammatory disorders and tumours.
**P56 Vladimir Riabov**

**Re-programming of macrophage phenotype for therapeutic immunomodulation in implantation**

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Currently, titanium-based biomedical devices are a mainstream solution for implantation in dentistry and orthopedics due to the biocompatibility and favorable mechanical properties of titanium and its alloys. Nevertheless, adverse immune response related effects such as chronic inflammation, allergy and osteolysis are sometimes observed upon implantation. Macrophages and their pro-inflammatory mediators are central players in implant-associated chronic inflammation. Local induction of anti-inflammatory macrophage phenotype is one of the strategies to minimize such adverse effects. However, long-term maintenance of anti-inflammatory state in macrophages is a challenging task due to plasticity of macrophage phenotype. In this study we characterized the effect of potent anti-inflammatory cytokine combination (M2 cocktail, M2ct) on cytokine production by human monocyte-derived macrophages in long-term culture and after re-polarization with pro-inflammatory stimuli. The results demonstrated that M2ct-stimulated macrophages sustained anti-inflammatory phenotype up to 12 days in *in vitro* culture even after stimulation with high dose of LPS. Moreover, after deprivation of cytokines from culture medium followed by challenge with LPS for 6 other days, TNFα release by M2ct-differentiated macrophages was strongly suppressed compared to prototypical M2 stimulator IL-4. Restoration of pro-inflammatory phenotype was possible only when macrophages were deprived of M2ct and stimulated with combination of IFNγ and LPS. Overall, this data demonstrate availability of potent anti-inflammatory cytokine combinations for local modulation of macrophage phenotype in case of implant-induced inflammatory complications.

**P57 Rocio Rojo**

**Examining the role of an intronic enhancer in the expression of CSF1R in macrophages**

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Macrophages perform fundamental roles for the maintenance of tissue homeostasis through the lifespan of an organism. The survival, proliferation and differentiation of these cells depend on the colony stimulating factor 1 (CSF1), which signals through the CSF1 receptor (CSF1R). CSF1R is a tyrosine kinase type III receptor encoded by the *c-fms* proto-oncogene, and alterations in its sequence are associated with neurological/reproductive defects, myeloid malignancies and carcinomas. The transcription of Csf1r is partially controlled by the *fms* intronic regulatory element (FIRE), a highly conserved sequence across species with binding sites for multiple myeloid transcription factors, which has the ability to perform a dual promoter/repressor function, depending on its orientation. Since deregulation of the expression of CSF-1R has clinical relevance, we aim to investigate whether the deletion of FIRE in its native context alters macrophage homeostasis/function; hypothesising that FIRE is required to achieve the physiological expression levels of Csf1r necessary for the development of CSF-1-dependent macrophages. We have generated homozygous deletions of FIRE in the Csf1r locus using the CRISPR/Cas9 system in 129/Ola-derived E14 embryonic stem cells (ESC) and the potential of these ESC clones to differentiate into macrophages has been tested by comparing them to the wild type phenotype. Preliminary results indicate that the absence of FIRE prevents the cells from becoming ESC-derived macrophages. Further work will involve the development of an animal model with this genotype and the characterisation of its phenotype.
P58 Kristin A. Sauter

Potential of CSF1-Fc in regenerative medicine.


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Macrophage colony-stimulating factor (CSF1) is the main growth factor responsible for the maintenance of tissue macrophages. We have produced a novel Fc conjugate form of CSF1 with an improved circulating half-life. Using either the native protein, or the more stable CSF1-Fc, we have shown that CSF1 administration can increase blood monocyte and tissue macrophage numbers, and has therapeutic potential in mouse models of liver regeneration, kidney regeneration, non-conjoined fracture healing and engraftment following bone marrow transplantation. We also showed that CSF1-Fc treatment can promote clearance of microbes from the circulation, and thereby reduce one of the major causes of pathology associated with liver failure. In the present study, we extended the analysis of CSF1-Fc efficacy to pigs. As observed in the mouse model, CSF1-Fc increased blood monocyte and tissue macrophage numbers and promoted a rapid increase in the size of the spleen and liver. There was no adverse impact of the treatment on liver function. Additionally, treatment with CSF1-Fc may expand the myeloid progenitor pool in the bone marrow as well as cause a shift towards a larger proportion of mature monocytes. CSF1 has therapeutic potential in regenerative medicine in multiple organs. The CSF1-Fc conjugate retains this potential, and may permit daily delivery by injection rather than continuous infusion required for the core molecule.

P59 Sonali Singh

The Impact of Surface Chemistry on Macrophage Polarisation

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Macrophages are master immunoregulators, adopting a spectrum of activation states in response to microenvironmental cues. These include the physicochemical properties (e.g. topography and chemical composition) of the substrates they interface with. Understanding how different substrate properties modulate macrophage polarisation provides opportunities for developing surfaces/biomaterials with immune-instructive properties, with applications in the management of chronic inflammation and fibrosis often associated with indwelling medical devices.

To assess the impact of different surface chemistries on macrophage polarisation, human monocyte-derived macrophages were generated by culturing peripheral blood monocytes for 6 days on untreated polystyrene (PS, hydrophobic surface) or on O2 plasma-etched polystyrene (O2-PS, hydrophilic surface). Our data showed that macrophages generated on the hydrophobic O2-PS surface were polarised towards a pro-inflammatory phenotype, as evidenced by significantly higher expression of calprotectin (p=0.0075, n=6) and production of pro-inflammatory cytokines IL-6 (p=0.0184, n=4) and IL-1β (p=0.0047, n=4). This was in contrast to macrophages generated on the hydrophilic O2-PS surface, which exhibited a more anti-inflammatory phenotype, evidenced by significantly higher production of anti-inflammatory cytokines such as IL-10 (p<0.0001, n=6) and CCL18 (p=0.0052, n=3). Preliminary data also indicate that macrophages generated on O2-PS surfaces are more phagocytic than those on PS surfaces. Additional characterisation (oxidative burst and transcription factor expression) of macrophages on the two surfaces is currently underway.

These data suggest that changes in surface wettability can be used to modulate the phenotype and functional properties of macrophages.
P61 Dagmara Wiatrek

Alterations in zinc transporters expression during dendritic cell maturation
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Zinc deficiency is known to cause defects in cellular immunity, therefore we have studied the expression of Zip and ZnT transporters in the key antigen presenting cell type, dendritic cells (DC). Using real time PCR we have found dynamic changes in zinc transporter expression upon LPS induced DC maturation. Expression of both zinc importer (Zip 10) and exporter (ZnT 1) decreased dramatically after 6h of LPS treatment, an effect that was sustained even at 72h post LPS treatment. This time period is likely to replicate the period of in vivo post antigen stimulation when DC have left the site of pathogen exposure and migrated to the nearest lymph node to initiate an immune response. Our data now implicates Zinc homeostasis as important part of antigen presentation process. Our current studies are concentrating on building a full picture of the behaviour of Zip and ZnT transporters during this crucial time period for DC.

P62 Alicia Wong

The modulation of TLR-9 mediated plasmacytoid dendritic cells activation by citrullinated forms of LL-37
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The cationic bactericidal peptide LL-37, the only human cathelicidin plays an important role in innate immunity due to its immunomodulatory properties. The LL-37 peptide triggers the response of plasmacytoid dendritic cells (pDCs). It forms complexes with free nucleic acids facilitating the delivery of oligonucleotides to TLR-9 in pDCs. Such mechanism is not only important in pathogen recognition but also in the development of autoimmune diseases. The latter reaction can be initiated during inflammatory conditions by LL-37 citrullination exerted by peptidylarginine-deiminase (PAD). This posttranslational modification alters the immunomodulatory functions of the peptide. Thus, the aim of our study was to investigate the role of citrullinated LL-37 on DNA recognition by pDCs. Our data revealed that the increase number of citrullinated arginine residues in LL-37 diminishes the peptide efficiency for oligonucleotides binding. Moreover, citrullination of LL-37 decreases DNA uptake and sensing by pDCs, manifested by changes in INFα and IL-6 secretion. Taken together, we postulate that upon citrullination of LL-37, pDCs become less sensitive in recognition of released pathogen’s DNA during infection, however it may be beneficial in autoimmunological disease development where the self-DNA recognition leads to immunotolerance breakdown.
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