EMDS
European Macrophage &
Dendritic Cell Society

25th Annual **EMDS** Meeting

Clinical and Fundamental Aspects of Monocyte, Macrophage and DC Plasticity

22-24 September 2011

Brussels, Belgium

www.emds2011.eu

LAST-MINUTE CHANGES IN THE PROGRAM SINCE PRINTING OF THE ABSTRACT BOOKS

- ***Matteo Iannacone***, who had been selected for an oral presentation in Session 3 on Friday 22 September, has informed us recently that he will unfortunately not be able to attend the EMDS 2011 meeting.
  A short talk will instead be given by **Chao Shi** (abstract C04)
- ***Thorsten Hagemann***, who had been scheduled as an invited speaker in Session 4 on Friday 22 September, has informed us recently that he will unfortunately not be able to attend the EMDS 2011 meeting.
  Jo Van Ginderachter (VIB – Vrije Universiteit Brussel) will instead give a presentation on "Heterogeneity of tumor-associated macrophages in mouse tumor models".

IMPORTANT REMARKS ABOUT THE VENUE

- The oral presentations will be in **the Salle Dupréel lecture room, which is at the first floor level of the venue building**.
- Coffee, lunch and poster sessions will be in the hall outside the Salle Dupréel lecture room and will be spread over the ground and first floor levels of the hall. We want to urge delegates to **make optimal use of the available space and spread over the two floors of the hall.**
- Please be advised that the ground floor and first floor hall are public areas in which we have to allow passage of those wanting to reach any of the 15 floors of the building. Therefore, it is best **not to leave valuable items unattended**.
- As a service to those coming straight to the venue with their luggage, we will aim to organise a luggage drop-off at the venue to the best of our ability. However, using the luggage drop-off implies you accepting that **any damage, loss or theft of items in the luggage drop-off remains at your own risk**.
- Upon registration, you will be provided your personal EMDS congress badge. Make sure to **wear your congress badge at all times**. Entrance to the Salle Dupréel lecture room and participation to the coffee and lunch breaks and to the reception will be restricted to those wearing an EMDS congress badge.
- **Your congress badge should also be worn as a proof of reservation for the congress dinner at the Comic Strip Center**. The EMDS congress badge will carry a purple dinner sign for those having reserved a regular meal and a green dinner sign for those having reserved a vegetarian meal.

IMPORTANT POSTER AND ORAL PRESENTATION GUIDELINES

- At least 1 author should be at the poster during the Poster Session on Friday 23 September.

- Each selected oral presentation is allocated 15 minutes. It is crucial to ensure that your talk lasts for 10-12 minutes maximum, so that time remains for questions.

- Bring a Powerpoint presentation of your talk to the meeting and upload it **at least 30 minutes before the start of the session** in which your talk is scheduled:
  - Presenters of talks on the 1st day of the meeting are urged to proceed to uploading their presentation as soon as possible after registration.
  - Presenters of talks on the 2nd and 3rd day of the meeting are encouraged to already upload their presentation the day(s) before.

WELCOME

We are happy to welcome you to the capital of Europe for the 25th Annual Meeting of the European Macrophage and Dendritic Cell Society. We are looking forward to an exciting meeting focused around the theme of Clinical and fundamental aspects of monocyte, macrophage and dendritic cell plasticity.

Throughout the preparations for the EMDS 2011 meeting, we have encountered a high level of enthousiasm. First, there was the enthousiasm with which the invited speakers responded to our invitation for the meeting. Based on the attractive list of top-level invited speakers, registrations have then poured in so overwhelmingly that we have had to close online registrations already at the end of May. Taking into account the capacity of the venue, the meeting has been topped off at 300 registered participants. These come mainly from 19 European countries, but also from Brazil, Australia, Canada, U.S.A., Israel, Japan and Singapore.

Enthousiasm is also what we encountered when we asked for members of the Local Scientific and Organising Committee, members of the Abstract Selection Committee and session chairs. We are thereby grateful for the fruitful interactions with the current and newly elected EMDS council members.

The registered participants have also been very eager to contribute to the meeting, since we have received over 170 submitted abstracts. The Abstract Selection Committee members were impressed with the overall quality of the submitted abstracts and it was quite a challenge to select only 22 abstracts to fill the slots for short oral presentations. Taking into account an optimal diversity of contributions among the invited speakers and selected abstracts, we have come to a meeting program that looks very appealing and promises a high level of scientific discussions. And considering the large number of high quality abstracts that could not be selected for oral presentation, we certainly encourage you to actively take advantage of poster discussion opportunities.

And we would be forgetting one of the biggest sources of enthousiasm if we would not acknowledge the eagerness with which the members of our lab have agreed to provide all sorts of logistical, administrative and practical support for the organization of the EMDS 2011 meeting.

We are grateful for the support by the central administrative services of the Vrije Universiteit Brussel. Our “Alma Mater” is an offshoot of the French-speaking Université Libre de Bruxelles (ULB) and since 1970, the Vrije Universiteit Brussel and the Université Libre de Bruxelles are officially two separate legal, administrative and scientific entities. But still they share the same founding principles of democracy and dogma-free scientific research and they have recently joined forces to offer multilingual course programmes under the name “Brussels University Alliance”. It is therefore not that surprising that we have chosen an auditorium on the ULB Solbosch campus as scientific venue for the EMDS meeting.

In addition to the Vrije Universiteit Brussel, our laboratory is also affiliated to VIB, an interuniversity life sciences research institute, joining groups which perform basic research with a strong focus on translating scientific results into pharmaceutical, agricultural and industrial applications. We would like to acknowledge the material support for the meeting which we have received from VIB.

Despite the economical crisis, we are also grateful that we have been able to obtain significant financial support for the meeting, including sponsoring by various life science companies and scientific publishers. We encourage you to visit the sponsors having an exhibition booth at the venue to scout for services, products, equipments and/or journals that can be useful for your research.

And while you are here, we also invite you to discover Brussels: a vibrant, multi-cultural city at the heart of Europe. Experience its characteristic blend of different cultures, languages and styles of art and architecture. Cover this with the Belgian “joie de vivre” and love of good food and you have a destination that can offer something for everyone, be they an architecture fan, an art-lover, a gourmet or a night owl. Or a scientist of course! We are happy to offer you a taste of Brussels during the congress dinner at the Belgian Comic Strip Center. As a museum dedicated to the comic strip (sometimes referred to as the "Ninth Art") in a building that is a masterpiece of Art Nouveau architecture designed by Victor Horta, this venue offers a unique marriage of the Ninth Art and Art Nouveau as two fine examples of Belgian/Brussels art and culture.

We hope you all have a successful conference, with plenty of networking and sharing of information that can stimulate ideas and collaborations.

Patrick De Baetselier, Geert Raes and Jo Van Ginderachter

LOCAL SCIENTIFIC AND ORGANIZING COMMITTEE

CONGRESS CHAIRS
Patrick De Baetselier, Geert Raes & Jo Van Ginderachter
Vrije universiteit Brussel – VIB

OTHER COMMITTEE MEMBERS
- Patrick Matthys (Rega Institute, Katholieke Universiteit Leuven)
- Muriel Moser (Université Libre de Bruxelles)
- Benoît Van den Eynde (Ludwig Institute for Cancer Research, de Duve Institute, Université Catholique de Louvain)

ABSTRACT SELECTION COMMITTEE

Abstracts were reviewed by an Abstract Selection Committee consisting of 14 members of the Local Scientific and Organising Committee and the EMDS council. To avoid possible conflicts of interest, each abstract has been assigned to 4 reviewers not affiliated to the same country as the presenting author and has been independently scored by these reviewers. Based on these abstract scores and taking into account an optimal diversity of contributions at the meeting, 22 abstracts have been selected for a short oral presentation.

Abstract Selection Committee members were: Patrick De Baetselier (Belgium), Manfred Lutz (Germany), Patrick Matthys (Belgium), Muriel Moser (Belgium), Amaya Puig Kröger (Spain), Geert Raes (Belgium), Ulrike Schleicher (Germany), Maciej Siedlar (Poland), Silvano Sozzani (Italy), Alexander Steinkasserer (Germany), Benoît Van den Eynde (Belgium), Jo Van Ginderachter (Belgium), Guenter Weiss (Austria) and Loems Ziegler-Heitbrock (Germany).

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EMDS 2011 IN NUMBERS

- Invited speakers: 16
- Registered participants: 300 (excl. invited speakers)
- Submitted abstracts: 171
- Abstracts to be considered for oral presentation: 122
- Selected short talks: 22
- Sponsor booths at venue: 14

Membership status of registered participants

EMDS member (non-student)	86	28.67%
Non-EMDS member (non-student)	111	37.00%
Student	103	34.33%

Registrations per country

Belgium	114	38.00%	Canada	3	1.00%
Germany	48	16.00%	Hungary	3	1.00%
United Kingdom	25	8.33%	Poland	3	1.00%
The Netherlands	21	7.00%	Serbia	3	1.00%
Italy	12	4.00%	Israel	2	0.67%
Spain	11	3.67%	Japan	2	0.67%
France	10	3.33%	U.S.A.	2	0.67%
Austria	7	2.33%	Finland	1	0.33%
Denmark	7	2.33%	Greece	1	0.33%
Sweden	7	2.33%	Lithuania	1	0.33%
Brazil	6	2.00%	Norway	1	0.33%
Australia	4	1.33%	Portugal	1	0.33%
Switzerland	4	1.33%	Singapore	1	0.33%

INTERNET ACCESS

FlowJo has kindly offered to bring a number of laptops to their exhibition booth that delegates can use to browse the internet and check their e-mail.

In addition, delegates that brought their own laptop or smartphone can use free wireless internet access via the Brussels Urbizone network:

- First connect to the Urbizone network in your computer's network and sharing center.
- Then open your internet browser (Internet Explorer, Firefox, opera, safari…).
- In your internet browser, the Urbizone login page should appear (see picture).
- In case you do not yet have an Urbizone login, select the menu "Registration" and then "New user" below.
- Choose a login name and password and enter the requested identification information. If you enter your e-mail address, this can be used later on to recover your password should you have forgotten it.
- Indicate that you accept the usage conditions and click "Save". When you get the message that your data are recorded, clicking "Continue" will bring you back to the Urbizone login page.
- Enter the Urbizone login name and password that you have chosen and click "Login".
- You should now be able to browse the internet and conntect to web-based e-mail clients.

VENUE ADDRESS

SCIENTIFIC VENUE

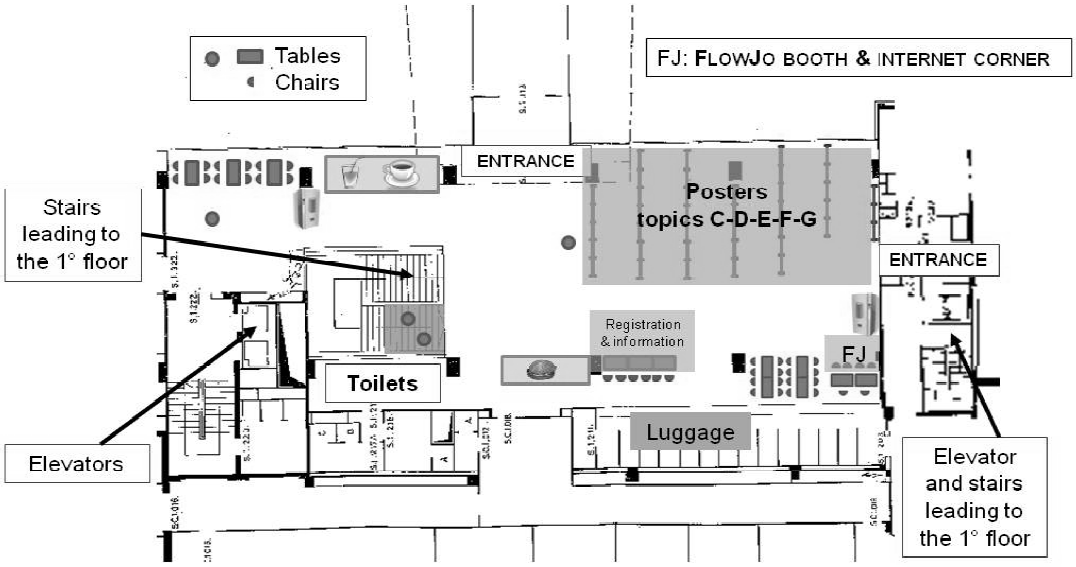
Salle Dupréel
Université Libre de Bruxelles (ULB)
Solbosch campus – Building S
Avenue Jeanne – Johannalaan 44
1050 Brussels (Ixelles – Elsene)
Belgium

CONGRESS DINNER VENUE

Belgian Comic Strip Center
Rue des Sables – Zandstraat 20
1000 Brussels
Belgium

VENUE FLOOR PLAN

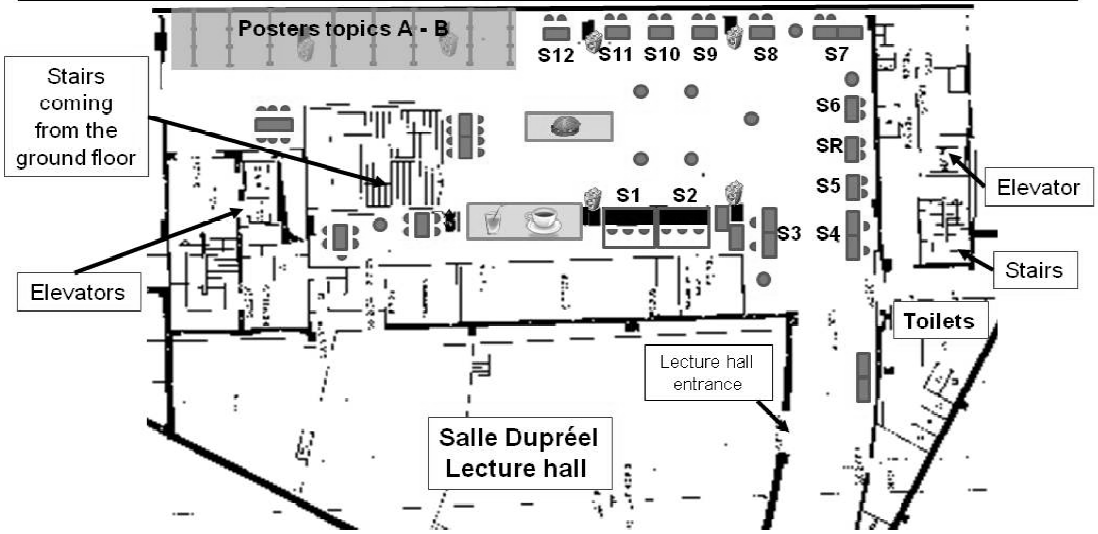
Ground floor



1° floor

SPONSOR BOOTHS

S1: Ovizio Imaging Systems	S4: Becton Dickinson	S7: Imtec	S10: Sigma-Aldrich
S2: GE Healthcare	S5: eBioscience	S8: Miltenyi Biotec	S11: PeproTech
S3: Bio-Rad	SR: R&D Systems	S9: Mabtech	S12: Merck Millipore
	S6: Gentaur	FLOWJO ON GROUND-FLOOR LEVEL	



GENERAL REMARKS

- Each session will consist of invited talks *(30 min., including questions)* and short talks selected from abstracts *(15 min., including questions).*
- Industrial exhibition by congress sponsors and display of posters is scheduled to be permanent throughout the meeting.
- English is the official congress language for the EMDS meeting. All oral and poster presentations should be in English. No (simultaneous) translation to other languages will be foreseen.

THURSDAY 22 SEPTEMBER 2011

10.00 – 13.00 **REGISTRATION & POSTER MOUNTING**

13.00 – 13.15 **OPENING & WELCOME ADDRESS**

13.15 – 15.15 **SESSION 1: REGULATION OF THE MONOCYTE, MACROPHAGE AND DC POOL**
Session chairs: ***Frédéric Geissmann*** *(U.K.)* & ***Patrick De Baetselier*** *(Belgium)*

(INV01) Monocytic differentiation and self renewal
Michael H. Sieweke *(France)*

(INV02) The Mononuclear Phagocyte System, as seen from the CX3CR1 angle
Steffen Jung *(Israel)*

(A02) SuperSAGE characterization of human monocyte subsets
Adam Zawada *(Germany)*

(A19) CD4+ Innate Lymphoid Cells Control CD8α- Dendritic Cell Homeostasis via the Lymphotoxin-β Receptor Pathway
Carl De Trez *(U.S.A./Belgium)*

(B20) The balance between monocyte-derived cells and conventional migratory dendritic cells determines the severity of T-cell-mediated colitis
Martin Guilliams *(France)*

15.15 – 15.45 **COFFEE & POSTERS**

15.45 – 18.00 **SESSION 2: MONOCYTES, MACROPHAGES AND DCS IN DISTINCT ORGANS AND TISSUES**
Session chairs: ***Muriel Moser*** *(Belgium)* & ***Patrick Matthys*** *(Belgium)*

(B02) Semaphorin 7A negatively regulates intestinal inflammation by IL-10 production of macrophages via αvβ1 integrin signalling
Kang Sujin *(Japan)*

(B06) Resolving the Local Microglial Response From the Inside: Orchestrated Monocyte Trafficking Is Pivotal for Spinal Cord Recovery
Catarina Raposo *(Israel)*

(B12) Intestinal Dendritic Cells Are Specialized to Activate TGF-β and Induce Foxp3+ Regulatory T-Cells via Integrin αvβ8
Mark Travis *(U.K.)*

(INV03) Monocytes in liver inflammation and liver fibrosis
Frank Tacke *(Germany)*

(INV04) Myeloid cells and intestinal homeostasis
Fiona Powrie *(U.K.)*

(INV05) Lung dendritic cell subsets in allergic asthma
Bart Lambrecht *(Belgium)*

18.00 – 19.00 **WELCOME RECEPTION**

FRIDAY 23 SEPTEMBER 2011

08.30 – 10.15 **SESSION 3: MONOCYTES, MACROPHAGES AND DCS IN INFECTIOUS DISEASES**
Session chairs: ***Christian Bogdan*** *(Germany)* & ***Alexander Steinkasserer*** *(Germany)*

(INV06) Novel DC targeting strategies for improved vaccination
Yvette van Kooyk *(The Netherlands)*

(INV07) Macrophages, iron and infection -- a classical triad!
Guenter Weiss *(Austria)*

(C04) Inflammatory monocytes but not neutrophils are essential for defense against systemic Listeria monocytogenes infection
Chao Shi *(U.S.A.)*

(C31) Epigenetic Control of Th2 Induction by Dendritic Cells
Andrew MacDonald *(U.K.)*

(C33) *Leishmania*-infected macrophages are resistant to NK cell cytotoxicity, but susceptible to NK cell-derived activating cytokines
Ulrike Schleicher *(Germany)*

10.15 – 10.45 **COFFEE & POSTERS**

10.45 – 12.30 **SESSION 4: MONOCYTES, MACROPHAGES AND DCS IN CANCER**
Session chairs: ***Maciej Siedlar*** *(Poland)* & ***Benoît Van den Eynde*** *(Belgium)*

(INV08) Cross-talk between myeloid-derived suppresser cells (MDSC) and macrophages modulates the tumor microenvironment and promotes tumor progression
Suzanne Ostrand-Rosenberg *(U.S.A.)*

(INV09) A target for cancer therapy: tumour associated myeloid cells
Thorsten Hagemann *(U.K.)*

(A13) Differential response to hypoxia by M1 and M2 macrophages: Role of EGLN3
Maria Escribese *(Spain)*

(D13) Human CLEC4C/BDCA-2/CD303 is a Receptor for Asialo Galactosyl Oligosaccharides
Carmen Parola *(Italy)*

(D14) A role for CD32B and humoral immunity in the polarization of monocytes and macrophage in human cancer
Subhra Biswas *(Singapore)*

12.30 – 14.00 **LUNCH & POSTERS**

14.00 – 16.00 **SESSION 5: INFLAMMATORY SIGNALING IN MACROPHAGES AND DCS**
Session chairs: ***Silvano Sozzani*** *(Italy)* & ***Loems Ziegler-Heitbrock*** *(Germany)*

(INV10) Myeloid cell expression of the deubiquitinating enzyme A20 controls inflammation and immunity
Rudi Beyaert *(Belgium)*

(A08) PPARγ-regulated cathepsin D is required for lipid antigen presentation by dendritic cells
Laszlo Nagy *(Hungary)*

(A20) NLRP12 drives steady-state granulopoiesis
Kate Schroder *(Australia)*

(E08) IRF3-dependent activation of inflammatory dendritic cells by extracellular host DNA mediates the adjuvant activity of alum on TH2 responses
Christoph Desmet *(Belgium)*

(E13) Human dendritic cells reprogramming by hypoxia: induction of a proinflammatory phenotype and identification of TREM-1 as a novel hypoxic marker
Luigi Varesio *(Italy)*

BI-ANNUAL EMDS PRIZE LECTURE ***(Prize sponsored by Becton Dickinson Biosciences)***

(INV11) G-protein-coupled receptors and sepsis
Nicole Kaneider *(Austria)*

16.00 – 18.00 **POSTER SESSION**
REMARK At least 1 author should be at the poster during the Poster Session

18.00 – 19.00 **EMDS MEMBERS' GENERAL ASSEMBLY**

19.30 **CONGRESS DINNER at the Belgian Comic Strip Center**

SATURDAY 24 SEPTEMBER 2011

08.30 – 10.15 **SESSION 6: MACROPHAGE ACTIVATION STATES**
Session chairs: ***Amaya Puig Kröger*** *(Spain)* & ***Jo Van Ginderachter*** *(Belgium)*

(INV12) The p50 NF-kB subunit is a key regulator of both polarized inflammation and adaptive immune response
Antonio Sica *(Italy)*

(INV13) Th2 immunity and macrophage activation: where inflammation is anti-inflammatory
Judith E. Allen *(U.K.)*

(A17) Macrophages as cellular targets for immune modulation in experimental autoimmune type I diabetes
Hannelie Korf *(Belgium)*

(E10) IRF5 and RelA in setting up pro-inflammatory macrophage phenotype
Irina Udalova *(U.K.)*

(F04) Macrophages programmed by apoptotic cells promote angiogenesis through prostaglandin E2
Andreas Weigert *(Germany)*

10.15 – 10.45 **COFFEE & POSTERS**

10.45 – 13.00 **SESSION 7: IMAGING THE BEHAVIOUR OF MONOCYTES, MACROPHAGES AND DCS**
Session chairs: ***Manfred Lutz*** *(Germany)* & ***Geert Raes*** *(Belgium)*

(G05) Antigen stored in Dendritic Cells after macropinocytosis is released unprocessed from late endosomes to target B cells
Florence Niedergang *(France)*

(G08) A critical requirement of the actin capping activity of Eps8 in dendritic cell migration
Gianluca Matteoli *(Belgium)*

(G09) Macrophage mannose receptor-specific nanobody-based targeting and in vivo imaging of tumor-associated macrophages
Steve Schoonooghe *(Belgium)*

(INV14) Macrophage / dendritic cell interaction with collecting lymphatic vessels in the adipose tissue outside of lymph nodes
Gwendalyn J. Randolph *(U.S.A.)*

(INV15) Dynamics of effector T cell interactions during infection
Philippe Bousso *(France)*

(INV16) Origin and fate of macrophages: analysis in context
Mikael Pittet *(U.S.A.)*

13.00 – 13.15 **CLOSING & FAREWELL ADDRESS**

13.15 – 14.30 **FREE SATELLITE LUNCH SEMINAR ON THE USE OF FLOWJO FOR ANALYSIS OF MULTI-PARAMETER FLOW CYTOMETRY DATA**
(Lunch for the seminar attendants will be provided by Celeza GmbH)

INVITED SPEAKER ABSTRACTS

INV01 Monocytic differentiation and self renewal

Michael H. Sieweke
Centre d'Immunologie de Marseille-Luminy, Marseille, France

Extended self-renewal capacity is usually considered to be restricted to stem cells or transformed progenitors, whereas terminal differentiation is typically linked to cell cycle exit. Despite the proliferative potential of certain subpopulations under specific inflammatory conditions, this is generally also the case for macrophages. The sustained proliferative response of myelo-monocytic progenitors to the cytokine M-CSF is thus lost upon differentiation to macrophages, despite the continued ability of the mature cells to sense the cytokine. The non-proliferative state of terminally differentiated cells is assured by robust mechanisms but it has been unclear what renders differentiated cells refractory to the very mitogen signals that stimulate the proliferation of their direct precursors. We observed that in the monocytic lineage expression of the transcription factors MafB and c-Maf is induced upon differentiation and inversely correlates with proliferative capacity. As a consequence combined MafB and c-Maf deficiency (Maf-DKO) enables extended M-CSF dependent expansion of mature monocytes and macrophages in culture without loss of differentiated phenotype and function. Upon transplantation, expanded Maf-DKO cells are non-tumorigenic and contribute to functional macrophage populations in vivo. Our results indicate that MafB/c-MafB deficiency renders extended self-renewal compatible with terminal differentiation of macrophages. It thus appears possible to amplify functional differentiated cells indefinitely without malignant transformation or stem cell intermediates. We will discuss the possibility to use Maf-DKO cells to dissociate molecular mechanisms of self-renewal and differentiation. Towards this end we have developed an inducible system of gene expression for MafB in theses cells to indentify relevant direct MafB target genes. We will present preliminary data of genome wide ChIP-Seq experiments revealing MafB binding sites and differential histone modifications in Maf-DKO and wt cells with the aim to identify an epigenetic signature of self-renewal in differentiated macrophages.

INV02 The Mononuclear Phagocyte System, as seen from the CX3CR1 angle

Simon Yona, Ki-Wook Kim, Alex Mildner and Steffen Jung
Department of Immunology, The Weizmann Institute of Science, Rehovot, Israel

While the last decade yielded major advances in our understanding of the mononuclear phagocyte system, many of its developmental and functional aspects remain poorly understood. Compared to the study of B and T cells, which profits from well-defined promoter/ enhancer elements that allow *Cre-lox* mediated gene ablations, mononuclear phagocytes, and in particular myeloid progenitors have remained largely refractory to such approaches. Moreover, the exceeding short half-life of monocytes and classical dendritic cells represent an additional challenge to conditional genetic manipulations. Here, we will report our efforts to exploit the activity of the CX3CR1 promoter to target the system. Specifically, we will discuss recent insights into monocyte dynamics gained from the use of CX3CR1-Cre and CX3CR1-CreERT2 mice.

INV03 Monocytes in liver inflammation and liver fibrosis

Frank Tacke
Dept of Medicine III, University Hospital Aachen, Germany

Liver diseases are a major health problem worldwide, with a characteristic progression from chronic hepatitis to hepatic fibrosis to end-stage cirrhosis and hepatocellular carcinoma. Sustained inflammation in the injured liver is a highly regulated process involving several innate and adaptive immune cell compartments. Experimental models of liver fibrosis highlight the importance of hepatic macrophages, so-called Kupffer cells, for perpetuating inflammation resulting by releasing proinflammatory cytokines and chemokines as well as activating collagen-producing hepatic stellate cells. Recent studies in mice demonstrate that these actions are only partially conducted by liver-resident macrophages, but largely depend on the recruitment of monocytes into the liver, namely of the inflammatory $Gr1^+$ ($Ly6C^+$) monocyte subset as precursors of tissue macrophages. The chemokine receptor CCR2 and its ligand MCP-1/CCL2 promote hepatic monocyte subset accumulation upon liver injury, while the chemokine receptor CX_3CR1 and its ligand fractalkine (CX_3CL1) are important negative regulators of monocyte infiltration by controlling their survival and differentiation into functionally diverse macrophage subsets. The infiltration of proinflammatory monocytes into injured murine liver can be specifically blocked by novel anti-MCP-1 directed agents. In patients with liver cirrhosis, 'non-classical' $CD14^+CD16^+$ monocytes are found activated in blood as well as liver and promote pro-inflammatory along with pro-fibrogeneic actions by the release of distinct cytokines and direct interactions with stellate cells. However, experimental animal models also indicate that monocytes/macrophages are not only critical for fibrosis progression, but also for fibrosis regression, because macrophages can also degrade extracellular matrix proteins and exert anti-inflammatory actions. The recently identified cellular and molecular pathways for monocyte subset recruitment, macrophage differentiation and interactions with other hepatic cell types in the injured liver may therefore represent interesting novel targets for future therapeutic approaches in liver inflammation and fibrosis.

INV04 Myeloid cells and intestinal homeostasis

Fiona Powrie
Translational Gastroenterology Unit, Experimental Medicine Division- Nuffield Dept of Clinical Medicine, University of Oxford, UK

The gastrointestinal (GI) tract is home to a large number and vast array of bacteria that play an important role in nutrition, immune system development and host defense. In inflammatory bowel disease (IBD) there is a breakdown in this mutualistic relationship resulting in aberrant inflammatory responses to intestinal bacteria. Studies in model systems indicate that intestinal homeostasis is an active process involving a delicate balance between effector and immune suppressive pathways. This presentation will focus on the role of haematopoietic progenitor cells and myeloid cell populations in promoting tolerance versus immunity in the intestine.

INV05 Lung dendritic cell subsets in allergic asthma

Bart N. Lambrecht
Laboratory of Immunoregulation and Mucosal Immunology, University Ghent, Belgium

Allergic asthma is characterized by airway wall infiltration with eosinophils, mast cells and Th2 cells that lead to goblet cell hyperplasia, bronchial hyperreactivity and airway wall remodelling. The ways in which Th2 cells get activated during sensitization and during recall responses have been intensively studied. Antigen-presenting dendritic cells are crucial not only in the initiation of T cell responses, but also for their maintenance. Targeting DCs using genetic strategies in mice with acute allergic inflammation, as well as those with chronically remodelled airways illustrated that interfering with the function of DCs holds therapeutic perspectives. Therefore, we have recently extensively studied how DCs get activated in response to inhaled allergens. Exogenous danger signals like LPS are commonly found in allergens like HDM. Strikingly, airway DCs get activated in response to LPS in HDM, but do so indirectly, via signals derived from bronchial epithelial cells, that release GM-CSF, TSLP, IL-25 and IL-33. Different DC subsets seem to perform different tasks in the process of allergic sensitization. Under conditions of Th2 development, basophils are also recruited to the lymph node and help DCs to sustain Th2 development. We have also found that endogenous danger signals like ATP and uric acid control the activation of DCs in response to allergen challenge or in response to Th2 adjuvants, that are commonly used for inducing experimental asthma, like the Th2 adjuvant alum. On the contrary, there also exist endogenous anti-inflammatory signals, like prostaglandins, that suppress the function of DCs and dampen Th2 development and effector functions. Thus, a fine balance exists that sets the level of DC activation in vivo and could be exploited to the design of novel forms of anti-inflammatory therapies.

INV06 Novel DC targeting strategies for improved vaccination

Ingeborg Streng-Ouwehand, Manja Litjens, Astrid van Beelen, Hakan Kalay, Sven Bruijns, Wendy Unger and Yvette van Kooyk
Department of Molecular Cell Biology and Immunology, VU medical Center, v.d. Boechorststraat 4, 1081 BT Amsterdam, the Netherlands
Y.vanKooyk@vumc.nl

Dendritic cells (DC) are specialized in the recognition of pathogens and play a pivotal role in the control of immunity. DC express several C-type lectins, that function as innate receptors that recognize pathogens, and facilitate antigen uptake and presentation. Yet many of these receptors also modify responses through signalling interference with TLR. We and others have shown that the C-type lectins DC-SIGN, MGL and Siglecs recognize specific glycan structures on many pathogens, and modulate DC mediated responses. We have shown that the glycan composition of the pathogens that is recognized by DC plays a dominating role in directing the immune response that they induce.
We recently started to modify antigens with specific glycans to favour direction of antigens to DC in situ, in particular to DC specific receptors that also enhance processing and presentation of antigen to T cells. Glycan modification of antigen with DC-SIGN or MGL binding glycans can strongly affect antigen uptake and presentation capacity of DC and instructs antigen specific CD4 and CD8 T cell responses and Th1 differentiation. We have shown that uptake of specific glycosylated antigen is differently routed intracellularly and favours cross-presentation without the need of any TLR signalling. We show that glycan modified antigens as protein/peptide or particulate compositions target these innate receptors on DC which leads to tailored immune responses both in-vitro and in-vivo that control immunity against cancer and infectious diseases.

INV07 Macrophages, iron and infection - a classical triad!

Günter Weiss
Department of Internal Medicine, Clinical Immunology and Infectious Diseases, Medical University, A-6020 Innsbruck, Austria
Email: guenter.weiss@i-med.ac.at

The control over iron availability is of central importance in host-pathogen interaction because mammalian cells and microbes have an essential demand for the metal, which is required for many metabolic processes and for microbial pathogenicity(1). In addition, cross-regulatory interactions between iron homeostasis and immune function are evident. Cytokines and the acute phase protein hepcidin affect iron homeostasis leading to the retention of the metal within macrophages. This is considered to results from a defense mechanism of the body to limit the availability of iron for extracellular pathogens while on the other hand the reduction of circulating iron results in the development of anemia of inflammation. Opposite, iron as well as the anemia inducible hormone erythropoietin affect innate immune responses by influencing IFN-γ mediated (iron) or NF-κB inducible (erythropoietin) immune effector pathways in macrophages(1, 2). Thus, macrophages loaded with iron lose their ability to kill intracellular pathogens via IFN-γ mediated effector pathways such as nitric oxide (NO) formation. Accordingly, macrophages invaded by the intracellular pathogen *Salmonella typhi murium* increase the expression of the iron export protein ferroportin thereby reducing the availability of iron for intramacrophage bacteria while on the other side strengthening anti-microbial macrophage effector pathways via increased formation of NO or TNF-α. In addition, certain innate resistance genes such as natural resistance associated macrophage protein function (NRAMP-1) or lipocalin-2 exert part of their antimicrobial activity by controlling host and/or microbial iron homeostasis. In a line of this, pharmacological modification of cellular iron trafficking e.g. by the calcium antagonist nifedipine(3) enhances host resistance to intracellular pathogens via limitation of iron availability(4). Thus, the control over iron homeostasis is a central battlefield in host-pathogen interplay influencing the course of an infectious disease in favor of either the mammalian host or the pathogenic invader.

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INV08 Cross-talk between myeloid-derived suppresser cells (MDSC) and macrophages modulates the tumor microenvironment and promotes tumor progression

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Tumor growth is facilitated by a variety of tumor-secreted molecules that drive the accumulation and recruitment of tumor-promoting host cells. These cells infiltrate tumor masses, form the stroma of the tumor, and circulate in the host. They contribute to tumor progression through a number of mechanisms including promoting angiogenesis, suppressing anti-tumor immunity, and/or providing growth factors for tumor cells. The myeloid compartment is a major contributor of tumor-promoting cells, with myeloid-derived suppressor cells (MDSC) and macrophages being particularly prevalent. With increasing tumor burden and increasing amounts of tumor-secreted factors, MDSC and macrophages are phenotypically and functionally altered, and are co-opted to inhibit, rather than activate, anti-tumor immunity. Many of the alterations that occur are due to pro-inflammatory mediators that are ubiquitously present in tumor microenvironments such as the bioactive lipid prostaglandin E2 (PGE2), the pro-inflammatory cytokines IL-1β and IL-6, complement component C5a, growth factors such as GM-CSF and VEGF, as well as the alarmin S100A8/A9. The accumulation and immune suppressive activity of MDSC and macrophages are not only regulated by tumor-secreted factors, but are also driven by cross-talk between the two cell populations. Cross-talk between MDSC and macrophages also regulates the production of numerous pro-inflammatory and anti-inflammatory cytokines and chemokines, thus further altering the tumor microenvironment and facilitating tumor progression. This talk will discuss the impact of MDSC-macrophage cross-talk on the accumulation and function of these cell populations, and how cell-cell interactions alter the inflammatory tumor environment, increase immune suppression, and support tumor growth. *(Supported by NIH RO1CA115880 and RO1CA84232)*

INV09 A target for cancer therapy: tumour associated myeloid cells

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Tumour progression is characterized by massive cellular proliferation associated with alterations of the tumour microenvironment. The tumour microenvironment has a fundamental impact on the growth and spread of malignant disease and contributes – at least partially - to the resistance of malignant disease to chemo- and targeted- therapy. The interaction in this microenvironment is complex and involves a multitude of factors and cells. Monocytes and macrophages are substantial part of the tumour microenvironment of many solid malignancies in men and mice. However, although recent data provided more insights into their role within primarily murine tumour models we are still uncertain about the magnitude of their heterogeneity, their human counterparts, and the distinctive markers.
The adaptation of tumour cells to the changing environment is a decisive driving force in the clonal selection that, ultimately, results in a more invasive and aggressive tumour phenotype. In this context, the tumour microenvironment causes a number of crucial effects on various cellular and physiologic functions, including angiogenesis, cell proliferation, immunosurveillance, metabolism, DNA replication and protein turnover. The inherent plasticity of the tumour microenvironment adds another layer of complexity, such that the challenge includes not only targeting the right cells and mechanisms in the right place, but also at the right time.
Recent data provides evidence that inhibition of myeloid influx is a suitable strategy to enhance therapeutic success in cancer bearing patients. Although this is certainly encouraging, many questions are still remain unanswered such as which population to target, for how long and when?

INV10 Myeloid cell expression of the deubiquitinating enzyme A20 controls inflammation and immunity

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NF-κB dependent gene expression plays a key role in inflammation and immunity. Increased or sustained NF-κB activity has been linked with many autoimmune and inflammatory diseases. Multiple molecular mechanisms normally ensure the proper termination of NF-κB signaling. In this context, the intracellular protein A20 (also known as TNFAIP3) is a key player in the negative feedback regulation of NF-κB signaling in response to proinflammatory cytokines and pattern recognition receptor stimulation. In addition, A20 negatively regulates IRF3 activation and type I interferon production in response to infection. A20 exerts its inhibitory function by acting as a deubiquitinating enzyme that targets specific NF-κB and IRF3 signaling proteins and is regulated by several A20-binding proteins. Human *A20* is a susceptibility locus for common inflammatory diseases such as Crohn's disease, rheumatoid arthritis, and lupus, suggesting that A20 deficiency contributes to the development and progression of human autoimmune and inflammatory diseases. A20 deficient mice die early after birth due to severe multi-organ inflammation. To understand the physiological function of A20 in myeloid cells we have generated myeloid cell specific A20 knockout mice. The characterization of these mice will be presented.

INV11 G-protein-coupled-receptors and sepsis

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Severe sepsis is a leading course of acute hospital admissions and often complicates the clinical course of individuals treated for other diseases. At the onset of sepsis bacteria and bacterial products stimulate macrophages and the endothelium to release an array of pro-inflammatory mediators. Among these pro-inflammatory mediators is the most important chemokine for neutrophils, interleukin-8, which attracts neutrophils to the sites of inflammation. In the case of sepsis the activation of neutrophils does not only help to clear the bacteria but also leads to the destruction of organ tissue including endothelium. This overzealous immune response often results in multi-organ failure, disseminated intravascular coagulopathy (DIC) and septic shock, which are potentially deathly sequelae of sepsis. The endothelium loses its anti-thrombotic function and along with the up-regulation of tissue factor, DIC is initiated. Thrombin receptors, namely PAR1 and PAR2, play a pivotal role in the onset of DIC but also mediate septic shock depending on the stage and severity of sepsis.
We developed a new class of G-protein-coupled receptor antagonists consisting of a peptide and a lipid moiety, called pepducins. Pepducins enter the inner layer of the cell membrane and selectively inhibit receptor signaling of any given G protein-coupled receptor they are designed for. Via pepducin technology, we investigated the role of specific G-protein-coupled receptors in sepsis.
We demonstrated that activated and transmigrated neutrophils appear to be the most critical cell type required for the progression of disease in a murine model of sepsis. Specifically, modulation of specific G protein coupled receptor signaling involved in innate immune pathways, like CXCR1 and CXCR2, PAR1 and PAR2, is effective in preventing as well as treating murine sepsis. Moreover, we were able to provide a mechanistic framework why activation of PARs causes septic shock and DIC in early stages of sepsis, but is protective in late and severe stages of the disease.

INV12 The p50 NF-κB subunit is a key regulator of both polarized inflammation and adaptive immune response

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We recently identified the p50 subunit of NF-κB as a key regulator of M2-driven inflammatory reactions in vitro and in vivo and demonstrated that p50-deficient mice show exacerbated M1-driven inflammation and defective capacity to mount allergy and helminth-driven M2-polarized inflammatory reactions. Accumulation of the NF-κB subunit p50 in macrophages as induced by microbial products, such as bacterial LPS, has been previously demonstrated to induce tolerance to the same agonist. Hence, the tolerogenic role of p50 NF-κB was explored also in dendritic cells (DC). I will discuss evidence showing that p50 NF-κB is a master regulator of both innate and adaptive immunity and speculate that proper modulation of the p50 NF-κB activity may be instrumental to reinstate protective inflammatory programs in disease, as well as in increasing the immunostimulatory potential of tumor vaccines based on antigen-pulsed DC.

INV13 Th2 immunity and macrophage activation: where inflammation is anti-inflammatory

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We have been using models of helminth infection to study the functional roles of macrophages activated by the Th2 cytokines IL-4 and IL-13 as well as investigating their origin and regulation. In these models, high numbers of macrophages expressing alternative activation markers such as RELMalpha, Ym1 and arginase are seen at the sites of infection. We have recently demonstrated that in contrast to macrophages during 'classical' inflammation, macrophage accumulation at the site of tissue helminth infection does not involve recruitment of blood monocytes but results from local expansion of the resident F4/80hi population. The absence of recruitment from the blood suggests that the "anti-inflammatory" nature of the Th2 response goes beyond the release of downregulatory molecules and is an intrinsic part of the process itself. The canonical Th2 cytokine IL-4 is the critical factor that drives macrophage expansion in a variety of body tissues and we have now defined the IL-4-responsive cells. Although both alternative activation and proliferation result from IL-4 receptor signalling to macrophages, the processes are independent and likely reflect very different functional pathways and evolutionary origins.

INV14 Macrophage / dendritic cell interaction with collecting lymphatic vessels in the adipose tissue outside of lymph nodes

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Molecules and cells that comprise lymph enter blind-ended lymphatic capillaries for further transport by muscularized collecting lymphatic vessels that can actively pump lymph. Beyond having a pivotal role in antigen transport to lymph nodes for the initiation of adaptive immune responses, collecting vessels are not known to influence innate or adaptive immunity. Anatomically, collecting vessels are prominent within white adipose depots outside the parenchyma of organs. Immunological or inflammatory diseases such as Crohn's, type II diabetes, or HIV are characterized by expansion of particular white adipose depots and by adipose inflammation. Here, using murine models and intravital imaging, we show that endocytic macrophages/dendritic cells localize within the collecting vessel muscular wall and adjacent lumen in a CCR7-dependent manner, allowing them to acquire lymph-derived antigens and in turn mediate inflammatory $CD11c^+$ macrophage accumulation and recall T cell responses in surrounding adipose. Lymph-sampling DCs were also recruited to inflamed lymph nodes from the adipose tissue. Finally, we show that lymph-sampling macrophages/DCs regulate the phenotype of adipocytes in the adipose tissue outside of lymph nodes.

INV15 Dynamics of effector T cell interactions during infection

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CD4 T cells play a crucial role in the control of *Leishmania major*, a protozoan intracellular pathogen. Clearance of the parasite is mainly attributable Th1 effector T cells that induce the production of reactive nitrogen compounds in infected phagocytes in order to degrade the parasite. However, the interactions that pathogen-specific T cells need to establish with infected cells in order to induce these responses are not completely understood. We have investigated the interactions of effector T cells with *Leishmania*-infected phagocytes in a cutaneous infection model. Using two-photon imaging, we could show that antigen-specific T cells undergo long lasting contacts with a minority of infected cells, suggesting limited antigen presentation at the site of *L.major* infection. Nonetheless, we provide evidence that only a fraction of the infected phagocytes was needed to present parasite antigen in order to induce an efficient antiparasitic response at the site of infection. Our results indicate that CD4 T cell responses, in contrast to CD8 T cell responses, largely rely on bystander activity during their effector phase.

INV16 Origin and fate of macrophages: analysis in context

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Tumors can promote the expansion and recruitment of a variety of circulating immune cells. Among them, mononuclear phagocytes can accumulate in large numbers in the tumor stroma, and participate actively in cancer growth. We have developed approaches to interrogate the behavior and function of immune cells in vivo and at different scales, from the whole animal to a single cell. Here I will present some recent findings on cancer-induced mechanisms that orchestrate the mononuclear response in conditional genetic mouse models, and discuss the possibilities to translate these findings clinically.

SUBMITTED ABSTRACTS TOPIC A: REGULATION OF THE MONOCYTE, MACROPHAGE AND DC POOL

A01 The effect of mast cells depletion on thioglycollate-induced peritoneal cells phenotype and function in two inbred rat strains

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The aim of the study was to clarify whether strain differences in the phenotype and function of inflammatory peritoneal exudate cells (PEC) from two inbred rat strains, Dark Agouti (DA) and Albino Oxford (AO), might be connected to their diverse regulation by mast cells. While thioglycollate injection enhanced proportion of granulocytes among PEC in DA rats paralleled by the increase in the PEC phagocytosing ability and the decrease in their capacity to produce nitric oxide (NO) and tumor necrosis alpha (TNFalpha), thioglycollate injection decreased the percentages of granulocytes in AO rats, followed by the increase in both NO and TNFalpha production of PEC. Mast cells depletion during peritonitis in DA rats, opposite to thioglycollate alone, diminished PEC yield and ability to produce hydrogen peroxide, and diminished proportion of granulocytes, ED1+ cells and ED2+ cells bearing H1 receptors. In contrast, mast cell depletion during ongoing peritonitis in AO rat strain exerted mostly additive effects to thioglycollate, observed in the additional increase of the PEC yield, phagocytosis, hydrogen peroxide, NO and TNFalpha production, and the supplementary decrease in the percentages of peritoneal granulocytes. Thus, differences in the regulation by peritoneal mast cells might contribute to the variations in the peritoneal inflammation of DA and AO rat strains (Supported by Ministry of Science, Serbia, Grant 175050).

A02 SuperSAGE characterization of human monocyte subsets

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Monocytes are a heterogeneous cell population with subset-specific functions and phenotypes. The differential expression of CD14 and CD16 distinguishes classical CD14++CD16-, intermediate CD14++CD16+ and non-classical CD14+CD16++ monocytes. Current knowledge on human monocyte heterogeneity is still incomplete: while it is increasingly acknowledged that CD14++CD16+ monocytes are of outstanding significance in two global health issues, namely HIV-1 infection and atherosclerosis, CD14++CD16+ monocytes remain the most poorly characterized subset so far.
We therefore developed a method to purify the three monocyte subsets from human blood and analyzed their transcriptomes using SuperSAGE in combination with high-throughput sequencing. Analysis of 5,487,603 tags revealed unique identifiers of CD14++CD16+ monocytes, delineating these cells from the two other monocyte subsets. Gene Ontology (GO) enrichment analysis suggests diverse immunological functions, linking CD14++CD16+ monocytes to antigen processing and presentation (e.g. CD74, HLA-DR, IFI30, CTSB), to inflammation and monocyte activation (e.g. TGFB1, AIF1, PTPN6), and to angiogenesis (e.g. TIE2, CD105). Functionally, we confirmed proangiogenic capacity, highest ROS-levels and highest capability of CD14++CD16+ monocytes to induce CD4+ T cell proliferation.
In conclusion, we provide genetic evidence for a distinct role of CD14++CD16+ monocytes in human immunity. After CD14++CD16+ monocytes have earlier been discussed as a potential therapeutic target in inflammatory diseases, we are hopeful that our data will spur further research in the field of monocyte heterogeneity.

A03 *Yersinia enterocolitica* impairs dendritic cell development.

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Yersinia enterocolitica (Ye) is a Gram-negative predominantly extracellularly located bacterium that causes food borne acute or chronic gastrointestinal and systemic diseases.
In mice, Ye infection reduces the number of splenic CD8α$^+$ and CD4$^+$ conventional dendritic cellls (cDCs) by 50 and 90%, respectively. The decreased number of cDCs is dependent on TLR4 and TRIF signalling and the result of both faster turn over and suppressed de novo cDC generation.
To address the mechanisms of the suppressed de novo cDCs generation, we analyzed whether Ye infection causes an inhibition of the development of DC-precursors. We analyzed *monocyte and DC precursors* (**MDPs**), *common DC progenitors* (**CDPs**) and *direct precursors for cDCs* (**pre-cDCs**) in bone marrow (BM), blood and spleens of infected mice. Our results show that Ye infection led to a decreased number of all cDCs-precursors analyzed in BM and spleen. This decrease was partially TLR4-dependent and not due to increased cell death. MDPs and CDPs did not migrate out from BM before differentiation. Moreover, BM DC precursors from infected mice proliferated stronger and at the same time there was an increase in BM-monoblasts and pro-monocytes, blood-circulating monocytes and splenic monocytes.
All in all, our results indicate that Ye infection causes a partial depletion of cDC precursors in BM and spleen, but an increase in other myeloid cells that share same precursors, like monocytes. Further experiments are needed to elucidate if Ye induced cDCs-precursors depletion is due to a shift into the production of monocytes by myeloid precursors.

A04 Control of Ly6Chigh monocyte traffic and immunosuppressive activities by atypical chemokine receptor D6

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The atypical chemokine receptor (ACR) D6 is a decoy and scavenger receptor for most inflammatory CC chemokines and prevents the development of exacerbated inflammatory reactions. Here we report that mice lacking D6 expression in the stromal/lymphatic compartment have a selective increase in the number of Ly6C^{high} monocytes in the circulation and in secondary lymphoid tissues. Under inflammatory conditions, Ly6C^{high} and Ly6G^{+} myeloid cells, both recognized by anti-Gr1, accumulate in increased number in secondary lymphoid organs of D6$^{-/-}$ mice. Gr1^{+} myeloid cells derived from D6$^{-/-}$ mice have enhanced immunosuppressive activity, inhibit the development of adaptive immune responses and protect mice from the development of Graft-versus-Host Disease (GvHD). Thus, D6 differentially regulates the traffic of monocyte subsets and controls their differentiation into suppressor cells.

A05 Unraveling the differential impact of 1,25-dihydroxyvitamin D_3 and dexamethasone on human dendritic cells through proteomics, protein networks and pathway analysis

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Tolerogenic dendritic cells (DC) that are maturation-resistant and locked in a semi-mature state are promising tools in clinical applications for tolerance induction through vaccination intervention strategies. Different immunomodulatory agents have been shown to induce a tolerogenic DC phenotype, such as the active form of vitamin D ($1,25(OH)_2D_3$) and glucocorticoids. In this study, we aimed to characterize the protein profile, function and phenotype of DCs in the presence of $1,25(OH)_2D_3$, dexamethasone (DEX), and a combination of both compounds, in view of investigating the protein alterations occurring in these DCs. Human CD14^{+} monocytes were differentiated towards DCs, with/without $1,25(OH)_2D_3$ (10^{-8}M) and/or DEX (10^{-6}M) (n=4). Protein samples were analyzed by 2-dimensional gel electrophoresis and differentially expressed spots ($p<0.05$) were identified using mass spectrometry (MALDI-TOF/TOF). In parallel, morphological and phenotypical analysis was performed, revealing that $1,25(OH)_2D_3$- and combi-DCs are closer related to each other than DEX-DCs. This was translated in their protein profile, indicating that $1,25(OH)_2D_3$ is more potent than DEX in inducing a tolerogenic profile on human DCs. Moreover, we demonstrate that combining $1,25(OH)_2D_3$ with DEX induces a unique protein expression pattern very close to the one of $1,25(OH)_2D_3$-DCs. Finally, protein networks and pathway analysis suggest that $1,25(OH)_2D_3$ has a severe impact on metabolic pathways involving lipids, glucose and oxidative phosphorylation which may affect the production of or the response to ROS generation. These findings provide new insights on the molecular basis of DC tolerogenicity induced by DEX and/or $1,25(OH)_2D_3$, which may lead to the discovery of new pathways involved in DC immunomodulation.

A06 Accumulation of myeloid cells with suppressive functions in mice after exposure to chronic psychosocial stress

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Chronic psychosocial stress has long been recognized as a risk factor for various immunological disorders. Glucocorticoids (GC) and chatecholamines are thought to be major effector molecules affecting the immune state during stress. However, experimental evidence for an altered susceptibility to inflammation and infection due to stressor exposure is still marginal. Chronic subordinate colony housing (CSC) has recently been established as an animal model of chronic psychosocial stress. A decrease in overall GC signaling due to adrenal insufficiency and GC resistance and the development of spontaneous colitis identified CSC as a significant model to investigate stress-induced immune alterations. In the present study we investigated the effect of chronic stress on myeloid cells in primary and secondary lymphoid organs to reveal cellular and molecular mechanisms underlying stress induced alterations in the immune state. In the spleen CD11b^{+} cells increased during CSC. No substantial changes in the composition of myeloid cell were seen in the bone marrow. Further analysis of CD11b^{+} cells revealed an increase of cells depicting phenotypic and functional characteristics of immature myeloid cells; the cells expressed CD11b^{+}Gr1^{+} and suppressed T cell proliferation in vitro.

Immature myeloid cells are discussed as potent immune suppressive cells in inflammation and cancer. Our data show that chronic psychosocial stress resulted in accumulation of CD11b^{+} Gr1^{+} cells with suppressive activity in spleen and bone marrow. Thus, stress-induced immune suppression might contribute to the break-down of the intestinal barrier function and the development of spontaneous colitis.

A07 MACROPHAGE SKEWING BY PHD2 HAPLODEFICIENCY PREVENTS ISCHEMIA BY INDUCING ARTERIOGENESIS

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PHD2 serves as an oxygen sensor that rescues blood supply by regulating vessel formation and shape in case of oxygen shortage[1-5]. However, it is unknown whether PHD2 can influence arteriogenesis. By using hindlimb ischemia as a model, we here studied the role of PHD2 in collateral artery growth, a process that compensates for the lack of blood flow in case of major arterial occlusion. We show that PHD2 haplodeficient ($PHD2^{+/-}$) mice displayed preformed collateral arteries that preserved limb perfusion and prevented tissue necrosis in ischemia. Improved arteriogenesis in $PHD2^{+/-}$ mice was due to an expansion of tissue-resident, M2-like macrophages and their increased release of arteriogenic factors, leading to enhanced smooth muscle cell (SMC) recruitment and growth. Both chronic and acute deletion of one PHD2 allele in macrophages was sufficient to skew their polarization towards an arteriogenic phenotype. Conversely, depletion of M2-like macrophages prevented collateral artery preconditioning and protection against ischemia in $PHD2^{+/-}$ mice. Mechanistically, collateral vessel preconditioning relied on activation of the NF-κB canonical pathway in $PHD2^{+/-}$ macrophages. These results unravel PHD2 control of blood flow and tissue oxygenation by skewing macrophages towards an arteriogenic phenotype in ischemia.

A08 PPARγ-regulated cathepsin D is required for lipid antigen presentation by dendritic cells

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It is well established that dendritic cells (DCs) take up, process and present lipid antigens in complex with CD1d molecules to invariant natural killer T (iNKT) cells. The lipid activated transcription factor, PPARγ, has previously been shown to regulate CD1d expression in human monocyte derived DCs, providing a link between lipid metabolism and lipid antigen presentation. We report that PPARγ regulates the expression of a lysosomal protease, cathepsin D (Catd), in human monocyte derived DCs. Inhibition of CatD specifically reduced the expansion of iNKT cells, and furthermore, resulted in decreased maturation of saposins, a group of lipid transfer proteins (LTPs) required for lysosomal lipid antigen processing and loading. These results reveal a novel mechanism of lipid antigen presentation and identify CatD as a key component of this machinery and firmly place PPARγ as the transcriptional regulator linking lipid metabolism and lipid antigen processing.

A09 Multiple sclerosis is associated with irregular numbers of circulating dendritic cells

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Dendritic cells (DC) belong to the innate immunity and are widely known as professional antigen-presenting cells. Due to their specialized antigen-presenting capacity an important link is provided to the adaptive immune system where they regulate the balance between immunity and tolerance. Recent studies have shown that DC can control autoreactive T cells and even induce regulatory T cells. We hypothesize that a disturbance in DC can ultimately lead to the induction or perpetuation of an autoimmune disease like MS. To test this, an *ex vivo* analysis was performed on DC in peripheral blood of MS patients and healthy controls with flow cytometry.
The frequency of myeloid DC (mDC, $p<0.05$) is changed during the course of MS as compared to healthy controls. Relapsing-remitting MS patients show a reduction of mDC in the peripheral blood, whereas chronic progressive MS patients have an increase in this subtype. Moreover chronic progressive MS patients showed a decrease in CD62L expression on mDC compared to healthy controls. The number of pDC remains the same between MS patients and healthy controls. But a decrease of pDC can be found in a subtype of MS patients which have the haplotype 1 of the IL-7R ($p<0.001$). Furthermore, Patients that are carriers of the HLA-DR15 showed a lower frequency of mDC in the peripheral blood than their negative counterparts. These observations suggest that patients with a particular risk factor could further influence the immunopathogenesis.

A10 ActivinA skews macrophage polarization by promoting a pro-inflammatory phenotype and inhibiting the acquisition of anti-inflammatory macrophage markers

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M-CSF favors the generation of folate receptor β–positive (FRβ), IL-10–producing, immunosuppressive, M2-polarized macrophages [M2 (M-CSF)], whereas GM-CSF promotes a pro-inflammatory, M1-polarized phenotype [M1 (GM-CSF)]. In the present study, we found that activin A was preferentially released by M1 (GM-CSF) macrophages, impaired the acquisition of FRβ and other M2 (M-CSF)–specific markers, down-modulated the LPS-induced release of IL-10, and mediated the tumor cell growth–inhibitory activity of M1 (GM-CSF) macrophages, in which Smad2/3 is constitutively phosphorylated. The contribution of activin A to M1 (GM-CSF) macrophage polarization was evidenced by the capacity of a blocking anti–activin A antibody to reduce M1 (GM-CSF) polarization markers expression while enhancing FRβ and other M2 (M-CSF) markers mRNA levels. Moreover, an inhibitor of activin receptor-like kinase 4/5/7 (ALK4/5/7 or SB431542) promoted M2 (M-CSF) marker expression but limited the acquisition of M1 (GM-CSF) polarization markers, suggesting a role for Smad2/3 activation in macrophage polarization. In agreement with these results, expression of activin A and M2 (M-CSF)–specific markers was oppositely regulated by tumor ascites. Therefore, activin A contributes to the pro-inflammatory macrophage polarization triggered by GM-CSF and limits the acquisition of the anti-inflammatory phenotype in a Smad2-dependent manner. Our results demonstrate that activin A–initiated Smad signaling skews macrophage polarization toward the acquisition of a pro-inflammatory phenotype.

A11 Myeloid-derived suppressor cells isolated from the spleen and tumor of different tumor models display distinct suppressive capacities

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Background: Despite the fact that the immune system recognizes and kills tumor cells, spontaneous tumor regression is rarely observed. One of the mechanisms that counteract tumor-specific immune responses involves so-called myeloid-derived suppressor cells (MDSC), a heterogeneous population of immature myeloid cells. Here, we compared the suppressive capacities of MDSC subpopulations in three different tumor models.
Methods: EG7-OVA, LLC or MO4 cells (10^6) were injected subcutaneously into C57BL/6 mice. After 14 days, single cell suspensions of the spleen and tumor were prepared. MDSC subpopulations ($CD11b^+Ly6G^+$ and $CD11b^+Ly6C^+$ cells) were isolated by cell sorting and cultured in the presence of anti-CD3/CD28 stimulated splenocytes after which the proliferation of $CD4^+$ and $CD8^+$ T cells was evaluated.
Results: In all models, $Ly6G^+$ MDSCs suppress $CD4^+$ T cell proliferation at a 1/8 (MDSC/T cell) ratio. The suppressive effect on $CD8^+$ T cells is less pronounced since proliferation is restored at a 1/4 ratio. Splenic $Ly6C^+$ MDSCs isolated from EG7-OVA tumor-bearing mice do not suppress $CD4^+$ or $CD8^+$ T cell proliferation, while $Ly6C^+$ cells isolated from the spleen of LLC or MO4 tumor-bearing mice can suppress T cell proliferation. In MO4 tumors only $Ly6C^+$ MDSCs can be found and these MDSCs have a stronger suppressive capacity compared to MDSCs isolated from the spleen of MO4 tumor-bearing mice.
Conclusions: We have shown that MDSCs isolated from different tumor models possess distinct suppressive capacities. These results show that it is important to take into account the tumor model and the effector cell population when evaluating the suppressive capacities of MDSCs.

A12 Phenotypical M1 macrophage polarization is positively regulated by MAP kinase ERK1/2

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To investigate the role of ERK1/2 in the phenotypical and functional polarization of human M1(GM-CSF) and M2 (M-CSF) macrophages, the expression of a panel of thirty-three genes, which hallmark both M1 and M2 phenotypes, was analyzed on RNA from macrophages that had been polarized in the presence of the MEK-ERK1/2 inhibitor UO126. ERK1/2 inhibition exerted a more pronounced effect on M1 macrophages, with relevant changes in eleven genes, whereas milder changes were seen on M2 macrophages. These results correlated with a stronger ERK1/2 phosphorylation in M1 macrophages and were confirmed by using an alternative MEK1/2 pharmacological inhibitor, PD98059. UO126 treated-M1 macrophages exhibited higher levels of typical M2 (M-CSF) markers (*c-MAF*, *SERPINB2*, *IGF1*), whereas the M1 marker *INHBA,* which codifies for the M1-polarizing cytokine Activin A, was remarkably downregulated at RNA and protein level. In fact, activin A expression was also reduced upon ERK1/2 inhibition in murine M1 bone marrow-derived macrophages and *ex vivo* isolated thymic macrophages.
The functional relevance of these changes was evaluated by determining K562 tumor growth inhibitory ability and LPS-induced cytokine release of macrophages exposed to the MEK-ERK1/2 inhibitor. UO126 inhibited the tumor growth inhibitory ability of M1 macrophages, in agreement with the lower levels of Activin A produced in the presence of the inhibitor. Besides, IL-6 and TNF-α were also significatively lower in UO126-treated LPS-activated M1 macrophages, thus correlating with their weaker M1 gene expression profile. Therefore ERK1/2 MAPK activation shifts human macrophage towards the acquisition of a pro-M1 phenotypical and functional polarization state.

A13 Differential response to hypoxia by M1 and M2 macrophages: Role of *EGLN3*

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The macrophage functional plasticity allows them to adapt to the surrounding environment. In an effort to elucidate novel therapeutic targets, and since macrophages operate under hypoxic conditions in a number of physiological and pathological settings, we decided to analyze the contribution of hypoxia to macrophage polarization.
To that end, pro-inflammatory and anti-inflammatory macrophages were generated in the presence of either GM-CSF [M1(GM-CSF) macropgages] or M-CSF [M2(M-CSF) macrophages], exposed to hypoxia for 24h and analyzed for their expression of a panel of M1 and M2 markers previously described by our lab (*INHBA*, *FOLR2*, *MAFB*) and their functional capabilities.
We found that the *EGLN3* gene, which codes for the HIF-1α–regulating enzyme Prolyl hydroxilase 3 (PHD3), is a marker for M1 pro-inflammatory macrophages under normoxic conditions. *EGLN3* was induced in M2-macrophages by a 24-h hypoxia treatment, reaching similar levels to those seen in M1 macrophages. The acquisition of *EGLN3* expression in M2 macrophages exposed to hypoxia correlates with changes in macrophage polarization such as 1) Decrease of M2-marker (*FOLR2* and *MAFB*) and acquisition of M1-marker (*INHBA*) expression; 2) Increase in pro-inflammatory cytokines (IL-12 and TNFa) secretion and diminished production of M2 cytokines (IL-10 and CCL2) upon exposure to TLR ligands; and 3) reduced proliferation of KM12c tumor cells by M2-hypoxic conditioned medium. Therefore, EGLN3 expression can be considered as a novel marker for pro-inflammatory M1 (GM-CSF) macrophages, thus implying that M1 and M2 macrophages differ in their ability to adapt to hypoxic environments, and that oxygen levels critically contribute to macrophage polarization.

A14 Human CD1c+ dendritic cells promote tolerogenic T-cell responses

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Background: The small intestinal mucosa of mice contains several dendritic cell (DC) subsets. The CD103+ DCs have been indicated to induce tolerogenic T cells by a TGF-β- and retinoic-acid (RA)-dependent mechanism.
Recent data suggest that DCs residing in human duodenal mucosa mainly originate from circulating myeloid CD1c+ DCs and CD14+ monocytes.
Aim: To assess the potential of blood derived CD1c+ DCs and CD14+ monocytes to induce tolerance in response to TGF-β and RA.
Methods: Myeloid CD1c+ DCs and CD14+ monocytes were pretreated with TGF-β and RA, and their capacity for RA production by the ALDH enzyme was analyzed. CD1c+ DCs and CD14+ monocytes, with or without pretreatment, were co-cultured with allogeneic naïve T cells, followed by analysis of Foxp3 expression and cytokine secretion.
Results: Compared with untreated CD1c+ DCs, RA- and TGF-β1-treated CD1c+ DCs displayed an enhanced ALDH activity. Treated CD1c+ DCs also induced higher Foxp3 expression in naïve T cells, and the co-culture supernatants contained higher levels of IL-10 and the Th2 cytokines IL-5 and IL-13, but no increase of TNF-α, IL-12 or IFN-γ was detected. In contrast, RA- and TGF-β1-treated CD14+ monocytes showed no increase in ALDH activity, induced no Foxp3 expression in naïve T cells, and the cytokine production maintained low.
Conclusion: Our findings demonstrated that RA- and TGF-β1-treated myeloid CD1c+ DCs favour Treg- and Th2 responses, whereas these factors had little or no effect on CD14+ monocytes. Together, this suggests that CD1c+ DCs residing in the human duodenal mucosa may promote tolerogenic T-cell responses under homeostatic conditions.

A15 LEPTIN AFFECTS THE BALANCE BETWEEN TH17 AND REGULATORY T CELLS THROUGH MODULATION OF DENDRITIC CELLS

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Leptin links metabolism and immunity, affecting a variety of immune cells. Our aim is evaluate if leptin affects the balance between Th17 and regulatory T cells (Treg) through modulation of dendritic cells (DC). $Lep^{ob/ob}$ iDC and mDC had lower expression of co-stimulatory molecules and MHCII and by array analysis modified gene expression pattern compared with WT. This phenotype was reversed by leptin. $Lep^{ob/ob}$ iDC induced higher frequency of Treg, Th1 and Th2 and lower Th1 from naïve precursors, compared with WT. *In vivo*, $Lep^{ob/ob}$ displayed higher frequency of Treg compared with WT. $Lep^{ob/ob}$ mDC induced lower $CD4^+$ T cells proliferation and Th1 cytokines production compared with WT. MOG_{35-55} peptide plus Leptin immunized mice showed higher DTH and $CD11c^+$ and lower Treg, Th17 frequency compared with control. $Lep^{ob/ob}$ mice displayed lower EAE. $Lep^{ob/ob}$ presented higher skin graft survival compared to WT. $Lep^{ob/ob}$ mice displayed lower frequency of total $CD4^+$ and $CD8^+$, $IFNg^+CD4^+$ T cells and higher $IL\text{-}4^+CD4^+$, $IL\text{-}17^+CD4^+$, $CD4^+Foxp3^+$ and $CD4^+GATA\text{-}3^+$ compared with WT. Foxp3 mRNA expression were higher in $Lep^{ob/ob}$ grafted skin. $Lep^{ob/ob}$ displayed lower alloreactivity. Leptin receptor deficient ($Lep^{db/db}$) $CD4^+$ reconstituted $Rag^{-/-}$ grafted mice showed higher graft survival compared with WT. $Lep^{db/db}$ $CD4^+$ cells displayed poor competitive homeostatic proliferation in lymphopenic host. In conclusion, $Lep^{ob/ob}$ DC exhibited a tolerogenic phenotype and modulates both Treg and Th17 T cells, *in vitro* and *in vivo*. Leptin affected allograft immune responses and a modified function in both CD4+ and DC cells in $Lep^{ob/ob}$ mice leaded to the higher graft survival.

A16 Definition of histone modification patterns in M1 and M2 primary human macrophages

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Activation of macrophages in response to exogenous signals is guided by transcriptional programs allowing these cells to specifically and adequately respond to these stimuli. Central to transcriptional re-programming is the regulation of histone modifications (HM), which can be interrogated on a global scale by chromatin immunoprecipitation combined with next generation sequencing (ChIP-seq). Applying multiplex ChIP-seq for permissive (e.g. H3K4me3, H4Ac) or repressive (e.g. H3K27me3) HM, we assessed differential epigenomic regulation during classical (M1) and alternative (M2) macrophage activation. To decrease for individual-specific HM, macrophages were generated from at least 3 individuals and data pooled prior to analysis. In both populations (M1 and M2) we identified a significant enrichment for permissive HM 1000 bp upstream of transcription start sites in 5'UTR regions of known genes and to a lesser extend in coding exons. However, when comparing HM in M1 versus M2 macrophages there was an obvious increase of gene loci with permissive HM in M2 macrophages, while the distribution to genomic regions was similar in both populations. Increased permissive HM in M2 was true for promoter regions, 5'UTR regions, and coding exons. To address, whether permissive HM is also associated with increased transcription leading to mature mRNA, we also assessed global gene transcription in these cells. In fact, the total number of transcripts in M2 macrophages exceeded that seen in M1 macrophages by 25%. Further integrating ChIP-seq and transcriptome data support a model of M1 macrophages being more terminally differentiated while M2 macrophages show a more versatile and flexible transcriptional program.

A17 Macrophages as cellular targets for immune modulation in experimental autoimmune type I diabetes

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Antigen presenting cells (APCs) are a focal point in the delicate balance between T cell tolerance and immune responses contributing to the onset of type I diabetes (T1D). Here, we investigated whether macrophages from non obese diabetic (NOD) mice featured an aberrant hyper-inflammatory status which might contribute to the amplification of pathogenic autoreactive T cell responses towards pancreatic β-cells. Indeed, NOD macrophages showed elevated mRNA levels of IL-12p40, TNFα and iNOS as well as effector T cell-recruiting chemokines, CXCL9, CXCL10 and CXCL11, when exposed to danger signals. Similarly, monocytic $Ly6C^{high}CD11b^{high}CD74^{pos}$ cells, exhibited increased responsiveness and intracellular TNFα production upon activation. Moreover, the increasing hyper responsiveness of NOD macrophages as the disease progresses, translated in the elevating ability to activate islet-antigen reactive $CD4^+$ T cells. The physiological importance of this cell subset is further supported by their increased abundance within inflamed pancreatic islets and the prominence of a similar pro-inflammatory signature also in peripheral blood-derived monocytes from T1D patients. In view of interfering in these pathogenic pathways in T1D, natural compounds with known suppressive actions specifically on APCs, were tested to counteract these processes. Preconditioning of macrophages with the bioactive form of vitamin D, $1,25(OH)_2D_3$, resulted in the inhibition of activation-induced pro-inflammatory mediators in an IL-10-dependent fashion and decreased the antigen-specific T cell-stimulatory capacity of the cells both *in vitro* and *in vivo*. Combined, these results highlight the possible therapeutic applicability of this natural immunomodulator in autoimmune diseases, due to its ability to counteract macrophage inflammatory and T cell-activating pathways.

A18 Analysis of Dendritic cell subpopulation migration while pathogenic recognition processes

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Dendritic Cells (DCs) are potent antigen presenting cells which are responsible for initial pathogenic recognition processes. DCs recognize different pathogen associated molecular patterns (PAMPs) expressed by various microorganisms through Toll-like receptors (TLR) and C-type lectin receptors (CLR). Here, we present data on the functional consequences of TLR1, TLR2, TLR3, TLR4, TLR5, TLR7/8, and TLR9 in vivo engagement in comparison to anti CD40 antibody treatment for the two main conventional DC subpopulations in the murine spleen (CD8-33D1+ and CD8+DEC205+). By confocal immunofluorescence analysis we found that bridging area and red pulp localized CD11c+CD8- DCs migrate into the outer T cell area close to the B cell area. In contrast, T cell area localized CD11c+CD8+ DCs migrate even deeper into the T cell area after TLR ligand administration. FACS analyses and CBA assays demonstrate the maturation state and inflammatory cytokine and chemokine production in the sera. Depending on the TLR ligand different migration times and activation states could be defined. We are now investigating the role of these different immune responses in regard to T cell response and humoral immune responses in naïve mice. Our data provide also evidence of a change in antigen presentation by mature tissue DCs.

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A19 CD4+ Innate Lymphoid Cells Control CD8α- Dendritic Cell Homeostasis via the Lymphotoxin-β Receptor Pathway

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Antigen-presenting CD8α- dendritic cells (DC) proliferate within lymphoid tissues under control of the Lymphotoxin-β receptor (LTβR), however the cellular networks providing the trophic signals remain unknown. Using both genetic and pharmacological approaches, we demonstrate the CD4+IL7Rα+CD3- innate lymphoid cells (ILC), also called lymphoid tissue inducer cells, control the homeostasis of CD8α- DC subsets through the LTβR pathway. $RAG^{-/-}$ and wild-type mice exhibited a similar ratio of splenic CD8α+ versus CD4+ DC subsets. However, RAG mice lacking the IL-2, -4, -7, -9, -15 and -21 common cytokine receptor-γc chain ($RAG\gamma c^{-/-}$) exhibited a specific decrease in the CD8α- DC subsets, phenocopying LT-deficient $RAG^{-/-}$ mice, implicating their participation in a common pathway controlling DC homeostasis. Enforced LTβR signaling using an agonist antibody in $RAG\gamma c^{-/-}$ mice restored the proliferative capacity of CD8α- DC subsets, indicating γc-deficiency impacts a LTαβ-expressing non-T and -B cell population in lymphopenic mice. LTβR-signaling further induced clustering of CD4+ DC around the arteriole, wherein ILC reside in intimate contact with DC, revealing a cellular network controlling DC homeostasis. IL-7-induced signaling via γc chain and the nuclear hormone receptor RORγt are required for integrity of the CD4+ ILC population in the spleen. $RAG^{-/-}$ mice deficient for IL-7 and RORγt expression also exhibited a specific defect in CD8α- DC subsets. Transfer of RORγt-expressing CD4+ ILC in $RAG\gamma c^{-/-}$ mice restored splenic CD8α- DC subsets. Together, our results demonstrate a positive role of CD4+ ILC in the homeostasis of conventional CD8α- DC subsets.

A20 NLRP12 drives steady-state granulopoiesis

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The recent identification of cytokine-activating 'inflammasomes' has revolutionised our understanding of molecular circuits linking pathogen/danger sensing, cytokine production and immune system activation in host defence, and dysregulation of these processes in human heritable and acquired disease. Only 3 members of the nod-like receptor (NLR) family are characterised to form inflammasomes, and the functions of many of their close homologs within the NLR family are unknown. A novel NLR, NLRP12, is the closest phylogenetic relative of the inflammasome scaffold NLRP3, shares an identical domain structure, and patients with NLRP12 mutation show similar clinical presentation to NLRP3-dependent hereditary fever syndromes. NLRP12 expression is highly enriched in human and mouse neutrophils, prompting us to examine a role for NLRP12 in granulopoiesis. *Nlrp12* deficiency causes a reduction in neutrophil abundance and maturity in the bone marrow and the circulation. The impact of NLRP12-dependent granulopoiesis on inflammatory responses was investigated by *in vivo* infection with *Leishmania major*, a potent stimulus for neutrophil influx. Indeed, *Nlrp12*-deficient mice exhibited defective neutrophil infiltration upon *Leishmania* infection and decreased lesion size, indicating that NLRP12 performs important and non-redundant functions in host immune responses by supporting granulopoiesis. This is the first study to suggest a function for any NLR in supporting hematopoiesis.

SUBMITTED ABSTRACTS TOPIC B: MONOCYTES, MACROPHAGES AND DCS IN DISTINCT ORGANS AND TISSUES

B01 Phenotypic characterization of antigen-presenting cells in developing prenatal human dermis

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Skin macrophage and dendritic cell precursors migrate into embryonic skin showing a primitive surface marker profile that subsequently matures into the profile of adult antigen presenting cells. Thus, the study of their ontogeny can provide interesting clues about their differentiation.
$CD36^{+}HLA\text{-}DR^{+/-}$ cells of unknown nature have previously been identified in human embryonic skin. To further characterize their phenotype, the expression of selected markers was evaluated in single cell suspensions and on frozen sections of embryonic and fetal human skin. Using flow cytometry, we found that 65.3% of $CD45^{+}$ leukocytes exhibit the scavenger receptor CD36 at 9 weeks estimated gestational age. Expression of CD14 on embryonic $CD36^{+}$ leukocytes is comparable, while HLA-DR is significantly lower than in adult skin. Immunofluorescence staining of embryonic skin sections locates $CD45^{+}CD36^{+}$ cells predominantly in the dermis. Various subsets of dermal $CD36^{+}$ leukocytes can be identified in developing prenatal and adult skin with regard to the expression of CD14, HLA-DR and selected pattern recognition receptors. Similar to adult skin, expression of the C-type lectin receptors CD206 and CD209 is restricted to dermal cells during all stages of prenatal development. Flow cytometric analysis showed that all $CD206^{+}$ leukocytes coexpress CD36 and that they express comparable levels of CD14 but lower levels of HLA-DR than in adult skin.
Taken together, the study of leukocyte ontogeny provides fascinating insights into the differentiation of skin leukocytes. In analogy to what has been found in various mouse models our data suggest that immature skin antigen-presenting cells acquire HLA-DR during development in the skin.

B02 Semaphorin 7A negatively regulates intestinal inflammation by IL-10 production of macrophages via αvβ1 integrin signaling

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The intestinal immune system is constantly challenged by commensal bacteria, so it has to maintain the quiescence with several regulatory mechanisms. Although intestinal macrophages have been implicated in repression of excessive inflammation, it remains unclear how their functions are regulated during inflammation. Here, we demonstrate that Sema7A, a glycosylphosphatidylinositol-anchored semaphorin and expressed in intestinal epithelial cells, induces IL-10 production by intestinal macrophages to regulate the intestinal inflammation. Sema7A-deficient mice showed severe signs of dextran sodium sulfate (DSS)-induced colitis due to reduce IL-10-levels in the intestine. Conversely, administration of recombinant Sema7A proteins to DSS-fed mice ameliorates the severity of colitis, of which effects were diminished by blocking antibodies against IL-10. We further identified $MHCII^{int}F4/80^{hi}CD11b^{hi}$ macrophages as a main producer of IL-10 in response to Sema7A, in which αvβ1 integrin functions as a receptor. Of note, we found that Sema7A is predominantly expressed on the basolateral side of epithelial cells. In addition, bone-marrow chimera mice with Sema7A-deficient intestinal epithelial cells showed severe colitis as it was the case for Sema7A-null mice. Collectively, these findings not only indicate that Sema7A plays crucial roles in suppressing intestinal inflammation through αvβ1 integrin, but also provide a novel inducer for IL-10 through interactions between intestinal epithelial cells and macrophages.

B03 Lung DCs induce TH17 cells that produce TH2 cytokines, express GATA-3 and promote airway inflammation.

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Background: Dendritic cells (DCs) are crucial to shape the adaptive immune response. Extensive *in vitro* manipulation reprograms Th2 and Th17 cell lines into Th1 cells, leading to the concept of $CD4^{+}$ Th cell subset plasticity. The conversion of memory Th17 cells into Th2 or *vice versa* remains to be clarified. **Objective**: We examined the localization of Th17/Th2 cells *in vivo*, their cellular origin (Th2 versus Th17) and the underlying mechanisms that drive the generation of these double Th producers. **Methods:** Ag-loaded-bone marrow-derived DCs (OVA-DCs) were repeatedly administered locally (intra-tracheally) or systemically (intravenously) to naïve mice to elicit chronic airway inflammation. Inflamed lungs and mLNs were examined for the presence of $IL\text{-}17^{+}IL\text{-}13^{+}IL\text{-}4^{+}$ $CD4^{+}$ T cells that co-expressed RORγt and GATA-3 (Th17/Th2). **Results**: We here show that repetitive administration of inflammatory OVA-DCs, locally or systemically, promoted the development of Ag-specific Th17/Th2 cells in lungs and mLNs. Immunized mice developed IgE-independent and steroid-resistant airway inflammation with a mixed neutrophil and eosinophil infiltration of the BALF. Airway inflammatory $SIRP\text{-}\alpha^{+}$ DCs reprogrammed *in vitro*-generated Th17 but not Th2 cells, as well as lung effector Th cells, into Th17/Th2 cells. **Conclusion**: We demonstrate the existence of Th17/Th2 cells that express GATA-3 in inflamed tissues and their Th17 origin. We further propose that repeated immunization with inflammatory DCs dominates over the route of DC administration to drive Th17/Th2-associated chronic lung inflammation. *This work was supported by the Canadian Institute for Health and Research (CIHR Grant, MOP-53152).*

B04 Langerin$^+$ dendritic cell subset in HMD-driven allergic airway inflammation.

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Lung DC bridge innate and adaptive immunity, and depending on the context, induce Th1, Th2, Th17 or tolerogenic responses to inhaled antigens. In our view, these outcomes are not the result of a single population of DC, and instead, subsets of DC might perform specialized functions. In the lung, a distinction can be made between cDC and pDC, where cDCs can be further divided into CD11b$^-$ langerin$^+$ and CD11b$^+$ cells. Here, we studied the role of both DC subsets in the lung in response to inhaled antigens. We found that both subsets (CD11b$^-$ and CD11b$^+$) are able to capture and present inhaled antigen to CD4$^+$ as well as CD8$^+$ T cells in lung draining nodes. Next we addressed the capacity of both subsets to induce allergic asthma. When langerin$^+$ DCs were depleted prior to sensitization with low dose house dust mite (HDM), using Langerin-DTR mice, an increase in eosinophils is seen, compared to a non depleted control mice. Interestingly, our data also show that unlike CD11b$^+$ DCs, CD11b$^-$ DCs sorted from the LNs of HDM-administered animals and adoptively transferred into the airways of naïve recipients, were unable to induce features of allergic airway inflammation. These findings suggest that the CD11b$^-$ subset might have a tolerogenic role and therefore, interfering with their function could constitute a novel form of treatment for allergic diseases.

B05 Down-regulation of the tumor suppressor p16^{INK4a} contributes to the polarization of human macrophages towards an ATM-like phenotype

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Human adipose tissue macrophages (ATMs) display an alternatively activated (M2) phenotype, but are still able to produce excessive inflammatory mediators. However, the processes driving this particular ATM phenotype are not fully understood. Recently, genome-wide association studies associated the CDKN2A locus, encoding the tumor suppressor p16^{INK4a}, with the development of type 2 diabetes. In the present study, p16^{INK4a} expression levels in human ATMs and its role in acquiring the ATM phenotype was assessed.
Hereto, p16^{INK4a} expression was analyzed in ATMs and compared with monocyte-derived macrophages (MDMs) from obese patients or during macrophage differentiation and polarization of monocytes isolated from healthy donors. The role of p16^{INK4a} in MDMs from healthy donors was further investigated through silencing by siRNA or by adenoviral-mediated over-expression of p16^{INK4a}.
In comparison with MDMs, ATMs from obese patients expressed lower levels of p16^{INK4a}. *In vitro*, IL4-induced M2 polarization resulted in lower p16^{INK4a} expression upon differentiation of monocytes from healthy donors in macrophages. SiRNA-mediated silencing of p16^{INK4a} expression in MDMs increased the expression of M2 marker genes and enhanced the response to LPS, resembling the ATM phenotype. By contrast, adenovirus-mediated over-expression of p16^{INK4a} in MDMs diminished M2 marker gene expression and the response to LPS. Western blot analysis revealed that p16^{INK4a} over-expression acts by inhibiting LPS-induced NF-κB signaling.
These results show that p16^{INK4a} inhibits the acquisition of the ATM phenotype. The age-related increase of p16^{INK4a} expression may thus influence normal ATM function and contribute to type 2 diabetes risk.

B06 Resolving the Local Microglial Response From the Inside: Orchestrated Monocyte Trafficking Is Pivotal for Spinal Cord Recovery

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Sequestered from the circulation by the Blood-Brain-barrier (BBB), the central nervous system (CNS) parenchyma, an immune privileged site, was thought to be deprived of the benefits of immune surveillance. The observations of breaches in the BBB together with immune cell invasion and tissue damage in many CNS pathologies, resulted in the assumption that these phenomena are linked and have a detrimental effect. We show that, in contrast to current models that assume uncontrolled monocyte invasion due to impaired BBB function, monocytes are actively recruited to the injured spinal cord through a well-regulated selective mechanism. This orchestrated trafficking of monocytes to the injured spinal cord, which involves specific chemokines and integrins, selects the type of cell recruited and further provides the entering cells with a controlled microenvironment. Close examination of the specific contribution of monocyte-derived cells to the repair process at the site of the trauma revealed that in contrast to the well documented pro-inflammatory and destructive contribution of the microglia (the CNS resident macrophages), the infiltrating monocytes not only do not contribute to the local inflammation but, rather, are needed for the resolution of the innate macrophage response. These monocyte-derived cells display a unique local immunoregulatory role, which is critically dependent upon their expression of the anti-inflammatory cytokine, interleukin 10. This novel function cannot be provided by their resident counterparts, the microglia. These findings are of therapeutic relevance, as increasing the pool of monocytes in the circulation, as well as in the CSF, benefits motor function recovery.

B07 Glial Scar-Monocyte Interplay: A Pivotal Resolution Phase in Spinal Cord Repair

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Monocytes undergo polarized activation, driven by their microenvironment. Given the unfavorable pro-inflammatory milieu within the traumatized spinal cord, a pertinent question is how parenchymal-invading monocytes acquire resolving properties essential for healing. By inhibiting the production of chondroitin sulfate proteoglycan (CSPG), a major constituent of the glial scar, we demonstrated that this matrix, mainly known for its growth inhibitory properties, is in fact a critical component skewing the encountering monocytes towards IL-10 producing (resolving) macrophages. In apparent feedback loop, the monocytes were not only affected by this matrix, but in turn, also regulated its resolution; monocytes were found to produce matrix degrading enzymes and determine scar resolution as demonstrated by conditional ablation of the monocyte-derived macrophages with diphtheria toxin. This apparent cross-regulation between the glial scar and monocytes thus primes the resolution phase of spinal cord repair, thereby providing a fundamental platform for the dynamic healing response. Therefore, refinement of this endogenous self-containing process may open new therapeutic avenues in the treatment of spinal cord injury and other CNS pathologies.

B08 Characterizing dendritic cell sub-populations in the onset of spontaneous colitis in Muc2$^{-/-}$ mice

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The intestinal mucus layer normally keeps luminal bacteria physically separated from intestinal epithelial cells and immune cells. However, mice lacking intestinal mucus (Muc2$^{-/-}$ mice) have bacteria in contact with the epithelial layer and develop spontaneous colitis, a situation that mimics ulcerative colitis. Although dendritic cells (DCs) of the colon likely initiate and maintain T cell-mediated inflammation, little is known about the role of DCs in ulcerative colitis. In this study we use Muc2$^{-/-}$ mice to understand the role of DCs in driving intestinal inflammation as a means to understand the causes underlying ulcerative colitis. We found an overall increase of immune cells, including CD4 T cells and IgA-producing B cells, in the colon of Muc2$^{-/-}$ mice compared to Muc2$^{+/-}$ and WT controls. A significant increase of neutrophils in the lamina propria was also found. Characterizing the lamina propria DC and macrophage compartment revealed significant shifts in cell numbers and ratios in colitic versus non-colitic mice. While colonic macrophages / monocytes and CD103^{+}/CD11b^{+} double positive DC increased, CD103^{+} "migratory" DC decreased in colitic mice. Overall these results provide insight into the immune mechanisms driving colitis and suggest that CD103^{+} DCs leave the lamina propria and drive the chronic inflammation that characterizes ulcerative colitis.

B09 Myelin-phagocytosing macrophages modulate autoreactive T cell proliferation

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Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the central nervous system (CNS) in which macrophages play a central role. Initially, macrophages where thought to be merely detrimental in MS, however, recent evidence suggests that their functional phenotype is altered following myelin phagocytosis. Macrophages that have phagocytosed myelin may be less inflammatory and may exert beneficial effects. The presence of myelin-containing macrophages in CNS-draining lymph nodes and perivascular spaces of MS patients suggests that these cells are ideally positioned to exert an immune regulatory role. Therefore we evaluated in this study the effect of myelin-phagocytosing macrophages on lymphocyte reactivity.
In this study we demonstrate that myelin-phagocytosing macrophages inhibit TCR-triggered lymphocyte proliferation in an antigen-independent manner. The observed immune suppression is mediated by an increase in NO production by myelin-phagocytosing macrophages upon contact with lymphocytes. Additionally, myelin delivery to primarily CD169^{+} macrophages in popliteal lymph nodes of OVA-immunized animals results in a reduced cognate antigen specific proliferation. In contrast to OVA-immunized animals, lymphocytes from MBP-immunized animals displayed an increased proliferation after stimulation with their cognate antigen, indicating that myelin-phagocytosing macrophages have dual effects depending on the specificity of surrounding lymphocytes.
Collectively our data show that myelin phagocytosis leads to an altered macrophage function that inhibits lymphocyte proliferation. Additionally, results from this study indicate that myelin-phagocytosing macrophages fulfill a dual role *in vivo*. On one hand they aggravate autoimmunity by activating myelin-reactive lymphocytes and on the other hand they suppress lymphocyte reactivity by producing NO.

B10 Functional evidence for TOLL-like receptors in modern bony fish

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The vertebrate Toll-like receptors can be subdivided into six major groups. Genetic analysis of several bony fish species, among which the zebrafish and common carp (both Cyprinidae), has confirmed the presence of most but not all mammalian TLR members and also identified some fish-specific TLRs. Although fish TLRs that fall within one of the six groups roughly recognize similar classes of pathogen-associated molecular pattern, there are several properties that are unique to fish TLRs. For example, genome analyses reveal that TLR6 and TLR10 might not be present in fish and suggest that TLR1 can be considered the common ancestor of these mammalian TLRs. Information on ligand recognition by TLR1 should be crucial in providing clues on the exact function of this receptor in fish. Functional data suggest that carp TLR2 may not recognize all ligands from Gram-positive bacteria to the same extent of mammalian TLR2. TLR4 seems present in Cyprinidae only and functional data indicate that it is not involved in LPS recognition. This suggestion is supported by the apparent absence from the zebrafish genome of essential co-stimulatory molecules (MD-2 and CD14). At present, we are studying expression of several TLR genes (TLR1, TLR2, TLR3, TLR4, TLR7, TLR9, TLR20, TLR22) in immune tissues of common carp, in neutrophilic granulocytes and in macrophages. We are expressing fluorescently tagged carp TLRs in fish cell lines to study receptor localization and are expressing carp TLRs in human (HEK) cell lines to study ligand recognition.

B11 Carbon nanotube instillation in mice causes sustained pulmonary inflammation and apoptosis of alveolar macrophages
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Inhalation of nanoparticles has been associated with acute and chronic pulmonary inflammation. The underlying pathways however are mostly unknown. Due to their phagocytotic activity, alveolar macrophages (AM) are considered to play an important role in triggering the toxicological response to particles.
Here, we show that a single exposure of mice to carbon nanotubes (CNT) but not spherical carbon particles (CNP) induces sustained pulmonary inflammation and cell death of AM. Immunohistochemical detection of different apoptotic marker proteins - the general effector caspase-3, as well as the extrinsic Fas-receptor and initiator caspase-8 and the intrinsic caspase-9 - on lavaged cells revealed a significant expression merely in AM of CNT exposed mice. Signal intensity per cell increased for both caspase-dependent pathways over time: initiator caspase-9 and effector caspase-3 show the highest expression after 90 days while Fas expression peaked on day 14. Intriguingly, cell death related protein expression was most abundant for CNT-laden AM (macrophages colocalizing with CNT agglomerates), although particle laden AM where detected till day 90 after CNT and CNP exposure. Similar results where observed in lung sections.
Our data suggest a causal relation of CNT induced apoptotic macrophage cell death and non-resolving, persistent lung inflammation, initiated by phagocytotic uptake of particle agglomerates. Since apoptosis is considered a programmed process of autonomous cellular dismantling that actively inhibits inflammation, further analysis shall focus on the occurrence of necrosis or pyroptosis upon CNT phagocytosis, and their impact to trigger chronic lung inflammation.

B12 Intestinal Dendritic Cells Are Specialized to Activate TGF-β and Induce Foxp3+ Regulatory T-Cells via Integrin αvβ8
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The immune system must be tightly regulated to prevent harmful responses to self or innocuous antigens in health, but be poised to rapidly respond to and eliminate harmful pathogens that enter the body. Such regulation is particularly crucial in the gut, where the bacterial flora constantly challenge the immune system. A key cytokine in maintaining immune tolerance, particularly in the gut, is TGFβ. TGFβ is produced by many cell types, but always as an inactive cytokine that must be activated to have biological function. However, the cellular and molecular pathways that mediate TGFβ activation and maintain intestinal immune tolerance are poorly defined.
Our data now shows that a recently described tolerogenic intestinal dendritic cell (DC) subset, marked by expression of CD103, is specialized to activate latent TGFβ, and that increased TGFβ activation is responsible for enhanced Foxp3+ Treg induction by these cells. Elevated Treg induction induction can occur independently of the vitamin A metabolite retinoic acid (RA), a molecule previously implicated in enhanced Treg induction by CD103+ gut DCs. Importantly, we find that the TGFβ-activating integrin αvβ8 is significantly upregulated on CD103+ intestinal DCs, and that cells deficient in αvβ8 expression lose both their elevated ability to activate latent TGFβ, and their enhanced ability to induce Foxp3+ Tregs *in vitro* and *in vivo*.
Our results therefore identify an important pathway by which TGFβ is activated and regulated in the gut by DCs, which is of great importance in the maintenance of immune homeostasis.

B13 PROTECTIVE ROLE OF APOLIPOPROTEIN E IN AN EXPERIMENTAL MODEL OF ACUTE RENAL ALLOGRAFT REJECTION
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Numerous leukocytes accumulate in the vasculature of rat renal allografts during acute rejection. Most of them are activated, cytotoxic monocytes. Previous microarray data from our group suggested that Apolipoprotein E (ApoE), an anti-inflammatory protein also involved in lipid metabolism, is up-regulated in graft blood leukocytes during reversible acute rejection. In this study, we test the hypothesis that ApoE attenuates acute renal allograft rejection. The Dark Agouti or Brown Norway to Lewis rat strain combination was used to investigate fatal acute rejection. In addition, Fischer 344 kidneys were transplanted to Lewis rats to study reversible acute rejection. Isograft recipients and untreated Lewis rats were used as controls. ApoE mRNA expression was tested in intravascular leukocytes accumulating in blood vessels of renal grafts and in graft tissue. ApoE protein levels were assessed in graft tissue and in plasma. In line with the microarray data, intravascular graft leukocytes and renal tissue obtained from animals undergoing reversible acute rejection expressed increased levels of ApoE mRNA, whereas during fatal rejection, ApoE expression remained unchanged. On the protein level, no changes in ApoE were seen in graft tissue and in plasma. However, we do not know if local leukocytic ApoE expression results in increased ApoE concentrations inside graft blood vessels. To test the protective potential of ApoE, recipients of Brown Norway kidneys were treated with ApoE-mimetic peptide. Preliminary data suggest that this treatment can reverse fatal acute rejection. ApoE may play a protective role in acute organ rejection and may have a therapeutic potential.

B14 Context Dependent Differentiation of Resident and Inflammatory Intestinal Macrophages from Identical Monocyte Precursors

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Macrophages (mϕ) are essential for homeostasis and protective immunity in the intestine, but are also important drivers of the pathology in inflammatory bowel diseases. It is not clear whether these different functions are due to distinct populations of mϕ, or if the same cell can alter depending on the circumstances. We show here that two populations of mϕ exist within the resting mouse colon, characterised by the levels of CX3CR1 expression. Most mϕ in resting mouse colon express CX3CR1 at much higher levels than any other tissue mϕ and also express CD11c. These resident mϕ produce a balanced mixture of TNFα and IL10 constitutively, but respond poorly to further stimulation via TLR ligation, despite expressing high levels of all TLR. During acute DSS colitis, mϕ expressing lower levels of CX3CR1 come to dominate. These "inflammatory" mϕ produce TNFα predominantly and respond to TLR ligation. Using a combination of gene profiling, immunophenotyping and adoptive transfer experiments, we show that CX3CR1^{int} mϕ in both healthy and inflamed intestine are derived from Ly6C^{hi} CCR2^{+} monocytes that upregulate CX3CR1 after entering the intestine, acquiring F4/80 and class II MHC expression in a sequential transition. However under resting conditions, the newly arrived monocytes undergo further differentiation, becoming CX3CR1^{hi}, producing IL10, expressing CD163 and CD206 and becoming TLR unresponsive. These results provide the first evidence that "resident" and "inflammatory" mϕ in the intestine may be derived from the same monocyte precursor whose ultimate fate is determined locally and depends on the presence or absence of inflammation.

B15 Antibody Binding To Porcine Sialoadhesin Reduces Phagocytic Capacity Without Affecting Other Macrophage Effector Functions

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Sialoadhesin (Sn, CD169, Siglec-1) is a macrophage-restricted receptor involved in cell-cell, cell-matrix and cell-pathogen interactions and was recently shown to be involved in signaling. Lately, sialoadhesin is gaining interest as a potential target for immunotherapy. A variety of ligands is proposed, however, little is known about the effect of ligand binding to sialoadhesin on macrophage effector functions. Therefore, this study aimed to determine whether ligand binding to sialoadhesin can alter macrophage effector functions. We investigated the effect of monoclonal antibody (mAb) binding to porcine sialoadhesin on macrophage viability, reactive oxygen species production, phagocytosis of microspheres, uptake and processing of soluble antigens, MHC I and MHC II cell surface expression and cytokine production *in vitro*. This was done by a treatment of porcine primary alveolar macrophages with the sialoadhesin-specific mAb 41D3, or an isotype-matched control mAb 13D12. Both dose- and time-dependent studies were performed. In this study, it was shown that antibody binding to porcine sialoadhesin has no significant effect on most macrophage effector functions studied, except for a significant reduction in phagocytic capacity. With increasing dose of the sialoadhesin-specific mAb, the percentage of macrophages phagocytosing beads decreased markedly. It was observed that antibody binding to sialoadhesin caused a decrease, maintained over time, in the percentage of macrophages phagocytosing beads and the number of beads that were phagocytosed per cell. In conclusion, this study demonstrates that ligand binding to sialoadhesin can prime macrophages to undergo a downregulation in phagocytosis, which could have implications on homeostasis, infectious and immune diseases, and immunotherapy.

B16 Beta Cell Regeneration Depends on the Recruitment of M2-like Macrophages in the Injured Pancreas

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Diabetes mellitus is a disease characterized by an absolute (type 1) or relative (type 2) insulin deficiency which results in subsequent hyperglycemia and increased risk for cardiovascular disease. The current treatment via oral antidiabetic drugs and/or insulin injections is insufficient to adequately control glycemia, resulting in secondary complications that severely lower the quality of life of diabetic patients. Cues to protect the residual beta cells and stimulate the generation of new ones in the adult pancreas are therefore urgently needed.
Recently, we discovered that intrapancreas transplantation of a GFP-labeled hematopoietic stem cell line completely restored the beta cell mass of diabetic mice. Interestingly, CD11b+GFP- myeloid cells infiltrated the injured pancreas shortly after stem cell transplantation and were located in the vicinity of SDF1+ seRM26 cells, SDF1+ duct cells, and injured beta cells. Flow cytometry analysis showed that the vast majority of these myeloid cells were F4/80+ macrophages expressing the chemokine-receptor CXCR4 and the M2 markers CD206 and MGL. In addition, qRT-PCR illustrated that these cells abundantly expressed the M2-associated gene arginase, while almost completely lacking expression of the M1-associated gene iNOS. Moreover interfering with the recruitment and retention of these macrophages via administration of clodronate-loaded liposomes and the CXCR4 antagonist AMD3100, respectively, completely inhibited the beta cell regeneration process.
Overall, these results indicate for the first time that M2 polarized macrophages play an essential role during beta cell regeneration.

B17 Effects of airway epithelium on dendritic cell antigen-uptake and maturation - assessed by an *in vitro* model system

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Airway epithelial cells (AECs) are among the first cells to encounter inhaled antigens and are suggested to be involved in immune homeostasis. To define the molecular mechanisms involved, we have investigated the impact of AECs on LPS-stimulated dendritic cells (DCs) together with uptake of allergen through the epithelial cell layer.
16HBE14o$^-$ epithelial cell lines are allowed to polarize and form a monolayer on culture inserts. Polarization is verified by confocal microscopy analysis and Trans-Epithelial-Electrical-Resistance (TEER) measurements.
Monocyte derived DCs were either allowed to adhere to the basolateral side of the AEC or cultured in AEC-conditioned supernatant with LPS or FITC-labeled allergen added to either apical or basal side of AECs for 24h before flow cytometric analysis.
When DCs were maturated in contact with epithelial cells flowcytometric analysis showed a decrease of the positive co-stimulatory molecules CD86. Expression of inhibitory co-stimulatory marker PD-L1 was simultaneously upregulated. Initial cytokine analysis showed increased levels of IL-10 and IL-6, while TSLP production was lowered when DCs were LPS-stimulated with AEC compared to controls.
There is a time- and dose-dependent DC uptake of allergen through the AEC layer, showing that antigens and allergens are able to pass through the epithelium. The AECs do act as a competent physiological barrier and only allow only smaller quantities of allergen to be sampled by the DCs.
We conclude that co-culturing of DCs with polarized AECs leads to a cell-to-cell contact dependent dampening of DC maturation, which seems to lead to a more tolerogenic profile of DCs.

B18 Study of tolerogenic dendritic cells in autoimmune thyroid disorders

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Background: The autoimmune thyroid diseases (AITD) form a group of autoimmune, organ-specific diseases, including primarily Hashimoto's thyroiditis (HT) and Graves' disease (GD). Dendritic cells (DCs) interacting with T lymphocytes could play a part in the pathogenesis of these diseases.
Objectives: To assess, in peripheral blood cells (PBMC) and thyroid tissue from AITD patients, the role of tolerogenic DCs, including the expression and function of inhibitory receptors such as ILT/CD85, PDLs, GITRL, CD162, CD69, and the synthesis and action of the main cytokines involved.
Patients & Methods: We obtained PBMC from patients with HT (n = 20), GD (n = 30), and healthy subjects (n=20). In addition we isolated thyroid infiltrating cells from eight AITD patients. The presence of myeloid (mDCs) and plasmacytoid DCs (pDCs), as well as expression of inhibitory molecules was determined by flow cytometry.
Results: The population of pDCs in peripheral blood was significantly decreased in patients compared to controls (p=0.001), while no differences were detected between cases and controls for mDCs. Significant differences were also found in the expression of several inhibitory receptors.
Conclusions: Our results suggest that pDCs could play an important role in the progression of AITD. A comprehensive characterization of the tolerance inducing mechanism of DCs in AITD patients would increase our knowledge of the autoimmune processes involved and allow the development of better treatment strategies.

B19 Origin of the Large and Small Peritoneal Macrophages

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Background: Recently, two phenotypically and functionally distinct subsets of peritoneal macrophages were identified: namely large and small peritoneal macrophages (LPM and SPM) (Ghosn et al., 2009). While it has been shown that in inflammatory at least SPM originate from blood monocytes, the origin of LPM and whether the classic (Ly6C+CD62L+CCR2+CD43-CX3CR1-) or non-classic (Ly6C-CD62L-CCR2-CD43+CX3CR1+) monocyte subsets exclusively give rise to LPM or SPM is not known. Using transfer of sorted classic or non-classic monocytes into mice with thioglycollate-induced peritonitis we investigated role of each subset in the development of LPM and SPM.
Methods: Classic and non-classic monocytes from *Cx3cr1*-gfp mouse were isolated and injected (i.v.) into thioglycollate-injected mice. Blood, spleen and peritoneal cells were harvested and analyzed by flow cytometry 24 and 48 hours later.
Results: In the blood, 24 hours after transfer of the classic monocytes they were Ly6C+, however, 48 hours later they all have become Ly6C-. In the spleen, both classic and non-classic monocytes had phenotype of Ly6C^{low} monocytes/macrophages (CD11b+Ly6C-CD115-CD11c-NK1.1-Ly6G-). Most importantly, cells derived from both classic and non-classic monocytes were found in the peritoneal cavity. Classic monocytes gave rise to the cells with LPM phenotype while non-classic monocytes differentiated into SPM. The expected difference in size and expression of CD11b, F4/80, Ly6C and MHC II was observed.
Conclusion: Using the transfer of sorted classic and non-classic monocytes we found that upon entrance into inflamed peritoneal cavity they differentiate into LPM and SPM, correspondingly. This study provides additional information about the relationship between monocyte and tissue macrophages subsets.

B20 The balance between monocyte-derived cells and conventional migratory dendritic cells determines the severity of T-cell-mediated colitis

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The large intestine contains conventional dendritic cells (cDCs), monocyte-derived DCs (MoDCs) and monocyte-derived macrophages (MΦs), but a clear definition of these cells has been plagued by the lack of specific markers to delineate them. As a result, while proposed functional specialization of these cells calls for the evaluation of their respective role during pathogenic responses, it has been challenging to properly distinguish them during inflammation. Here, we describe a novel 10-color gating strategy, validated by the use of CCR2KO vs WT mixed-bone-marrow-chimera, that allows the unequivocal identification of cDCs and monocyte-derived cells in the steady-state and during inflammation. This analysis underlines that large intestine MHCII+ monocyte-derived cells constitute a homogeneous population that should not be split in MoDCs and MFs based on CD11c expression. By applying our gating strategy during T-cell mediated colitis, we conclude that Tregs do not impair the recruitment and differentiation of monocytes but efficiently limit their nitric-oxide production. Moreover, we demonstrate that retinoic-acid-producing cDCs remain poor inducers of effector T cells and that monocyte-derived cells are the most efficient inducers of IFNg production by effector T cells during T-cell-mediated colitis. Finally, impaired migration of cDCs to the mesenteric lymph nodes leads to elevated IFNg production by effector T cells, increased nitric-oxide production by monocyte-derived cells and the development of more severe colitis. Altogether, we conclude that the balance between tolerogenic retinoic-acid-producing cDCs and pro-Th1 monocyte-derived cells in the mesenteric lymph node determines the activation of effector T cells and the severity of T-cell-mediated colitis.

B21 MDSC subpopulations differentially affect distinct aspects of CD8$^+$ T-cell activation

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Both in humans and mice, tumour growth coincides with an accumulation of myeloid-derived suppressor cells (MDSC). In the case of mice, the aforementioned cells consist of two subpopulations: monocytic CD11b$^+$CD115$^+$Ly6G$^-$ MO-MDSC and granulocytic CD11b$^+$CD115$^-$Ly6G$^+$ PMN-MDSC. In the present research, we illustrate that EG7 tumor-induced MO- and PMN-MDSC affect distinct aspects of early CD8$^+$ T-cell activation, though each on a different manner. Although both MDSC populations suppress antigen-driven T-cell proliferation, only the PMN-MDSC augment IFN-γ production on a per cell basis. MO-MDSC, on the other hand, suppress IFN-γ production in early activated CD8$^+$ T cells. However, if cell-contact is abrogated, then MO-MDSC also augment IFN-γ-production while suppressing T-cell proliferation. In addition to modulating proliferation and cytokine production, MDSC also alter the expression levels of several pivotal membrane proteins. This implies that MO-MDSC augment Fas-expression which renders activated T cells more sensitive to Fas-mediated apoptosis. Furthermore, MO-MDSC result in a down-regulation of the IL-2Ra chain (CD25) on CD8$^+$ T cells which possibly contributes to their anti-proliferative capacity. In addition, both MDSC subpopulations differentially modulate CD43, CD44, CD62L and CD162, all crucial molecules involved in lymphocyte migration. Finally, while MO-MDSCs alter the expression of CD45RB and CD71, the presence of PMN-MDSCs leads to a transient upregulation of CD80. Functional consequences of the aforementioned alterations are under investigation. In short, our data demonstrate that MDSC subsets modulate distinct CD8$^+$ T-cell activation characteristics, some of which are even stimulated.

B22 Role of CCRL2 in the pathogenesis of mouse experimental arthritis

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Rheumatoid Arthritis (RA) is an autoimmune-mediated disease characterized by chronic inflammation and leukocyte recruitment into the inflamed joints. Chemokines and chemokine receptor activation are known to represent a major component of the effector proteins involved in the onset of RA.
CCRL2 [Chemokine (CC motif) receptor-like 2], also known as L-CCR, is a seven transmembrane protein that show a high homology degree with many members of the CC chemokine receptor family. CCRL2 possesses a non-canonical DRYLIVE motif in the second intracellular loop that makes it to resemble like a nonsignaling chemotactic receptor (e.g. D6, DARC, CCX-CKR). CCRL2 is expressed by LPS-stimulated murine macrophages, neutrophils, mast cells, dendritic cells, glial cells, astrocytes and microglia and is up-regulated in human RA synovial neutrophils.
In order to elucidate the role of CCRL2 in RA, CCRL2 KO mice were tested in the model of collagen-induced arthritis. Only few CCRL2 KO mice displayed the pathological signs characteristic of arthritis with reduced leukocyte infiltration, synovial hyperplasia and joint erosions and with a significant reduction in visual scoring and paw thickness. Moreover, CCRL2 KO mice showed a marked delay (about ten days) in the onset of the disease and histological features reminiscent of recent joint damage. Conversely, the levels of specific anti-collagen II IgG in the serum were similar in CCRL2 KO and control mice. The mechanisms underling the protection of CCRL2 mice in RA models are currently under investigation and will help to define whether CCRL2 may represent a new therapeutic target for RA.

B23 Expression of the complement receptor CRIG on intestinal macrophages

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A complement receptor of the immunoglobulin superfamily (CRIg, also described as Z39Ig) were recently identified as a novel receptor for complement fragments (C3b and iC3b). In human and murine tissues, it has been reported that CRIg expression is restricted on resident tissue macrophages such as kupffer cells, alveolar macrophages and peritoneal macrophages. The complement system serves an important role in clearance of pathogens, immune complexes, and apoptotic cells. However, complement receptors involved in this process have not been fully identified in intestine. In this study, we show the distribution of CRIg expressing macrophages in human and murine intestine and the role of these macrophages in the homeostasis of intestine.
We identified the expression of CRIg in human and murine large intestine but not in small intestine. These macrophages had the iC3b binding capacity through CRIg.
Intestinal macrophages from TNBS induced Crohn's disease model had decreased CRIg expression but increased CD11b expression. The intraperitoneal administration of the anti-CRIg antibody removed most macrophages in large intestine but not kupffer cells and caused interstitial edema, grandular dilatation, atrophic mucosa in large intestine.
These results show that CRIg is a new marker of macrophages and a dominant component of the phagocytic system responsible for rapid clearance of C3-opsonized particles in large intestine. Further studies concerning the function of CRIg expressing macrophages will contribute to a better understanding of pathophysiology in intestinal inflammatory diseases.

B24 DC accumulating in the NOD pancreas prior to insulitis are abnormal and easily turned into pro-inflammatory cells

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The non-obese diabetic (NOD) mouse is a widely used animal model of autoimmune diabetes. Before the start of the lymphocytic insulitis, dendritic cells (DC) accumulate at the islet edges. The phenotype of these early accumulating DC has not been studied and we here present a detailed phenotypic characterization of these local DC. Also we investigated the proneness of these cells to turn into pro-inflammatory cells.
The pancreas was isolated from 4 week old NOD and C57BL/6 control mice followed by flow-cytometric analysis. Isolated DC from NOD and C57BL/6 pancreati were cultured with LPS followed by analysis of mRNA expression for IL10, IL12, TGFβ and TNFα. TNFα protein expression was analyzed in pancreas lysates of NOD and C57BL/6 mice at different ages.
DC in the murine pancreas could be divided in a CD8α- and CD8α+ subset. In the NOD and C57BL/6 pancreas CD8α+ DC expressed CD11c, CD11b, CD103 and CCR7. However, CD8α+ DC in the NOD pancreas had a lower expression of CD86, Langerin and CD200R3. After LPS stimulation, CD8α+ NOD pancreas DC expressed less IL10 and more TNFα than C57BL/6 DC. Also the protein level of TNFα was significantly higher in NOD pancreas lysates than in C57BL/6 lysates at 5 weeks of age.
This data show that early accumulating CD8α+ DC in the NOD pancreas (prior to lymphocytic infiltration) are abnormal and more prone to turn on a pro-inflammatory program. This observation is in line with the view that abnormalities in DC drive the autoimmune process in this animal model.

B25 Elevated levels of free fatty acids are sensed by human primary macrophages thereby inducing specific transcriptional programs

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Chronic inflammation in adipose tissue is associated with macrophage infiltration and a switch from anti-inflammatory M2 to pro-inflammatory M1 macrophages. The impact of elevated expression levels of inflammatory cytokines including IL-6 and TNFα on early steps of obesity-associated inflammation is not well understood. Here we asked the question whether elevated levels of fatty acids, a hallmark of obesity, are sensed by macrophages thereby inducing their activation and whether such activation is associated with a M2 to M1 switch. Using human primary macrophages as the model, we exposed these cells to increasing concentrations of saturated (SFA) or unsaturated (USFA) fatty acids (FA). Uptake of FAs was demonstrated by a FA-associated dose-dependent increase of lipid droplets, development of foam cell characteristics and reduced metabolic activity. Transcriptome analysis 24 hrs post FA exposure clearly revealed that macrophages sensed FAs reacting with a FA-specific transcriptional program including the induction of several hundred genes. Hierarchical cluster and principle component analysis demonstrated that SFA- as well as USFA-induced cellular programs differed significantly from classical M1 and M2 programs. These data were corroborated by assessment of cell surface markers associated with the M1 or M2 phenotype. A hallmark of FA-mediated activation of human macrophages was the induction of genes associated with lipid uptake, handling and accumulation but also cytokine expression. We conclude that sensing of elevated FAs is directly inducing FA-specific transcriptional responses without the requirement of classical inflammatory stimuli. Therefore, conceptually the induction of obesity-associated autoinflammation might be a consequence of unphysiologically elevated fatty acids.

B26 Role of dendritic cells in the invasion and dissemination of *Yersinia enterocolitica*

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Yersinia enterocolitica (Ye) is an extracellular bacterium that enters the host via contaminated food and causes acute and chronic gastrointestinal diseases. After colonisation of the small intestine the bacterium invades the peyer's patches (PP) via M cells and disseminates to the mesenteric lymph nodes (MLN), spleen and liver.
We assume that Ye uses DCs to disseminate from the intestine. By means of an oral mouse infection model and various transgenic mice we analysed the different invading and dissemination routes via PP and CX_3CR1^+ cells in the lamina propria. Lymphotoxin beta receptor knockout ($LT\beta R^{-/-}$) mice that lack PP and MLN showed no difference in survival compared to wildtype mice after Ye infection, indicating that PP are dispensable for survival. $CX_3CR1^{-/-}$ mice (absence of phagocytes that extend their dendrites in the intestinal lumen to sample directly luminal antigens) infected with Ye survived longer compared to wt mice. In contrast, DC depleted mice died as early as 3 days post infection. Death of mice was associated with bacterial dissemination via the bloodstream to the spleen. Histological analysis of the small intestine showed RFP expressing Ye in close contact to CX_3CR1^+ DCs 1 h post infection.
Altogether these data indicate that dissemination of Ye takes place by an alternative route independent of PP and that DCs are essential for the survival upon oral Ye infection.

B27 Can *in vitro* alveolar macrophage models aid in early risk assessment?

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There are many regulatory hurdles specific to inhaled drug development and methods to assess risk early are important to save both costs and time.
Resident alveolar macrophages are a normal part of the lung infrastructure and are primarily involved in host defence from both microbial attack and from environmental pollutants, where such materials are phagocytosed and cleared from the lung.
To reassure regulatory authorities that these responses are part of the normal clearance mechanism and do not represent a risk to patients when delivering inhaled drugs to the lung, a better understanding of how macrophages behave in response to drug substance is required, both at the cellular and tissue level. This will enable selection of molecules with a reduced risk of inducing a pathological response.
The aim of the current work presented is to assess risk, prior to *in vivo* inhalation studies, based upon data obtained from *in vitro* alveolar macrophage models. The goal is to enable the identification of potential cellular liabilities early in the development of drug candidates and ultimately reduce attrition.
Two cell culture systems were used to study alveolar macrophage responses; a rat alveolar macrophage cell line NR8383 and human monocyte derived macrophages differentiated with M-CSF. These cells were exposed to a variety of inhaled compounds and assessed for changes in viability and morphology.
The data generated from *in vitro* assays will be compared to *in vivo* pathology outcomes to determine whether *in vitro* models can aid early candidate selection.

B28 Splenic $CD169^+$ macrophages facilitate the activation of adaptive immunity to blood-borne antigens

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The spleen is the lymphoid organ that generates adaptive immune responses to antigens present in the blood. The blood enters the spleen in the marginal zone, where two subsets of macrophages characterized by CD169 or SIGNR1 expression efficiently take up antigens. $CD169^+$ macrophages line the white pulp containing the B and T cells, whereas $SIGNR1^+$ macrophages are located towards the red pulp. Both type of macrophages have been implicated in the eradication of blood-borne pathogens, but have not been described to be involved in the activation of adaptive immune responses.
We have conjugated ovalbumin (OVA) antigen to monoclonal antibodies specific for CD169 and compared targeting to $CD169^+$ macrophages to targeting to dendritic cells with respect to activation of adaptive immune responses. Targeting OVA antigen to $CD169^+$ macrophages resulted in very efficient induction of OVA specific $CD8^+$ T cell, $CD4^+$ T cell and B cell responses. We showed that OVA targeted to $CD169^+$ macrophages was transferred to dendritic cells that can activate T cells. Currently, we investigate the process of $CD169^+$ macrophage mediated B cell responses.
Our results indicate an important role for $CD169^+$ macrophages in the capture of blood-born antigens and the activation of both cellular and humoral adaptive immune responses. We propose that $CD169^+$ splenic macrophages have a similar role as $CD169^+$ subcapsular sinus lymph node macrophages and take up antigens and transfer these to both dendritic cells and B cells and thereby facilitate the induction of adaptive immune responses.

B29 Development of pro-arteriogenic monocytes as therapeutic cell product for vascular regeneration and tissue repair

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Collateral artery formation (arteriogenesis) may prevent tissue damage caused by arterial stenosis and is critically important to limit the consequences of coronary and peripheral arterial disease. This naturally occurring process of neovascularization is supported by hematopoietic progenitor cells such as monocytes, which are recruited to sites of early ischemia, and can be boosted by mobilization or transfusion of these cells.
We previously described the generation of neovascularization-promoting pro-arteriogenic monocytes by conditioning cells with activated-T-cell-conditioned Endocult® medium. Transfusion of these pre-stimulated human monocytes, but not that of unstimulated monocytes, significantly improved blood flow recovery after hind limb ischemia in nude mice and increased both collateral size and number in the post-ischemic tissue. Pro-arteriogenic monocytes are phenotypically distinct from monocyte-derived macrophages and dendritic cells and form clusters that are comparable to colony-forming unit (CFU)-Hill colonies, a measure for vascular function and cumulative cardiovascular risk.
To develop pro-arteriogenic monocytes as applicable therapeutic cell product we aim to delineate those factors that drive pro-arteriogenic differentiation. Luminex analysis of the T-cell-conditioned medium combined with the parallel assessment of the effects of size-fractionated medium highlighted 8 potentially important factors involved in Hill-associated cluster formation. Interestingly, one of these factors was the pro-inflammatory cytokine interferon-γ (IFNγ), a central mediator of atherosclerosis-associated inflammation. Using purified IFNγ and IFNγ-neutralizing antibodies we found that the presence of IFNγ is essential for cluster formation. Our current attention is therefore on the importance of IFNγ in pro-arteriogenic cell differentiation and the ambivalent role this cytokine may play in neovascularization and tissue repair.

B30 Dynamic Regulation of Hyaluronan Binding by Macrophages

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Hyaluronan is a glycosaminoglycan present in virtually all tissues as a major component of the extracellular matrix. Injury and infection result in the breakdown of hyaluronan into fragments which can stimulate an inflammatory response. As part of the resolution of inflammation, these fragments are removed and full-length hyaluronan is restored. CD44 is the major surface receptor for hyaluronan on leukocytes and although CD44 is expressed on macrophages, their ability to bind hyaluronan is tightly regulated. The aim of this study is to understand when and why macrophages bind hyaluronan. Macrophages exhibit considerable plasticity, their phenotypic and functional heterogeneity is often influenced by changes in the surrounding environment. Tissue specific macrophages differ in their ability to bind hyaluronan; peritoneal and lung interstitial macrophages bind low or no detectable levels of hyaluronan whereas alveolar macrophages constitutively bind high levels of hyaluronan. In addition, the percentage of hyaluronan binding cells in the alveolar space is dramatically reduced during LPS induced lung inflammation and is restored upon resolution of inflammation, suggesting a function for hyaluronan binding under homeostatic conditions. However, bone marrow-derived macrophages do not bind hyaluronan but are induced to bind when activated with LPS/IFNγ (M1). Alternative activation of macrophages with IL-4 (M2) also induces hyaluronan binding, but to a lesser extent. Hyaluronan binding by peritoneal, M1 and M2 macrophages is dynamically regulated by both CD44 expression and chondroitin sulfation. Thus macrophage plasticity is accompanied by changes in hyaluronan binding, which is regulated by both the local environment and exposure to specific stimuli.

B31 Regulating Complement Expression in Retinal Pigment Epithelial (RPE) Cells by M1 and M2b Macrophages

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Age-related macular degeneration (AMD) is the largest cause of blindness in the elderly in developed countries. Complement activation is believed to play an important role in AMD pathology. The underlying mechanism, however, remains elusive. We have shown previously that retinal aging is accompanied by a low-grade of complement activation and a small population of macrophage activation at the retina-choroidal interface. Activated macrophages are in close contact with retinal pigment epithelial (RPE) cells at the retina-choroidal interface. The aim of this study is to understand how complement activation at the retina-choroidal interface is affected by macrophage-RPE cell interaction. When bone marrow-derived macrophages (BMDMs) were incubated in the eye-cups containing RPE cells, BMDMs expressed high levels of arginase-1, iNOS, IL-1b, IL-6 and IL-10 genes, a phenotype that is similar to M2b macrophages. In vitro cultured M1 (by LPS + IFN-g) and M2b (IL-4 + LPS) macrophages expressed high levels of complement components C1r, C3, C4 and factors B (CFB) the C1 inhibitor (C1INH), and lower levels of regulatory factors (CFH, CD59, and DAF) as compared to M0 and M2a macrophages. Furthermore, the supernatants of M1 and M2b macrophages significantly increased the expression of C1r, C1s, C2, C3, C4 CFB and CFH genes in RPE cells. Our results suggest that M1 and M2b but not M2a macrophages are involved in complement activation under inflammatory conditions. In the aging eye, subretinal macrophages are M2b-like cells, and together with RPE cells they play an important role in age-related retinal complement activation.

B32 Role of Macrophages and Endoplasmic Reticulum Stress in an Experimental Model of Renal Disease

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Tubulointerstitial nephritis (TIN) is a primary injury to renal tissue, resulting in decreased renal function, extensive tubular dilation, and inflammation; macrophages seem to be important in this disease. One of the mechanisms that could be involved in macrophage activation must be induction of endoplasmic reticulum stress (ERS). Heme oxigenase-1 (HO-1) is a cytoprotective molecule and its upregulation could be beneficial. The aim of this work was to evaluate the role of macrophages as well as its modulation on TIN. To generate experimental NTI we used the model of adenine enriched food. C57/Bl6 (WT) mice and MIP-1α, CCR2, CCR4 and CCR5 KO mice were fed with this modified food. WT animals also were treated with clodronate liposome or with Hemin, an HO-1 inducer. At sacrifice time, blood and renal tissue were collected for renal function, histopathologic, gene and protein expression, and flow cytometry analysis. Also, bone marrow derived macrophages were stimulated with ox-LDL and gene expression of ERS molecules and IL-6 were quantified. WT animals presented higher levels of serum creatinine and enhanced cellular infiltration and collagen deposition. They also showed higher expression of inflammatory cytokines and ERS markers. In contrast, KO mice showed renoprotection, and decreased macrophage infiltration. ox-LDL stimulated macrophages had a significantly increase on ERS, with higher levels of IL-6 production. Abrogation of macrophages with clodronate was striking protective and Hemin treatment was able to attenuate NTI as well. In conclusion, macrophages play a pivotal role on NTI and its negative modulation could be benefical. FAPESP/INCT Complex Fluids/CNPq.

B33 Uric acid signalling via adaptor molecule MyD88 and M1/M2 macrophage balance in the development of renal fibrosis

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Introduction The chronic renal failure is an immune mediated disease characterized by renal fibrosis. The injured tissue releases molecules, such as uric acid, resulting from extracellular matrix degradation or dying cells, which can activate Toll-like receptors (TLRs), and leads to translocation MyD88 in many cell types. This immune system modulation interferes in the macrophage and TCD4+ cell activity, with the Th1/Th2 paradigm considered a possible effector mechanism of fibrosis. **Objective** We aimed to investigate the role of uric acid signaling via TLR 2, TLR 4 and MyD88 and the function of M1/M2 macrophage in the development of renal fibrosis. **Methods** We used the Unilateral Ureter Obstruction (UUO), where the animals were sacrificed at seven days after the surgery. Some animals were treated with allopurinol, a xanthine oxidase inhibitor. Proteinuria and uric acid levels were measured in wild-type (C57Bl/6) and IL-12, IL-4, TLR2, TLR4 and MyD88 knockout (KO) mice. TGF-β Elisa assay and hydroxyproline quantification of kidneys tissues were done. Macrophage culture was supplemented with uric acid and Th1/Th2 cytokines was quantified by qPCR and Elisa assay. **Results** UUO increases macrophage entrance in obstructed kidneys, as seen by flow cytometry. IL-12 KO mice presented higher levels of TGF-β compared to WT mice. Besides, TGF-β and type 1 collagen mRNA was decreased in TLRs KO mice, compared to WT mice. Allopurinol treated animals showed preserved renal function and decreased fibrosis formation. MyD88 KO mice showed a renal protection. Uric acid stimulated pro-fibrotic cytokines production by macrophage *in vitro*. These data were corroborated by Sirius red staining and hydroxyproline quantification. **Conclusion** Uric acid crystals are responsible to stimulate Th2 immune response, which leads to fibrosis. This suggests future therapeutic strategies against renal fibrosis should be based on uric acid formation blockage and finally, in the Th1/Th2 balance. **Support** CNPq and Fapesp.

B34 CD14++CD16+ monocytes and cardiovascular outcome

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Introduction
Atherosclerotic vascular disease is the principal cause of death in industrialized countries. Monocytes are strongly involved in initiation and progression of atherosclerosis, which is nowadays considered a chronic inflammatory disease. The impact of monocyte heterogeneity has recently gained substantial interest. In line, we previously reported an association between CD14++CD16+ monocytes and cardiovascular disease in a highly selected cohort of patients with chronic kidney disease. The importance of monocyte heterogeneity in patients with cardiovascular disease and intact renal function is poorly understood in humans.
Therefore, we set out to initiate the prospective HOME SWEET HOMe study in order to assess the impact of elevated cell counts of CD14++CD16+ on cardiovascular outcome.
Methods
Between 2007 and 2010, we recruited more than 1000 patients who underwent elective coronary angiography for diagnosis of coronary artery disease. In a whole blood assay three monocyte subsets (CD14++CD16-, CD14++CD16+, CD14+CD16++) were defined via flowcytometry according to their differential CD14 and CD16 expression. Traditional cardiovascular risk factors were assessed. Patients were followed annually for the occurrence of the primary end-point, which is defined as myocardial infarction, non-hemorrhagic stroke, and death of cardiovascular origin.
Results
We are currently completing analysis of all follow-up data gathered from study initiation until June 30th, 2011. We are planning to share first longitudinal results with EMDS delegates.

B35 Characterization of Dendritic Cells in human lymphoid organs

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Dendritic Cells (DCs) play an important role as antigen presenting cells in the immune system. In peripheral tissues immature DCs continually recirculate as sentinels and search for invading pathogens. After encounter of antigen in the presence of microbial products maturation of the DCs is induced by pattern-recognition-receptors. Costimulatory molecules become upregulated while the endocytotic capacity of mature DCs is drastically diminished. In addition, mature DCs release proinflammatory cytokines to attract other inflammatory cells, and present antigens to T cells. In contrast to thoroughly investigated murine tissue DCs, human DC-subpopulations are far less studied. This is mainly based on the poor availability of human organ material. Here, we provide data on human lymphoid tissue DC-subpopulations. First, we developed a protocol to efficiently purify leukocytes from limited patient material. Second, in order to deepen the understanding of human DC-subpopulations and their potentially distinct functionalities, we compared the expression of numerous cell surface markers responsible for migration, antigen uptake or costimulation by extensive Multicolor-FACS-analyses of various lymphoid organs. Third, high resolution confocal-immunofluorescence-analyses were performed to identify DC-subset localization in lymphoid tissues in the steady state. Genearray analyses will provide additional evidence. With this study we aim to identify uniquely expressed, tissue- and subset-restricted surface molecules which can be utilized for future antibody mediated antigen targeting in humans.
This study (D.D.) is supported by DC-Thera, the German Research Foundation (SFB643-TPA7 and DU548/2-1). D.D. is a fellow of the 'Förderkolleg' of the Bavarian Academy of Sciences.

B36 Conventional CD11b+ DCs, not monocyte-derived DCs, are the major DC subset driving intramuscular alum immunization

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While most vaccines are administered intramuscularly, little is known about the dendritic cells (DCs) present within skeletal muscles. DCs can originate from pre-cDC-precursors (conventional DCs or cDCs) or monocytes (monocyte-derived DCs or MoDCs). However it has been challenging to properly distinguish these cells. Here, we describe a novel 10-color flow-cytometry gating strategy validated by the use of CCR2KO vs WT mixed bone-marrow chimera that allows the identification of CD8a-type cDCs, CD11b-type cDCs and MoDCs in the skeletal muscle and the draining lymph node (dLN). Next, we assessed the function of these cells during intramuscular Alum immunization. All DC subsets captured antigen, migrated to the dLN and activated naïve T cells. However, while MoDCs represented the major DC subset in the inflamed muscle, only a small fraction of these cells migrated to the dLN. Indeed, CD11b-type cDCs vastly outnumbered CD8-type cDCs or MoDCs in the dLNs, suggesting that they account for most of the T cell responses. This also indicates that although Alum induced massive recruitment of MoDCs to the inflamed muscle it did not license MoDCs to massively migrate to the dLN. Interestingly, adding LPS to the Alum induced an increased migration of MoDCs to the dLN. As MoDCs possessed the highest capacity to differentiate naïve T cells into IFNg producing effector T cells on a per cell basis, this suggests that adding TLR triggers to Alum might change the capacity of MoDCs to migrate to the LN and hereby influence the T cell responses generated.

B37 Antigen targeting to splenic CD169$^+$ macrophages induces strong humoral immune responses and CD4 T cell activation.

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Immune responses against blood-borne pathogens are raised in the spleen. The macrophages in the splenic marginal zone are strategically located to capture and phagocytose pathogens. We have recently shown a previously unappreciated role for CD169$^+$ macrophages in the induction of cytotoxic T cells.
Here we investigated the role of CD169$^+$ macrophages in B cell and T helper cell activation. We conjugated ovalbumin (OVA) to antibodies (mAb) specific for CD169 and DEC205 and used these mAb:OVA complexes to target OVA to CD169$^+$ macrophages or DEC205$^+$ DCs. Targeting to CD169$^+$ macrophages induced a robust antibody response at 9 and 28 days after immunization and after boosting. At day 28 and after boosting, antigen targeting to CD169$^+$ macrophages led to even stronger B cell responses compared to DC targeting. Since B cell responses are dependent on CD4 T cell help, we next investigated whether targeting to CD169$^+$ macrophages resulted in efficient CD4 T cell activation. We boosted mice with OVA-NP 28 days after antigen targeting and measured anti-NP antibodies, which is solely dependent on CD4 T cell help. Targeting to CD169$^+$ macrophages showed increased anti-NP titers compared to DC targeting.
Together these data leads us to suggest that antigen targeting to CD169$^+$ macrophages facilitates a more sustained B cell response compared to DC targeting, which is at least in part dependent on better CD4 T cell help. Whether CD169$^+$ macrophages can also transfer antigens directly to B cells, as CD169$^+$ macrophages in lymph nodes do, is currently the topic of our research.

B38 C-type lectin expressing myeloid cells in brain inflammation

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C-type lectins are carbohydrate receptors expressed mostly on myeloid antigen-presenting cells cells such as dendritic cells (DCs) and macrophages. C-type lectins bind exogenous ligands, like glycans on pathogens, but also endogenous ligands like glycans on native glycoproteins. Triggering of certain C-type lectins may inhibit inflammatory responses as was previously shown for DC-SIGN (Geijtenbeek et al., J. Exp. Med, 2003). In addition, C-type lectins are suggested to play an important role in maintaining immune homeostasis (Garcia-Vallejo and Van Kooyk, Immunol Rev, 2009). An organ that particularly benefits from a tightly controlled immune environment is the central nervous system (CNS). Here, many mechanisms have evolved to dampen unwanted immune reactions to prevent neuronal damage. We therefore argued that C-type lectins may exert immune homeostatic mechanisms in the CNS as well and could play a role in neuroinflammatory diseases like multiple sclerosis (MS). In the present study we analyze the expression of mannose receptor, DC-SIGN, MGL and other lectins in the human and/or mouse brain under control and neuroinflammatory conditions. In control human and mouse brain, mannose receptor is mainly expressed by perivascular and meningeal macrophages, corroborating previous reports. We show DC-SIGN expression on microglia and perivascular macrophages in the control human CNS. Whereas MGL is not expressed on microglia in human control CNS, we here show that MGL is abundantly present on activated microglia and macrophages in the rim of chronic active MS lesions. Further analysis on C-type lectin expression in the CNS as well as functional consequences are currently in progress.

B39 Selected macrophage functions during inflammatory phase of wound healing in rats

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Macrophages (MF) have the central role in inflammation which is important component of wound healing. In this study some features and activities of cell population from granulation tissue of 5-day full-thickness excisional wound in rats (enriched in MF content by enzyme digestion and density gradient centrifugation) were described. The wound MF population (WM) has a lower CD54 expression compared to peritoneal MF (PM), while the CD11b positive cells remained unaltered. It is assumed that the lower percentages of $CD54^+$ WM might be partly responsible for the lower observed costimulatory activity of WM as well as for lower levels of IL-2 activity in costimulatory-proliferation assay, compared to PM. Spontaneous, LPS-stimulated as well as T cell-stimulated NO production was lower compared to PM. WM population also exhibited lower spontaneous and PMA-stimulated oxidative metabolism, as well as ability of adhesion to plastic. Lower CD11b expression on WM compared to PM might account for the latter, which was supported by inhibition studies with anti-CD11b antibody. It seems not to be due to cell immaturity, as higher densities of this molecule were noted in WM *vs* peripheral blood monocytes. Majority of described activity of WM are altered in conditions of thermal injury. Also, we found generally higher activities of PM from surgical trauma and thermal injury in comparison to surgical injury solely. Data regarding basic aspects of wound MF functions and their alterations in conditions of severe injury might improve knowledge of the underlying processes during inflammatory phase of wound healing.

B40 Short chain fatty acids modulate CD40 expression in dendritic cells and inhibit lymphocyte proliferation *in vitro.*

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Short Chain Fatty Acids (SCFA) such as butyrate, acetate and propionate are produced through carbohydrate fermentation from intestinal microbiota. It has been attributed anti-inflammatory roles for these SCFA. The aim was to evaluate whether SCFA was able to modulate dendritic cell (DC) maturation and to inhibit lymphocyte proliferation *in vitro*. DCs were generated *in vitro* by harvesting bone marrow (BM) from C57BL/6, supplemented with GM-CSF (20ng/mL) for 6 days ($1x10^6$ cells/well). BMDC maturation was performed with LPS (20ng/mL) or Zymosan (25µg/mL) in the presence/absence of SCFA for 24h. BMDC were labeled with conjugated antibodies for phenotype studies. For the lymphocyte proliferation, spleen cells from de C57BL/6 OT-II mice were labeled with CFSE and stimulated with aCD3+aCD28 (1µg/mL each) or OVA (2µg/mL) for 4 days plus SCFA in plate 96 wells. In both experiments, cells were treated with butyrate (3.2mM) propionate (12mM) and acetate (25mM). Butyrate- and propionate-treated BMDC had decreased number of CD40+ cells after stimulus with LPS. A lesser reduction was observed in the number of CD80+ and CD86+ BMDC treated with butyrate and propionate. An inhibition of lymphocyte proliferation was observed after treatment with all SCFA in spleen cells stimulated with aCD28+aCD3 and with OVA peptide. In an ischemia and reperfusion model of renal injury, CD40 expression on renal DC was also observed after treating animals with SCFA prior to ischemia. In conclusion, SCFA treatment modulate expression of CD40 in BMDC and inhibit lymphocyte proliferation, being an important tool to modulate the immune response. Support: FAPESP and CNPq.

B41 Human airway mucosal DCs respond to TSLP and induce Th2 responses

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Thymic stromal lymphopoietin (TSLP) may control allergic Th2 inflammatory responses through induction of distinct activation programs in myeloid (m)DCs. TSLP-treated mDCs appear limited in their ability to induce Th1-polarization, but rather express the Th2-polarizing molecule OX40L. Furthermore, TSLP-stimulated mDCs produce the STAT6-inducible chemokines CCL17 and CCL22 that may attract Th2 cells. It has been shown that constitutive levels of TSLP-receptor (TSLPR) are low on mDCs in the blood, but it is strongly upregulated following DC activation. However, knowledge about TSLPR expression and functional consequences of receptor activation by DCs residing in the human respiratory tract is very limited. To address this, we first performed detailed immunophenotyping of DCs in the normal nasal mucosa. We found that the dominant population of DCs closely resembles mDCs in the blood, by being $CD1c^{pos}$, $CD11c^{hi}$, $HLA\text{-}DR^{hi}$, $CD103^{neg}$, $DC\text{-}SIGN^{neg}$. However, in contrast to mDCs in the circulation, the majority of mDCs isolated from the nasal mucosa exhibited high expression levels of TSLPR. Furthermore, phospho-flow analysis of purified airway mucosal DCs following TSLP-treatment revealed a very rapid (within minutes) phosphorylation of STAT-molecules, including STAT6. Following topical challenge of patients with nasal allergy with relevant allergen for 7 days, the number of mDCs increased in the mucosa during the allergic reaction, which strongly correlated with the reported symptoms. Furthermore, mDCs co-cultured with autologous T cells from peripheral blood of patients with upper airway allergy, revealed a potent induction of allergen-specific T cell proliferation and Th2 cytokine production when TSLP was added together with the allergen.

SUBMITTED ABSTRACTS TOPIC C: MONOCYTES, MACROPHAGES AND DCS IN INFECTIOUS DISEASES

C01 Influence of the Chlamydia psittaci Type III Secretion System on the innate immune response of chicken macrophages

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The Type III secretion system (T3SS) of *Chlamydiaceae* plays an important role at different stages of their biphasic developmental cycle like for instance i) during entry, when inducing actin recruitment to the entry site following translocation of the T3SS effector protein tarp, ii) during resistance to phagolysosomal fusion through modification of the inclusion membrane and iii) at the end of the developmental cycle when reticulate bodies detach from the inclusion membrane and differentiate to elementary bodies. The T3SS is highly conserved among several G^- bacteria and plays also a role in regulating the innate immune response of the host cell following infection with pathogens such as *Shigella* spp., *Pseudomonas* spp. and *Burkholderia* spp. as well as *Chlamydia trachomatis*.
Chlamydia psittaci also possesses a functional T3SS. Primary replication takes place in epithelial cells in upper respiratory tract. Later on, the bacteria can be found in epithelial cells and macrophages of the lower respiratory tract. Subsequently, *C. psittaci* can be found in plasma and blood monocytes, resulting in a systemic infection. Unfortunately, less is known about the underlying host innate immune response of *C. psittaci* infected macrophages and monocytes. As monocytes/macrophages play such an important role in the innate immune system, it is rather unique that *C. psittaci* as well as other *Chlamydiaceae* are able to survive and even replicate within those cells. In this way, the hypothesis arose that the T3SS might play a role in this process.
To investigate if *C. psittaci* T3SS plays a significant role in regulating innate immune response, HD11 chicken monocytes/macrophages, a well established "*in vitro*" model for studying bacterial host cell interactions were used. We determined the cytokine response following *C. psittaci* infection of HD11 cells by examining gene transcripts of IL-1β, Caspase 1, TNF-α, IL-6, MIF, IL-3, IL-10, IL-12p35, GM-CSF, chemokines (CXCLi2, CXCLi1, CCLi3 and IL-16) and toll like receptors (TLR2, TLR3, TLR4, TLR5, TLR7, TLR21) at different time points (2h, 4h, 8h, 12h and 18h) during an infection with the virulent *C. psittaci* strain 92/1293. Experiments were conducted in the presence and absence of the Type III secretion inhibitor INP0007. The results indicate that, dependent on the stage of the developmental cycle of *C. psittaci*, the T3SS has an influence on the host pro-inflammatory cytokine gene expression level (IL-1β, Caspase 1, TNF-α, MIF, IL-6 and IL12-p35), on the pro-inflammatory chemokine gene expression level (CXCLi2, CXCLi1 and CCLi3), on the growth factor GM-CSF gene expression level, on the expression level of the activation marker iNOS and on the NO production by HD11 cells. Furthermore, the T3SS do not regulate the anti-inflammatory response regarding to IL-10 and also the expression level of the pro-inflammatory chemokine IL-16. Interestingly, looking at the expression level of the toll like receptors, TLR21, which is a unique intracellular avian Toll like receptor for chickens with a broad DNA ligand specificity, is the most upregulated TLR. This TLR gene expression is also regulated by the T3SS at late time points in the infection.

C02 Lipoteichoic acid induced cytokine release is inhibited by apolipoprotein B100

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The initial immune response to pathogens depends on the presence of specialized receptors including Toll-like receptors and nucleotide-binding domain proteins. In addition to these cell associated immune receptors, a number of soluble serum proteins have been described to contribute to the recognition of lipopolysaccharide of Gram-negative bacteria. However, little is known about the role of serum proteins during immune recognition of lipoteichoic acid (LTA), an important immunostimulatory cell wall component of Gram-positive bacteria.
Comparative SDS-PAGE profiles of chromatography fractions of human plasma preincubated with staphylococcal LTA revealed an interaction between LTA and apolipoproteins (ApoA1, ApoA2, ApoA4 and ApoB100), which was confirmed by solid-phase binding assays. In the presence of ApoB100 but not ApoA1 or ApoA2 a significant inhibition of LTA-induced cytokine release from human peripheral blood cells (PBMC) was observed. Comparable to the human data, PBMCs and peritoneal macrophages of LDL-R. knockout mice, comprising increased ApoB100 levels, showed reduced cytokine induction compared to WT cells upon stimulation with LTA as well as with heat-inactivated *S.aureus* . Moreover, mice pretreated with a drug (4APP) that inhibits low-density lipoprotein secretion by the liver were significantly more susceptible to infection with heat-killed *S.aureus* that WT mice. The present study identifies apolipoprotein B100 as an important serum protein able to inhibit the cytokine induction by staphylococcal LTA. Furthermore the *in vivo* experiments indicate that ApoB100 in the blood essentially contributes to the innate immune recognition and modulation of *S.aureus* during an infection.

C03 IFN-α inhibits monocytes in their capacity to mount a Th1 immune response

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Both IFN-α and IFN-γ display immunomodulatory effects. IFN-γ is the main mediator of Th1 responses and essential in the control of infections with intracellular bacteria, such as non-tuberculous *Mycobacteria*, while IFN-α plays a major role during viral infections. Virulent *Mycobacterium tuberculosis* strains induce IFN-α and thereby may interfere with an effective Th1 response. Here we investigated the effects of IFN-α on the modulation of Th1 immunity by monocytes.
Human CD14+ monocytes were isolated from blood and stimulated overnight with various concentrations of IFN-α and IFN-γ in the presence or absence of LPS. IFN-α reduced the IFN-γ-enhanced CD54 and CD64 expression up to threefold. The LPS-induced IL-12p40 production was five-fold reduced by IFN-α. The IL-12p40, TNF and IL-1β production induced by the combination of IFN-γ and LPS were seven-, four- and threefold reduced by IFN-α.
To investigate by which mechanism IFN-α inhibits these Th1 immune responses we examined the influence of IFN-α on the expression of the IFN-γ receptor and on the IFN-γ induced signal transduction. We determined the kinetics of STAT1 and STAT2 phosphorylation and found that IFN-α can interfere directly with the IFN-γ induced STAT1 phosphorylation. After two hours of IFN-α stimulation the cell surface expression of IFN-γR1 was gradually reduced up to four fold. Currently, we are investigating the role of protein arginine methyltransferase (PRMT1), which is associated with the IFN-α receptor, in the inhibitory effects of IFN-α.
At the meeting we will present the various mechanisms by which IFN-α reduces the IFN-γ responsiveness of monocytes.

C04 Inflammatory monocytes but not neutrophils are essential for defense against systemic Listeria monocytogenes infection.

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Listeria monocytogenes is a facultative intracellular bacterium that causes systemic infections in immunocompromised hosts. Early recruitment of myeloid cells, including inflammatory monocytes and neutrophils, to sites of *L. monocytogenes* infection is essential for the control of infection and host survival. Because previous experimental studies used depleting or blocking antibodies that affected both inflammatory monocytes and neutrophils, the relative contributions of these cell populations to defense against *L. monocytogenes* infection remain incompletely defined. Herein, we used highly selective depletion strategies to either deplete inflammatory monocytes or neutrophils from *L. monocytogenes* infected mice and demonstrate that neutrophils are dispensable for early and late control of infection. In contrast, inflammatory monocytes are essential for bacterial clearance during the innate and adaptive phases of the immune response to *L. monocytogenes* infection.

C05 Phagocytosis of *Listeria monocytogenes* by Human PMA-derived Macrophage-like THP-1 Cells in Comparison to Primary Human M1 and M2 Macrophages

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The human food-borne pathogen *Listeria monocytogenes* (*Lm*) is the causative agent of listeriosis. After entering the human body via the intestinal tract, *Lm* spreads systemically mainly to the liver and spleen, where it is preferentially phagocytosed and eliminated by resident macrophages. This step is the major defence line of healthy mammalian hosts against listerial infection. By contrast, in immunocompromised individuals *Lm* can not be effectively eliminated and, in some cases, causes a life threatening disease characterized by a mortality rate of 30%.
For a better understanding of the infection progress and to identify novel targets to treat *Lm* infections the early listerial-macrophage interactions are of main interest. Throughout the body, macrophages are found to be polarized into two main populations: pro-inflammatory type-1 (M1) and anti-inflammatory type-2 (M2) macrophages. We analyzed the phagocytic behaviour of primary M1 (GM-CSF derived) and M2 (M-CSF derived) macrophages in comparison to PMA-derived THP-1 macrophages against *Lm* at different multiplicities of infection by standard gentamicin protection assay and fluorescence microscopy.
M1 and M2 macrophages tolerated higher numbers of infecting *Lm* and had a significantly lower phagocytic activity compared to THP-1 cells. These results clearly show that there are great differences in the infection of primary human macrophages and cultured cell lines with *Lm*. This suggests that the functional and phenotypic outcomes of infection also differ greatly. Thus, to produce physiological relevant data further studies on the interaction of *Lm* with macrophages will be carried out solely using primary cells.

C06 CXCR4 and CCR5 ligands cooperate in monocyte and lymphocyte migration and in inhibition of dual-tropic (R5/X4) HIV-1 infection

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One of the most important functions of chemokines and their receptors is the regulation of directional migration of leukocytes within tissues. In specific tissue compartments, cells are exposed to multiple chemokines presented in complex dimensional and temporal patterns. Therefore, a leukocyte requires mechanisms to integrate the various directional signals it receives from different chemoattractants. In this study, we report that CCL3, CCL5 and CCL8, three potent mononuclear cell chemoattractants, are able to synergize with the homeostatic chemokine CXCL12 in the migration of $CD14^+$ monocytes, $CD3^+$ T-lymphocytes or PHA-activated lymphoblasts. In addition, CCL5 augmented the CXCR4 ligand-driven ERK phosphorylation in mononuclear cells. Furthermore, the synergistic effect between CCL5 and CXCL12 in monocyte chemotaxis is inhibited in the presence of specific CCR1 antibody and AMD3100, but not by maraviroc. In HIV-1 infection assays, a combination of CXCL12 and CCL5 cooperated to inhibit the replication of the dual-tropic (R5/X4) HIV-1 HE strain. Finally, although the dual-tropic HIV-1 strain was barely suppressed by AMD3100 or maraviroc alone, HIV-1 infection was completely blocked by the combination of these two receptor antagonists. Our data demonstrate cooperation between CCL5 and CXCL12, which has implications in migration of monocytes/lymphocytes during inflammation and HIV-1 infection.

C07 *Trypanosoma cruzi* activates cord blood myeloid dendritic cells, promoting proliferation of $CD8^+$ T cells and IFN-γ secretion

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Immune responses in early life are of limited effectiveness and Th2-skewed. However, our previous works support the ability of *Trypanosoma cruzi* to favour neonatal type 1 immune responses. Indeed, human fetuses congenitally infected with *T. cruzi* develop an adult-like $CD8^+$ T cell response producing IFN-γ, and young infants from *T. cruzi*-infected mothers mount a stronger and type 1 polarized immune response to unrelated vaccines they have received in first months of life. To investigate the mechanisms allowing *T. cruzi* to induce type 1 immune response in early life, we have studied *in vitro* its ability to activate dendritic cells (DCs) from healthy newborns (cord blood cells). Our results show that *T. cruzi* trypomastigotes significantly up-regulate expression of co-stimulatory molecules (CD40, CD80 and mainly CD83) on cord blood CD11c+ myeloid DCs (mDCs). Interestingly, *T. cruzi*-specific IgG Ab (as those transferred during gestation from the mother) amplified the stimulating effect of parasites. Such activated mDCs were able to trigger T cell responses in allogeneic reactions. Indeed, circulating mononuclear cells enriched in mDCs ("eDCs") co-cultured with *T. cruzi* (Tc) stimulate proliferation of adult $CD4^+$ T lymphocytes and induce more potent proliferation of $CD8^+$ T cells. T cell proliferation was associated with IFN-γ release and down-regulation of IL-13 production. *T. cruzi*-activated eDCs also triggered proliferation of cord blood T cells containing mainly naïve lymphocytes. These data show that *T cruzi* activates human cord blood mDCs, priming $CD8^+$ T lymphocyte and favouring type 1 immune response, and that maternal antibodies can contribute to the development of mature DCs.

C08 Immuno-modulatory Impact of *Leishmania*-induced Macrophage Exosomes

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Exosomes are 50-100nm secretory vesicles released by various eukaryotic cells. During the recent years, immune cells have been shown to secreted exosomes that possess immunomodulatory effects. Interestingly, exosomes released by antigen-presenting cells, especially macrophages infected with bacteria have been shown to be pro-inflammatory. However, the impact of exosomes during the infection with protozoan parasites such as *Leishmania* remains uncharacterized. *Leishmania* are causative agents of leishmaniasis. These digenic parasites are transferred to their mammalian host via sandfly bite and phagocytozed by macrophages. *Leishmania* parasites utilize multiple virulence mechanisms to subvert the immune response and survive in the harsh conditions of the phagolysosome.
In order to study the effect of *Leishmania* infection on exosome release by infected macrophages, we performed a comparative proteomic analysis of the exosomes produced by untreated J774 macrophages, LPS-stimulated macrophages and *Leishmania mexicana*-infected macrophages. We observed that *Leishmania* infection results in modulation of the proteome content of macrophage exosomes. These modulations include up and down-regulation of protein levels as well as introduction of previously non-reported proteins into the exosomes. In addition, we observed that *Leishmania*-induced exosomes stimulate nuclear translocation of pro-inflammatory transcription factors NF-κB and AP-1 but not STAT-1 in naïve macrophages. Activation of these transcription factors seems to be concurrent with activation of MAP kinases such as ERK1/2. Overall, our results indicate that *Leishmania*-induced exosomes contain a unique set of proteins and that these exosomes can trigger pro-inflammatory signalling pathways in naïve macrophages, suggesting an important role for exosomes in pathology of leishmaniasis.

C09 Innate immune recognition of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an opportunistic pathogen that causes severe respiratory infections in susceptible individuals (e.g. cystic fibrosis patients). This study aims to identify the conditions required for *P. aeruginosa* PA01 clearance by one of the major innate immune players in bacterial infections – macrophages.
We have established an *in vitro* infection model looking at how human macrophages respond to *Pseudomonas aeruginosa* infection. Under non-opsonic conditions human macrophages generated in the presence of M-CSF eliminated 40-50% of PA01 within 4 hours of infection, but were themselves killed within 6 hours.
Our results to date are consistent with previous mouse work indicating that the protection against *Pseudomonas aeruginosa* infection conferred by a Th1-dominated response does not involve increased microbicidal activity by macrophages but the amplification of their pro-inflammatory response. Macrophages treated with IFN-γ have a microbicidal activity similar to that of untreated macrophages. IFN-γ treatment has a major effect on cytokine production leading to an increased pro-inflammatory/anti-inflammatory cytokine ratio, particularly in the presence of the inflammatory cytokine GM-CSF. Intriguingly, the neutrophil chemotactic factor IL-8 was consistently detected.
Thus, it would appear that activated macrophages alone will not clear *P. aeruginosa* infection but, very likely, will recruit other cells, such as neutrophils, and prime them for killing.

C10 Monocyte-derived dendritic cells collaborate with conventional DC to prime Th1 responses to Salmonella.

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Th1 differentiation of T cells is essential for the effective clearance of intracellular infections such as those caused by *Salmonella* Typhimurium (STm). The way these responses develop in vivo is only partially understood. Using an in vivo model of infection we observed a marked accumulation of monocyte-derived DCs (moDC) in the T-zone of the spleen by 24h after infection with live, but not killed, bacteria. This population is not only directly infected by live bacteria but are so at higher levels and frequencies than cDCs. We examined the capacity of this population to present antigen ex vivo. MoDC are sufficient to induce T cell priming but are less efficient at doing so than cDCs. Interestingly co-culturing both DC populations was most efficient at driving T cell proliferation and Th1 priming as measured by T cell CFSE dilution and INFγ production. The participation of moDCs in T cell priming in vivo was confirmed by depleting circulating monocytes using clodronate. In the absence of moDCs in vivo T cell INFγ responses are diminished, whilst IgG switching was unaffected, suggesting that moDCs play an important role in Th1 differentiation but not for the induction of follicular T cells. Our results highlight the importance of different DC subsets in T cell subset priming and their potential to cooperate to induce optimal T cell responses. This has implications for understanding how Th1 responses develop in response to complex viable antigens during intracellular infections such as those caused by STm.

C11 HIV-1 Infection in Polarized Primary Macrophages

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Macrophages are important targets for HIV-1 infection and are crucial for mucosal transmission of the virus. Previously we observed that the susceptibility of macrophages to HIV-1 infection is regulated by cytokines. In this study, the expression levels of HIV-1 restricting cellular factors in the different types of polarized monocyte-derived macrophages (MDM) was analyzed and their role in HIV-1 susceptibility was investigated. Macrophages differentiated in the presence of IFN-α, IFN-β, IFN-γ/TNF-α (M1), IL-4 (M2a) or IL-10 (M2c), differentially expressed characteristic membrane receptors, such as CD14, CD16, CD64, CD80, CD162, CD200R and CD206, confirming the polarized/activated phenotype. Unpolarized and M-CSF/GM-CSF-stimulated MDM were highly susceptible to infection, whereas IFN-α, IFN-β, IFN-γ/TNF-α, IL-4 or IL-10 treatment resulted in a significant inhibition of virus replication. Infection of these populations with a VSV-G pseudotyped virus indicated that HIV-1 replication was inhibited at a post-entry level. Expression of HIV-1 restriction factors like APOBEC3G, Trim5α, CyPA, tetherin, Trim22 and recently identified anti-HIV miRNAs was upregulated in MDM treated with type I IFNs, and to a lesser extend in M1 polarized macrophages. This suggests that these factors may contribute to inhibition of HIV-1 replication in MDM upon treatment with type I interferons. However, these factors are not likely involved in HIV-1 inhibition in M1 or M2 macrophages. Additional studies are necessary to identify other host factors involved in the resistance of polarized macrophages to HIV-1 infection.

C12 Antigen presenting cells involved in Th17 cell priming during Candidiasis

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The fungus *Candida albicans* lives as part of the normal microflora in healthy individuals without triggering any harmful effects. However, it can cause severe disease in immunocompromised individuals. The increased prevalence of mycoses such as orophangyngeal candidiasis (OPC) in AIDS patients provides evidence for $CD4^+$ T cells playing a key role in protection from fungal diseases. *C. albicans*-specific $CD4^+$T cells are primed efficiently in response to oropharyngeal infection in mice and these T cells produce high levels of IL-17A and other Th17-type cytokines. However, it remains unclear which subset(s) of antigen presenting cells (APCs) mediate this efficient T cells priming during OPC. Here we show that among others monocyte-derived DCs (MoDCs) infiltrate strongly into the draining lymph nodes during infection suggesting that they mediate directly or in cooperation with lymph node-resident APCs the priming of *Candida*-specific Th17 cells. MoDCs indeed present *Candida*-derived antigens in the draining lymph nodes of infected mice as they are able to activate *Candida*-specific T cell hybridoma *ex vivo*. The role of MoDCs for the activation of *Candida*-specific T cells during OPC *in vivo* is being confirmed. Together, this study shall illuminate and extend the prominent function of MoDCs as activators of the adaptive immune system that has recently been attributed to them in various tissues, including the lung, to the oral cavity, another prominent site of pathogen entry.

C13 The role of TGF-β1 and TGF-β1 RI in morphogenesis of granulomatous lymphadenitis in pigs naturally infected with *Mycobacterium avium complex*

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M. avium subsp. *avium* cause generalised tuberculosis in poultry and wild birds, while *M. avium* subsp. *hominissuis* is an opportunistic pathogen, infecting pigs and humans.
Study was performed on mesenteric lymph nodes samples of 100 pigs with positive tuberculin skin test, euthanized in quarantine. Real-time PCR was used to demonstrate that *M. avium* subsp *hominissuis* is present in mesenteric lymph nodes. Routine hematoxilyn-eosin staining, Masson trichrom specific staining for connective tissues, and Ziehl Neelsen staining for acid fast bacteria, were performed on formalin fixed and paraffin embedded tissue. LSAB2 and double staining immunohistochemical methods included TGF-β1, TGF-β1 RI, αSMA, desmin and CD3 as primary antibodies.
Microscopically granulomatous lesions compatible with mycobacterial infection were found in all mesenteric lymph nodes. In one third of the lymph nodes with histological changes no macroscopically visible changes were established. The granulomas were composed of focal accumulation of macrophages, epithelioid cells, multinucleated giant cells, lymphocytes, eosinophilic granulocytes and myofibroblasts. Ziehel Neelsen positive rods were detected in 5% of samples. Positive reactions for IS*1245* on real-time PCR confirmed presence of *M. avium* in 55% of the mesenteric lymph nodes. The main sources of TGF-β1 which induce proliferation of myofibroblasts are macrophages and eosinophilic granulocytes. Myofibroblasts express TGF-β1RI and αSMA and play a key role in morphogenesis of granulomatous lymphadenitis in swine caused by MAC. Formation and sustainability of the granuloma is the most important response to this infecton. Eosinophilic granulocytes express TGF-β, induce myofibroblast proliferation and participate in granuloma morphogenesis and defence of the host.

C14 Differential effects of the antimicrobial peptides LL-37 and hLF1-11 on monocyte-macrophage differentiation

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Due to their diverse properties antimicrobial peptides serve as potential candidates for the development of new agents to combat infections with (multidrug-resistant) pathogens. In this study we investigated the effects of two antimicrobial peptides of interest, i.e. the cathelicidin LL-37 and a peptide derived from human lactoferrin (hLF1-11), on monocyte-macrophage differentiation. For this purpose, human monocytes were cultured with GM-CSF (to differentiate into pro-inflammatory macrophages; MΦ-1) or with M-CSF (resulting in anti-inflammatory macrophages; MΦ-2) in the presence of these antimicrobial peptides or control peptides. Thereafter, the abilty of the resulting macrophages to produce pro- and anti-inflammatory cytokines and their antimicrobial activities were assessed.
Results revealed that monocytes cultured with M-CSF in the presence of LL-37, but not control peptide, led to macrophages displaying a pro-inflammatory signature, i.e. low expression of CD163 and little IL-10 and profound IL-12p40 production upon LPS stimulation. The effects of LL-37 on M-CSF-driven macrophage differentiation were dose- and time-dependent. This peptide enhanced the GM-CSF-driven macrophage differentiation. Exposure of fully differentiated MΦ-2 to LL-37 for 6 days resulted in macrophages that produced less IL-10 and more IL-12p40 upon LPS stimulation than control MΦ-2. In contrast, LL-37 had no effect on fully differentiated MΦ-1. Peptide mapping using a set of 16 overlapping 22-mer peptides covering the complete LL-37 sequence revealed that the C-terminal portion of LL-37 is responsible for directing the M-CSF-driven macrophage differentiation.
Incubation of monocytes with GM-CSF in the presence of hLF1-11, but not control peptide, resulted in macrophages characterized by increased pro- and anti-inflammatory cytokine production and enhanced responsiveness to microbial structures such as LPS, lipoteichoic acid and *Candida albicans*. Moreover, these macrophages were highly effective in phagocytosing and killing of *C. albicans* and *Staphylococcus aureus*. Thus, hLF1-11 directs GM-CSF-driven differentiation of monocytes toward macrophages with enhanced effector functions.
We conclude that LL-37 directs M-CSF-driven macrophage differentiation toward macrophages with a pro-inflammatory signature and that hLF1-11 directs GM-CSF-driven monocyte differentiation toward a macrophage subset with enhanced antimicrobial activities. Our results furthermore indicate that the effects of LL-37 and hLF1-11 on macrophage differentiation required internalization of the peptides. By modulation of monocyte-macrophage differentiation antimicrobial peptides may strengthen the immune response of the host to a subsequent infectious challenge.

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C15 Interleukin-6-deficient Dendritic Cells Promote Th2 Responses In Vivo

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How naive $CD4^+$ T cells commit to the T helper type 2 (Th2) lineage upon antigen loaded dendritic cell encounter remains poorly understood. In this work we show that inoculation of antigen-pulsed IL-$6^{-/-}$ bone marrow derived dendritic cells (BMDCs) strongly promoted Th2 responses in vivo and exacerbated recruitment of eosinophils in an experimental asthma model. Depletion of basophils had no effect on the Th2 response whereas iNKT were required for optimal Th2 differentiation. Activation of iNKT in the draining lymph nodes occurred independently of IL-6 secretion and CD1d1-mediated glycolipid presentation by the BMDCs, unrevealing an unconventional stimulation of iNKT during immunization with BMDCs. Co-injection of antigen-pulsed or unpulsed wild type BMDCs together with IL-$6^{-/-}$ BMDCs indicated that IL-6-competent BMDCs down regulated Th2 cytokine secretion in vivo through a TcR/Ag-MHC specific interaction at the level of T helper cells. We therefore propose a model whereby inflammatory BMDCs both initiate (through iNKT activation) and regulate (through IL-6 signaling at the level of antigen-specific Th cells) amplification of Th2 immunity.

C16 The role of iNOS in iron homeostasis and *Salmonella* infection

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Iron homeostasis and nitric oxide (NO) biology are closely connected to each other since the transcription of inducible NO synthase (iNOS) is controlled by iron while the post-transcriptional control of iron homeostasis via iron regulatory proteins (IRPs) is affected by NO, which links maintenance of iron homeostasis to optimal formation of NO for host defence.
We studied the effects of NO on the expression of iron metabolic genes in primary peritoneal macrophages infected with the intracellular pathogen *Salmonella* Typhimurium and evaluated cellular iron homeostasis in *iNOS*$^{+/+}$ and *iNOS*$^{-/-}$ cells.
iNOS disruption led to the accumulation of iron in peritoneal macrophages, paralleled by a significantly decreased ferroportin-1 (Fpn-1) mRNA expression in these cells. The cause-effect relationship between NO and Fpn-1 expression was underscored by the observation that the pharmacological NO donor Nor-5 increased Fpn-1 expression in macrophages by a transcriptional mechanism. Additionally, macrophages from *iNOS*$^{-/-}$ mice showed reduced TNF and IL-12p35 expression following *Salmonella* infection with. While *Salmonella*-infected *iNOS*$^{-/-}$ macrophages displayed increased bacterial load, addition of the iron chelator desferasirox as well as over-expression of Fpn-1 abrogated the differences observed between *iNOS*$^{-/-}$ and *iNOS*$^{+/+}$ macrophages and restored TNF and IL-12p35 production in *iNOS*$^{-/-}$ cells.
Our results demonstrate that NO is a central regulator of iron homeostasis and that its reduction results in an increased iron accumulation in macrophages due to down-regulation of Fpn-1 expression. The accumulation of iron in *iNOS*$^{-/-}$ macrophages reduces the expression of M1-type innate host response mechanisms which may partly underlie the impaired immune response of *iNOS*$^{-/-}$ mice.

C17 HIV-1 modulation of macrophage function: insights into pathways controlling cytokine production in mononuclear phagocytes

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Macrophages are critical effectors of inflammation and immunity, and a key mechanism by which they act is through production of cytokines which modulate the immune response. Innate immune activation of macrophages leads to induction of pro-inflammatory factors, coupled closely to anti-inflammatory regulation mechanisms. This is exemplified by induction of IL-10 as a component of innate immune macrophage responses to mycobacteria and fungal pathogens, wherein the anti-inflammatory action of IL-10 is postulated to have physiologically significant effects. In studies investigating the effect of HIV-1 infection on macrophage responses to these pathogens, we observe that the transcriptional response of IL-10 is attenuated by HIV-1 infection, but that those of other cytokines such as IL-6 and IL-1β are not similarly affected, suggesting dissociated mechanisms for regulation of pro- and anti-inflammatory responses. We demonstrate that this specific deficiency in IL-10 leads to exaggerated and sustained inflammatory macrophage responses, through lack of homeostatic IL-10 regulation. We have used this model to investigate the signalling pathways that are affected by HIV-1 to attenuate IL-10 responses specifically. Our findings suggest that HIV-1 infection of macrophages may contribute to the pathogenesis of mycobacterial or fungal co-infection in patients with AIDS, and furthermore may also provide greater insight into the molecular details of specific pathways for induction of innate immune IL-10 responses in macrophages.

C18 Interaction Between Vaccines and Afferent Lymph Dendritic Cells in a Sheep Lymphatic Cannulation Model

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Vaccines first interact with dendritic cells (DCs) at the site of injection and are subsequently transported to the local lymph node where immune responses are initiated. Hence, the lymph node draining the site of vaccine delivery largely defines the immune responses induced. However, the signals available to the lymph node at this early stage are generally difficult to investigate.
The sheep pseudo-afferent lymphatic cannulation model allows access to cells and soluble factors draining from the site of injection to the local lymph node. Using a 2-step procedure, the draining lymph node is first removed and the lymphatics are allowed to re-anastomose. Subsequently the newly formed pseudo-afferent duct is cannulated. In the absence of the lymph node the cells and soluble factors normally draining from the skin to the local lymph node are now intercepted and can be analysed.
Using pseudo-afferent cannulation we have investigated the interaction between vaccines and the cells draining the site of injection. Interactions between DCs and the vaccine occur both at the site of injection and within the draining lymph node as vaccine components are transported in afferent lymph in free and cell-associated form. Thus adjuvants have the potential to activate DCs in the skin, during transport in the lymphatics and within the draining lymph node. In contrast to cell-free vaccine components, which are able to migrate through the draining lymph node, DCs are unable to migrate past the lymph node into efferent lymph. Hence, only non-cell associated material can influence downstream lymph nodes.

C19 An innate antibody-independent antiviral role for B cells

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Neutralizing antibodies are long thought to be required for protection against acutely cytopathic viruses, such as the neurotropic vesicular stomatitis virus (VSV). Utilizing mice that possess B cells but selectively lack antibodies, we show here that survival upon subcutaneous VSV challenge is independent of neutralizing antibody production or cell-mediated immunity, but does require B cells. B cells are necessary to provide lymphotoxin (LT) $\alpha\beta$ that maintains a protective subcapsular sinus (SCS) macrophage phenotype within the virus draining lymph nodes (LNs). Indeed, macrophages within the SCS of B cell-deficient LNs, or of mice that lack LT$\alpha\beta$ selectively in B cells, display an aberrant phenotype, fail to replicate VSV and to produce IFN-I required to prevent fatal viral central nervous system (CNS) invasion through intranodal nerves. These data demonstrate that while B cells are required for survival following VSV infection, their contribution is innate, rather than adaptive.

C20 Infection of dendritic cells with herpes simplex virus type 1 induces rapid degradation of CYTIP, thereby modulating adhesion and migration

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Immune responses require spatial and temporal coordinated interactions between different cell types within distinct microenvironments. This dynamic interplay depends on the competency of the involved cells, predominantly leukocytes, to actively migrate to defined sites of cellular encounters in various tissues. Due to their unique capacity to transport antigen from the periphery to secondary lymphoid tissues for the activation of naïve T-cells, dendritic cells (DCs) play a key role in the initiation and orchestration of adaptive immune responses. Therefore, pathogen-mediated interference with this process is a very effective way of immune-evasion. CYTIP (cytohesin-interacting protein) is a key regulator of DC motility. It has previously been described to control LFA-1 deactivation and to regulate DC adherence. CYTIP expression is upregulated during DC maturation, enabling their transition from the sessile to the motile state. Here, we demonstrate that upon infection of human monocyte-derived DCs with herpes simplex virus type 1 (HSV-1), CYTIP is rapidly degraded and as a consequence beta-2 integrins, predominantly LFA-1, are activated. Furthermore we show that the impairment of migration in HSV-1-infected DCs is in part due to this increased integrin-mediated adhesion. Thus, we propose a new mechanism of pathogen-interference with central aspects of leukocyte biology.

C21 Human Cytomegalovirus (HCMV) Infection of M1- and M2- Macrophages Promotes a Pro-Inflammatory Signature and Antigen Presentation

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Macrophages (MØ) are first targets during HCMV infection and are thought to be crucial cells for viral persistence and dissemination. However, since they are also a first line of defence and key modulators of the immune response, MØ are at the crossroad between protection and viral pathogenesis. Since MØ may display opposite properties and functions depending on their polarization state, we characterized HCMV infection and the resulting effects in pro-inflammatory M1-MØ (driven by GM-CSF) as well as in anti-inflammatory M2-MØ (driven by M-CSF). In both MØ types the infection was productive and persistent, but infection rates were higher in M2-MØ. Upon HCMV infection both types of MØ displayed a proinflammatory signature, namely secretion of IL-1β, IL-6, IL-12, TNF-α as well as inflammatory chemokines. Notably, HCMV was as potent as the classical activation stimulus LPS and comparable amounts of proinflammatory factors were released by HCMV-infected or LPS-stimulated MØ. HCMV-infected M1- and M2- MØ showed features of classical activation such as up-regulation of CD80 and down-regulation of CD206 and CD36. Even though these features would indicate that both types of MØ have anti-microbial functions, HCMV was not eradicated and continued to replicate in infected MØ cultures. Moreover HCMV-infected MØ induce proliferation of autologous T cells obtained from HCMV seropositive donors but not from seronegative donors, thus indicating that MØ could not induce a naïve response to the virus. In summary, our data reveal that HCMV interferes with the normal M1-M2 polarization of MØ by inducing a pro-inflammatory signature.

C22 Varying effects of different beta-glucans on the maturation of porcine monocyte-derived dendritic cells

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Beta-glucans are well known for their immunomodulatory capacities in human and mice. For this reason together with the European ban on growth-promoting antibiotics, β-glucans are intensively used in pig feed. However, as described in the present study, there is much variation in the stimulatory capacity between β-glucans of different sources. As dendritic cells (DCs) are the first cells which are encountered after an antigen is taken up by the intestinal epithelial cell barrier, we investigated the effect of two concentrations (5 and 10 µg/ml) of five commercial β-glucanpreparations (laminarin, curdlan, Euglena gracilis, Macrogard and zymosan), differing in structure and source, on expression of maturation markers, antigen uptake, allogeneic mixed lymphocyte reaction and cytokine secretion by porcine monocyte-derived dendritic cells (MoDCs). Although all β-glucans gave rise to a significant reduction of the phagocytic activity of DCs, only Macrogard induced a significant phenotypical maturation. Besides Macrogard, also zymosan, another β-glucan derived from *Saccharomyces cerevisiae*, and curdlan significantly improved the T cell stimulatory capacity of MoDCs. Most interesting however, is the cytokine secretion profile of curdlan-stimulated MoDCs as only curdlan induced significantly higher expression levels of IL-1β, IL-6, IL-10 and IL-12/IL-23p40. As the cytokine profile of DCs influences the outcome of the ensuing immune response and thus may prove valuable in intestinal immunity, a careful choice is necessary when β-glucans are used as dietary supplement.
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C23 *Trypanosoma cruzi* trypomastigotes activate cord blood monocytes and myeloid DCs

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Immune response in early life is known to be immature, leading to a greater susceptibility of neonates and young children to infections. Although most cell types of the neonatal innate and adaptive immune system show functional deficiencies *in vitro* in comparison to adult cells, we have previously showed that fetuses congenitally infected with *Trypanosoma cruzi,* the protozoa agent of Chagas disease, were able to mount a strong, adult-like $CD8^+$ T cell immune response. In order to understand the mechanisms of initiation of this response, we studied the expression of activation markers and the production of IL-12 by cord blood (CB) monocytes and dendritic cells (DCs) from healthy neonates in response to *T. cruzi* live trypomastigotes.
Intracellular flow cytometry analyses showed that *T. cruzi* induced the production of IL12-p35 by 10% of neonatal monocytes, but not by neonatal DCs, after 6h incubation of CBMC with parasites. Although we could not detect the release of IL-12p70 in the supernatants, we detected its biological activity by its role in IFN-γ production by natural killer (NK) cells. Indeed, depletion of CD14+ cells (MACS magnetic beads) or neutralization of IL-12p70 (by blocking specific moAb) strongly impeded by more than 90% the IFN-γ production by NK cells that we described elsewhere. *T. cruzi* also upregulated surface expression of co-stimulatory molecules (CD40, CD80 and mainly CD83) on CB $CD11c^+$ myeloid (m)DCs and monocytes.
These data show that *T. cruzi* activates human CB mDCs and monocytes which could contribute to overcome the immunological immaturity associated with early life.

C24 ROLE OF CPG-ELICITED INFLAMMATORY DCS IN THE T-CELL RESPONSE TO SOLUBLE AND PARTICULATE ANTIGEN

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Dendritic cells (DCs) are the crucial initiators of adaptive immune responses and thus represent the key targets of new vaccine approaches. DCs are however far from a homogenous population, with multiple subsets having been identified harboring specialized functions. Inflammatory DCs (iDCs), characterized by a $CD11b^{hi}$ $Ly6C^{hi}$ phenotype, are typically recruited in case of infection/inflammation, and emerge from $Ly6C^{hi}$ monocytes in the bone marrow in a CCR2 dependent way. Besides having direct antimicrobial functions, iDCs also might play crucial roles in the initiation of adaptive immune responses. Following subcutaneous injection of the TLR9 ligand CpG, we observed a dramatic influx of iDCs in the draining lymph nodes. To address the role of these CpG-elicited iDCs in the initiation of adaptive immunity, mice were immunized with either soluble or microparticle encapsulated ovalbumin in combination with CpG. Immunization with the microparticulate OVA was far more potent in generating Th1 and CTL responses, likely reflecting the enhanced capacities of DCs to process and present microparticulate antigens. Immunizing CCR2-/- mice, which lack iDCs but display normal numbers of conventional DCs, resulted in an almost total abrogation of the Th1 and CTL responses. Using fluorescently labeled OVA, we could demonstrate that the vast majority of iDCs became OVA positive, suggesting iDCs might be the antigen presenting cells responsible for the initiation of the T cell response. Nevertheless, sorted iDCs were incapable of stimulating OT-I or OT-II proliferation, arguing against a direct role of iDCs in antigen presentation. In contrast, $Ly6C^{lo}$ $CD11b^{hi}$ dermal migratory DCs efficiently presented the antigen.

C25 ***Staphylococcus aureus*** **PSM peptides inhibit proinflammatory cytokine secretion by DCs and induce regulatory T cells**

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Virulence of the emerging community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) and other highly pathogenic *S. aureus* depends on Phenol-Soluble Modulin (PSM) peptide toxins, which are known to attract and lyse neutrophils. In human cells PSM peptides exert their function by binding to the Formyl-peptid Receptor 2 (FPR2). We demonstrate that the murin homologue mFPR2 is expressed on mouse bone marrow-derived dendritic cells (BM-DC), which makes them a possible target for PSM peptides. We further show that PSM peptides bind to mouse splenic DCs and granulozytes and BM-DCs. We demonstrate that PSM peptides are chemoattractants for BM-DCs but in contrast to neutrophils show no cell lysis at high peptide concentrations. Using BM-DCs we analyzed the effect of PSM peptides on cytokine secretion induced by a lysate of a Protein A and PSM peptide deficient *S. aureus* strain (Δspa), a TLR2 ligand. The addition of PSM peptides during Δspa stimulation of BM-DCs inhibits the secretion of TNF-, IL-12- and IL-6 whereas IL-10 secretion was increased. We demonstrate that PSM peptides also reduce the uptake of the model antigen OVA by BM-DCs. Further analysis of the T-cell responses induced by PSM peptide treated DCs revealed a reduced T-cell activation and proliferation. Characterization of the activated T cells showed an increased percentage of $FOXP3^+$ regulatory T cells. These data show immuneregulatory effects of PSM peptides changing the cytokine secretion of DCs towards an antiinflammatory response. This together with the induction of Tregs indicate that PSM peptides modulate the adaptive immune response by increasing the tolerance towards the pathogen.

C26 **REDUCED EXPRESSION OF MATURATION MARKERS IN BOVINE DENDRITIC CELLS AFTER INCUBATION WITH GIARDIA DUODENALIS TROPHOZOITES**

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Giardia duodenalis (syn. *G. intestinalis, G. lamblia)* is an important intestinal parasite in a wide range of mammals, including farm animals, companion animals and humans. The role of dendritic cells in the immune response against *G. duodenalis* is poorly documented and has only been studied in the mouse, which is not a natural host for this parasite. The objective of this study was to investigate whether *G. duodenalis* trophozoites or their excretion/secretion (ES) products activate and mature dendritic cells from cattle *in vitro*. Bovine monocyte-derived dendritic cells (MoDCs) were incubated with different concentrations of *G. duodenalis* trophozoites. An effect of ES products was investigated by preventing direct contact between the trophozoites and the MoDCs, using 0.4µm cell culture inserts. After 18h of stimulation, expression of the maturation markers CD80, CD40 and MHCII was measured and ovalbumin uptake was monitored. A dose-dependent decrease of ovalbumin uptake was observed in MoDCs incubated with trophozoites (but not ES), suggesting functional maturation. However, none of the maturation associated cell surface expressed molecules were upregulated after incubation of MoDCs with *Giardia* trophozoites or ES. In order to investigate whether or not MoDCs can induce lymphocyte proliferation after incubation with *Giardia* trophozoites, a mixed lymphocyte reaction will be performed with allogeneic $CD2^+$ lymphocytes. Selected cytokines will be determined by ELISA in the culture supernatant of the stimulated MoDCs and from the co-cultured MoDCs and T cells, to determine the phenotype of the immune response that is induced.

C27 **IDO-mediated bactericidal activity of human myeloid cells against Listeria monocytogenes is executed by toxic downstream tryptophan catabolites**

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Myeloid cells (MC) including dendritic cells (DC) and macrophages (Mφ) play an important role in the antimicrobial defense. The tryptophan (trp) degrading enzyme indoleamine 2,3 deoxygenase (IDO) has been suggested to be involved in pathogen defense albeit it remains unclear, whether its bactericidal activity is mediated by trp starvation or by toxicity of downstream catabolites. Lack of IDO induction in murine MC has further hampered to resolve this important question. In a model of Listeria monocytogenes (L.m.) infection of human MC we found that IDO is strongly induced after infection. Compared to IDO^- MC, IDO^+ MC show 70% less bacterial burden after infection suggesting that IDO-expressing MC control L.m. more efficiently. Most important, knock-down of IDO in human MC led to an uncontrolled bacterial growth. Surprisingly, when comparing trp deprived and enriched conditions, IDO^+ MC were unable to control L.m. growth in absence of exogenous trp clearly suggesting that trp deprivation is not responsible for bactericidal activity. These findings were corroborated by observation that purified trp catabolites demonstrated a significant bactericidal activity on L.m. Our results strongly suggest that IDO is a key mediator of the microbicidal activity observed in human but not in murine MC. Toxic trp catabolites rather than trp starvation are responsible for the observed microbicidal activity.

C28 Depletion of dendritic cells enhances innate anti-bacterial host defense through modulation of phagocyte homeostasis

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Dendritic cells (DCs) as professional antigen-presenting cells play an important role in the initiation and modulation of innate and adaptive immune responses. Here, we have analyzed the role of DCs and their impact on bacterial clearance in an experimental model of the extracellular bacterium *Yersinia enterocolitica* (Ye). We used CD11c-diphteria toxin (DT) mice to deplete DCs during an infection with Ye.
DC depletion significantly increased the survival after Ye infection. Interestingly, throughout the infection the bacterial load in the spleen of DC depleted mice was significantly lower than that of wild type control mice. This was accompanied by an increase in the serum chemokine levels of CXCL1, G-CSF, IL-1α, and MCP-1 (CCL3) upon DC depletion and an increase in the frequency and numbers of phagocytes, namely PMNs and monocytes. Splenocytes from DC depleted mice exhibited increased bacterial killing capacity compared to splenocytes from wild type control mice. Ye colocalized more frequently with the phagocytes in DC depleted mice compared to wild type mice. DC depletion improved phagocytic activity, production of reactive oxygen species and Ye killing capacity by PMNs. Adoptive transfer of Gr-1^{+} cells from DC depleted mice into wild type mice prior to Ye infection reduced the bacterial load to the level of Ye-infected DC-depleted mice, demonstrating that the increased number of effective phagocytes resulting from DC depletion accounts for the initial differences in the bacterial load. Beyong bacterial infection, our data indicate that DCs regulate the homeostasis of PMNs and monocytes into the spleen by a yet unknown mechanism.

C29 Crucial role of MIF in trypanosomiasis elicited inflammation-associated immunopathology

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

C30 NK-DC crosstalk activates an activin A-dependent negative regulatory pathway

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The importance of the bidirectional crosstalk between NK cells and DC during inflammatory responses has been demonstrated by a number of studies. These interactions involve both cell–cell contact and soluble factors and dramatically influence the quality and strength of immune responses. A detailed understanding of this crosstalk is therefore essential.
Activin A, a member of the TGF-beta superfamily, is a multifunctional cytokine expressed in response to inflammatory stimuli that has both anti- and pro-inflammatory properties.
In this study we found that activin A expression is induced in NK cell-DC co-cultures. This induction is contact dependent and is mainly mediated by TNF-alpha production. Interestingly, the presence of High-mobility group protein B1 (HMGB1), possibly released by NK cells and/or dying DC, potentiates activin A expression.
Using follistatin, a natural inhibitor of activin A, we demonstrated that the endogenously produced activin A down-modulates the expression of cytokines, such as TNF-alpha, IL-6 and IFNgamma. Additionally, activin A also inhibited NK cell-induced DC maturation evaluated as inhibition of the maturation markers CD83, CD86 and HLA-DR.
In conclusion, our study shows that activin A inhibits NK cell and DC activity in an autocrine and paracrine manner, and may thus provide a negative feedback mechanism to prevent excessive immune activation.

C31 Epigenetic Control of Th2 Induction by Dendritic Cells

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Dendritic cells (DCs) play a critical role in Th2 priming during helminth infection, yet the mechanism by which they direct Th2 polarization is poorly understood. Since Th2 inducing DCs display minimal transcriptional activation, we investigated whether Th2 priming by DCs is dependent on epigenetic regulation of gene transcription via methyl-CpG binding domain protein-2 (MBD2), which links CpG methylation to repressive chromatin structure. We found that global MBD2$^{-/-}$ mice mount an impaired Th2 response following injection of eggs from the medically important parasitic helminth *Schistosoma mansoni*. Then, to determine the impact of restriction of MBD2 deficiency to DCs alone, we generated mice with conditional deletion of MBD2 in CD11c+ cells (CD11c^{cre}MBD2$^{fl/fl}$). These animals displayed significantly impaired Th2 development in response to egg challenge, but in-tact Th1 development following injection of heat killed *Salmonella typhimurium*. This suggests that MBD2 has an important role in controlling Th2, rather than Th1, priming by DCs. To further address this possibility, we generated bone marrow derived DCs from global MBD2$^{-/-}$ mice. These MBD2$^{-/-}$ DCs displayed defective activation in response to a variety of stimuli *in vitro*, and MBD2$^{-/-}$ DCs activated with soluble egg antigen (SEA) from *S. mansoni* exhibited severely impaired Th2 induction ability following transfer *in vivo*. These data demonstrate that epigenetic regulation of DCs, via the action of MBD2, can be critical for Th2 induction and development. Ongoing work is investigating which genes are targeted by MBD2, with the aim being identification of specific mechanisms employed by DCs that are fundamental for Th2 promotion.

C32 Subset-specific function of RIG-I-like halicases in monocyte-derived dendritic cells

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Cytosolic RIG-I-like helicases (RLR) are pattern recognition receptors involved in type I interferon production and antiviral immunity. We studied the expression and functional role of signalling cascades associated to RLR in the previously identified CD14$^-$ DC-SIGN$^+$ PPARγ^{low} CD1a$^+$ and CD14^{low} DC-SIGN$^+$ PPARγ^{high} CD1a$^-$ human monocyte-derived dendritic cell (moDC) subsets. Our results revealed that the expression of RLR genes, proteins, and the activity of the coupled signaling pathways are significantly higher in the CD1a$^+$ subset than in its phenotypically and functionally distinct CD1a$^-$ counterpart. Specific activation of RLR in moDC by pI:C or influenza virus was shown to induce the secretion of IFNβ via IRF3, whereas the regulation of pro-inflammatory cytokine production was under exclusive control of the TLR3-NF-κB pathway challenging the current paradigm of the collaborative or dichotomical regulation of these cytokine responses. The requirement of RLR-mediated signaling in CD1a$^+$ moDC for priming naïve CD8$^+$ T lymphocytes and inducing influenza virus-specific cellular immune responses was confirmed by RIG-I/MDA5 silencing that abrogated these functions. Our results demonstrate the subset-specific activation of RLR and identify CD1a$^+$ moDC as an inflammatory subset with specialized functional activities. Furthermore, the presence of RIG-I/MDA5 positive cells with DC morphology in human lungs with adult respiratory distress syndrome caused by A(H1N1)-2009 influenza virus infection verified the *in vivo* importance of CD1a$^+$ RIG-I and MDA5 expressing DC. These findings not only describe the underlying mechanism of IFNβ production by moDC subsets but also identify the CD1a$^+$ DC subtype as a potential target for improving the efficacy of prophylactic and/or therapeutic vaccines against intracellular pathogens.

C33 *Leishmania-infected macrophages are resistant to NK cell cytotoxicity, but susceptible to NK cell-derived activating cytokines*

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NK cells are components of a protective immune response against intracellular pathogens such as *Leishmania* parasites that reside within myeloid cells. They are activated by conventional dendritic cells in a TLR9-, IL-12-, and IL-18-dependent manner during the early phase of infection and help to restrict the tissue parasite burden. Here, we tested whether NK cells contribute to the control of *Leishmania* infections by lysing or by activating infected host cells via cytokine release. As assessed by co-culture experiments and ^{51}Cr-release-assay activated NK cells from *L. infantum*-infected or poly(I:C)-treated mice did not lyse *Leishmania*-infected macrophages or dendritic cells but efficiently killed tumor target cells. Consistent with these *in vitro* data, CFSE-based *in vivo* cytotoxicity assays showed only a poor NK cell-mediated cytotoxicity against adoptively transferred infected or uninfected wild-type macrophages, while MHCI-deficient macrophages serving as a positive control were efficiently eliminated. This protection of infected macrophages against NK cell cytotoxicity is partly attributed to their inability to up- or down-regulate NK cell-activating (Rae1alpha, MULT-1, CD48) or inhibitory molecules (MHCI, Qa-1) on the host cell surface in response to *Leishmania* infection. Interestingly, NK cells stimulated by IL-12 and IL-18 helped macrophages to kill intracellular parasites *in vitro* in a cell contact-independent manner via the release of IFN-gamma and TNF and the induction of iNOS. We conclude that *Leishmania* parasites, unlike viruses, do not render infected myeloid cells susceptible to the cytotoxic activity of NK cells. Instead, soluble products of NK cells trigger the leishmanicidal activity of macrophages.

C34 IL-10 Limits the Pathogenic TNF Production by Liver M1 Myeloid Cells Through the Induction of Nuclear P50 NF-kB During Parasitic Infection

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The development of classically activated myeloid cells (M1) is a prerequisite for effective elimination of African trypanosomes. However, persistent activation of M1 and associated production of TNF and NO cause tissue injury, including liver cell necrosis, hereby negatively affecting survival of the infected host. In relatively tolerant models of trypanosome infection, such as *T. congolense* infection in C57Bl/6 mice, we observed an accumulation of bone marrow-derived CD11b+Ly6C+ inflammatory myeloid cells in the liver of infected mice, which were characterized as a main pathogenic M1 subpopulation that produces TNF and iNOS. Using *in vivo* anti-IL-10R antibody treatment we showed that IL-10 reduces liver injury and increases survival during *T. congolense* infection by limiting both recruitment and M1-type activation of these inflammatory cells. In addition, by using myeloid cell-specific IL-10 KO mice, we showed that IL-10 derived from myeloid cells is involved in limiting TNF production by CD11b+Ly6C+ inflammatory myeloid cells during *T. congolense* infection. Moreover, we provide evidence that this IL-10-dependent suppression of TNF production is regulated via the NF-kB family of transcription factors. Indeed, a preferential nuclear accumulation of the p50 NF-kB subunit was observed, which could block M1 activation in liver myeloid cells in an IL-10 dependent manner, providing a possible downstream mechanism for the anti-inflammatory role of IL-10 on liver M1 activation.

C35 Effects of the laminated layer of larval *Echinococcus granulosus* on dendritic cell and macrophage phenotype

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Helminths evade their hosts' immune systems by stimulating endogenous anti-inflammatory circuits, so that a regulatory component becomes superimposed onto the Th2 response. Infection with *Echinococcus granulosus* larvae is an interesting model to study this phenomenon, as this parasite has an extreme capacity to control inflammation after its establishment in appropriate hosts. Upon establishment, this larva deploys a massive acellular mucin-rich coat termed the laminated layer (LL). We are analysing how macrophages (Mϕ) and dendritic cells (DC) decode a particulate preparation of the LL (pLL). *In vitro*, exposure to pLL inhibits the conventional pro-inflammatory response of bone marrow-derived Mϕ/DC to TLR agonists (IL-12 secretion, induction of CD40), while potentiating IL-10 expression. Interestingly, pLL *per se* strongly stimulates expression of CD86, but not CD80 or MHCII. Therefore Mϕ/DC appear to respond to pLL with a suppressed phenotype. This response is dependent on protein components in pLL, since induction of the phenotypic changes is abrogated by proteolysis with pronase, but not by oxidation with periodate. In preliminary *in vivo* experiments we also observed that intraperitoneal injection of pLL in C57BL/6 mice elicited in lymphoid organs an antigen-specific IL-10 response, together with a moderate Th2 response that did not increase with increasing antigen dose. Locally in the peritoneal cavity, pLL injection caused a striking increase in the percentage of $CD4^+$ $FoxP3^+$ T cells. Altogether, these initial results suggest that pLL is able to induce tolerogenic responses, probably through specific Mϕ/DC phenotypes.
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C36 Antigen presenting cell requirements for Th2 induction *in vivo*

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We have previously shown that depletion of CD11c+ cells using diphtheria toxin (DTx) treatment of CD11c.DOG mice, which express the human DTx receptor under the control of the CD11c promoter, severely impairs Th2 response induction against *Schistosoma mansoni* eggs *in vivo*. In this system CD11c depletion significantly impaired, but did not completely ablate, the Th2 response. Further, coincident with reduced Th2 induction, CD11c depleted animals displayed intact or increased IFN-γ production. We have now gone on to assess the contribution of different types of antigen presenting cell (APC) in the Th2 induction process, and in priming of the residual Th2 response evident in CD11c.DOG mice, using strategies to deplete other potential APCs. Clodronate liposomes deplete phagocytic cells such as macrophages, which may have antigen presenting capacity. Clodronate liposome treatment combined with CD11c depletion has allowed us to test the relative contribution of CD11c+ DCs and other phagocytes in promotion of the Th2 response to *S. mansoni* eggs. In addition we have characterized which cell types are the major sources of IL-4 and IFN-γ following egg injection, and whether this is altered following APC depletion.

C37 The role of triggering receptor expressed on myeloid cells-2 in inflammation

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The triggering receptor expressed on myeloid cells (TREM) -2, is a type-1 transmembrane protein, expressed on macrophages and other myeloid cells. Next to a role in bone formation and brain function, TREM-2 negatively regulates inflammation by macrophages, by a yet poorly understood mechanism.
To identify interaction partners of the TREM-2 complex we decided to perform a pulldown experiment. Macrophages were stably transfected with streptavidin-hemaglutinin tagged TREM-2 or with a chimeric construct, consisting of the extracellular domain of TREM-2 fused to the intracellular domain of its adaptor protein DAP12. This chimera will allow distinguishing between proteins that directly interact with TREM-2 and secondary interactors, which associate with the complex via DAP12. Interactions partners will be analyzed by LC-MSMS and verified by overexpression and silencing strategies.
Since TREM-2 has been shown to be a phagocytic receptor for *E. coli* and to negatively regulate TLR responses, we hypothesized that TREM-2 importantly contributes to the host defense against bacterial infections, such as *E. coli* peritonitis. Preliminary experiments with wildtype (WT) and TREM-2 knockout mice showed an improved bacterial clearance in TREM-2-/- animals. Accordingly, proinflammatory cytokines were significantly reduced in gene deficient mice 16h after infection, thus indicating resolution of inflammation. However, peritoneal cell counts remained increased in TREM-2-/- animals and we did not observe any differences in survival. One potential explanation we are currently investigating is based on the hypothesis that TREM-2-/- mice might be incapable of clearing apoptotic cells, hence displaying prolonged inflammation despite an improved ability to eliminate bacteria.

SUBMITTED ABSTRACTS TOPIC D: MONOCYTES, MACROPHAGES AND DCS IN CANCER

D01 NGAL expression in tumor-associated macrophages as a marker of pro-tumorigenic activation

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Macrophages are critical players in tumor development and progression since they are, among others, capable of providing direct growth support to cancer cells and recruiting new blood vessels to developing tumors. Little is known about the interaction of cancer cells with infiltrating monocytes or tumor-derived signals that are able to re-program macrophages towards a pro-tumorigenic phenotype. However, a hallmark of tumor-associated macrophages is the transcription factor STAT3 that was previously linked to tumor-derived signals or autocrine IL-6. Neutrophil gelatinase-associated lipocalin (NGAL) is a member of the lipocalin superfamiliy that transports small lipophilic ligands and essential factors such as iron. NGAL is up-regulated in a number of pathological conditions and has been defined as a pro-survival factor for immune cells as well as cancer cells.
We provide emerging evidence regarding potential functions of NGAL in breast cancer and the molecular mechanisms that underlie its production. NGAL is secreted by primary human macrophages activated with tumor cell supernatants. Reporter assays of full length or deletion constructs of the NGAL promoter provided evidence that NGAL production in macrophages is STAT3-dependent, which is activated downstream of IL-10 signalling. Furthermore, IL-10-mediated NGAL expression not only supports pro-angiogenic macrophage polarization, but also growth and proliferation of the human breast cancer cell line MCF-7.
Our data point to a macrophage-dependent IL-10-STAT3-NGAL axis that might contribute to tumor progression. Understanding the regulation of relevant genes such as NGAL is of crucial importance for potential therapeutic approaches to complex diseases such as cancer.

D02 p50 NF-κB is a key orchestrator of cancer-related inflammation

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Colorectal cancer (CRC) is one of the best example of pathological association between chronic inflammation and cancer development. Tumor Associated Macrophages (TAM) represent the major leukocyte population present in tumors. Despite macrophages are potentially able to express M1 polarized anti-tumor functions, within the tumor microenvironement TAM undergo to phenotypic switch promoting an M2 polarized phenotype with tumor promotion properties. We have demonstrated that nuclear accumulation of p50 NF-κB promotes a tolerant pro-tumoral phenotype. Recently, we have shown that, endotoxin tolerance and M2 macrophage polarization are related processes orchestrated by p50. This may be relevant in the gut, where innate immune cells are central regulators of intestinal homeostasis and orchestrate the balance between immune response and tolerance. Based on this, here we investigated the role of p50-driven polarized inflammation in CRC development and progression, by using two distinct models of genetic- (Apc^{Min} mice) and colitis-associated cancer (CAC). Analysis of mice survival, tumor incidence, size and histopathological stage, in Apc^{Min} versus Apc^{Min}-$p50^{-/-}$ mice, demonstrates that the p50 NF-κB subunit is required to support cancer growth at different stages of the neoplastic process, including early (tumor initiation) and late stages of tumor progression. Strikingly, using a chemical model of CAC, we observed that p50-/- mice exhibit a dramatic intestinal inflammation (as scored by weight loss, intestinal bleeding and histological analysis of colon tissues) paralleled by reduced incidence of tumor development. Overall our results suggest that, irrespective of the etiological events triggering CRC development, the p50 NF-κB subunit is required to promote cancer development.

D03 CCL2-induced VEGF-A production by infiltrating macrophages sustains Kaposi sarcoma growth

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CCL2 is an inflammatory chemokine overexpressed in several tumors both at primary and metastatic sites. Beside monocyte recruitment at tumor site, CCL2 exerts other protumoral functions such as tumor-associated macrophage polarization toward a M2 phenotype and promotion of metastasis. Here we describe an in vivo model of Kaposi sarcoma using a cell line (KS-IMM) overexpressing the atypical chemokine receptor D6 that drives to degradation several inflammatory CC chemokines. D6 overexpression did not influence KS-IMM in vitro proliferation rate while significantly reduced its growth when subcutaneously injected in the flank of nude mice when compared to mock cells. Leukocyte infiltrate of D6 overexpressing tumors was mainly composed by $Ly6C^{high}/F480^{+}$ monocytes with strong reduction of both neutrophils and tumor-associated macrophages when compared to mock tumors. D6 overexpressing tumors had decreased amount of mVEGF-A and reduced angiogenesis compared to mock tumors. We found that CCL2 present in KS-IMM conditioned medium enhanced VEGF-A production by bone marrow-derived macrophages with a CCR2 and COX_2 dependent mechanism. Thus regulation of CCL2 bioavailability by D6 overexpression at tumor site inhibited monocyte differentiation and VEGF-A production resulting in reduced tumor growth.

D04 Interaction of monocyte subpopulations with tumour derived microvesicles

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Introduction: Tumor-derived microvesicles (TMV) are small membrane fragments released by tumor cells during proliferation, activation etc. They may modulate biological activity of monocytes both *in vitro* and *in vivo*. Blood monocytes are a heterogeneous population of cells involved in inflammatory and anti-tumour response. Based on CD14 and CD16 expression, in peripheral blood two main subpopulations of monocytes were described: $CD14^{++}CD16^{-}$ and $CD14^{+}CD16^{++}$. Now we ask if TMV interactions with these subpopulations of monocytes mimic tumor cell induced production of cytokines, RNI and ROIs.

Methods: TMV were isolated by centrifugation of tumor cell culture supernatants. Monocytes were isolated from blood leukocytes by elutriation and their subpopulations ($CD14^{++}CD16^{-}$, $CD14^{+}CD16^{++}$) by FACS sorting. Monocytes and their subpopulations were cultured with TMV. Cytokines production was measured by ELISA and real time RT-PCR. Intracellular production of ROIs and RNI was detected by flow cytometry.

Results: $CD14^{+}CD16^{++}$ monocytes stimulated with TMV showed significantly higher production of NO, TNF and IL-12, and lower secretion of IL-10 in comparison to $CD14^{++}CD16^{-}$ cells. H_2O_2 and O_2^{-} production after stimulation with TMV was significantly lower in $CD14^{+}CD16^{++}$ subpopulation. Now, we presented for the first time that TMV may interact with subpopulations of monocytes with a pattern similar to tumour cells. $CD14^{+}CD16^{++}$ subpopulation, in comparison to $CD14^{++}CD16^{-}$ monocytes responds to TMV with an increased proinflammatory cytokine and NO release (similar as after interaction with tumour cells) but not ROI production. We suppose that contact with TMV might be sufficient to trigger anti-tumour response of monocytes.

D05 Dendritic cells differentiated from breast cancer patients' monocytes (Mo-DCs) present a functional bias towards the induction of regulatory T cells via tgf-beta participation.

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We observed previously that immature Mo-DCs (Mo-iDCs) from breast cancer patients showed higher CD86 expression and induced significantly higher frequency of suppressive regulatory T cells (Tregs) in vitro, than healthy Mo-iDCs. Thereby, we designed this study to compare T cell stimulation by monocytes, Mo-iDCs and mature Mo-DCs (Mo-mDCs) from breast cancer patients, investigating the roles of TGF-beta and CD86 expression by Mo-DCs.
Mo-iDCs were differentiated from breast cancer patients' blood monocytes in presence of GM-CSF and IL-4 for seven days, TNF-alfa addition (day 5), was used to obtain Mo-mDCs. Monocytes and Mo-DCs were characterized by flow cytometry and co-cultured with $CD4^{+}CD25^{neg}$ lymphocytes. Cell activation (CD25 expression) and *de novo* Tregs ($CD4^{+}CD25^{+}Foxp3^{+}$) generation were analyzed in these co-cultures after 6 days. In Mo-iDCs-T lymphocyte co-cultures, we tested the effects of monoclonal anti-TGF-beta antibodies upon lymphocyte stimulation by Mo-iDCs (FACS-sorted in $CD86^{Low}$ and $CD86^{High}$ subpopulations).
Interestingly, patients' monocytes induced a significantly higher frequency of $CD25^{+}$ expression and a lower Treg frequency, inducing higher TNF-alfa and IFN-gamma levels. Surprisingly, we found no differences between Mo-iDCs and Mo-mDCs in Tregs induction or lymphocyte activation. Mo-DCs FACS-sorting showed that, when compared to $CD86^{Low}$, $CD86^{High}$ Mo-iDCs induced a higher frequency of $CD25^{+}$ lymphocytes, but also a higher number of Tregs. Antibody blocking of TGF-beta in unsorted Mo-iDCs –T cells co-cultures, caused a 50% decrease in Treg frequency, an effect that was not noted in sorted $CD86^{High}$ Mo-iDCs-T cells co-cultures.
Those apparent bias needs to be considered on the effectiveness of cancer immunotherapy based on patients' Mo-DCs.

D06 C-myc ablation in tumor-associated macrophages inhibits B16 melanoma progression.

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C-myc is a proto-oncogene whose deregulated expression is associated with the development of tumors in mice and humans and for this reason its role in tumor cell biology has been extensively investigated. In addition to the tumoral cells, we observed c-myc expression in tumor-associated macrophages (TAMs) which infiltrate tumors in the B16 melanoma model. To elucidate the role of c-myc in these TAMs, we bred *c-myc*$^{fl/fl}$ mice with *LysM*$^{cre/+}$ mice to yield mice with c-myc-null TAMs (*c-myc*$^{fl/fl}$ *LysM*$^{cre/+}$) and control counterparts (*c-myc*$^{fl/fl}$). Bone-marrow derived macrophages (BMDM) obtained from control mice acquire TAM-like phenotype and properties when stimulated *in vitro* with B16-conditioned medium. By contrast, BMDM from *c-myc*$^{fl/fl}$ *LysM*$^{cre/+}$ mice show reduced proliferation rates and only basal expression and activity of the pro-tumoral molecules MMP9, HIF1α and VEGF. To study *in vivo* the role of c-Myc in TAMs, we implanted s.c. $5x10^5$ B16 cells-luciferase into control and *c-myc*$^{fl/fl}$ *LysM*$^{cre/+}$ mice and tumor growth was followed for 2 weeks by in vivo-imaging (IVIS). We observed a significant delay in tumor growth in *c-myc*$^{fl/fl}$ *LysM*$^{cre/+}$ mice and consequently, smaller tumors and reduced lung metastasis at the time of sacrifice. This reduction in tumor size correlates with lower expression of mRNA for MMP9, HIF1α and VEGF within the tumor. All together, our results indicate that c-myc plays an important role in tumor growth by controlling the expression of several pro-tumoral factors in TAMs and suggests the use of c-myc inhibitors to interfere with TAM activation as a potential anti-tumoral therapy.

D07 Manipulating Tumour Associated Macrophages (TAM) in a mouse model of B-cell Non-Hodgkin Lymphoma (NHL)

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We are testing the hypothesis that TAM play an important role in the progression of NHL, and might therefore constitute a rational and effective therapeutic target.
Our aim is to investigate tumour-macrophage interactions by manipulating TAM numbers and phenotype in a transplantable mouse lymphoma.
As a model system to study B-NHL we used a B-cell lymphoma arising in Eμ-myc/bcl-2 transgenic mice, which, when intravenously injected into healthy C57BL/6 mice produced a disseminated lymphoma. Macrophage depletion was achieved by intravenous injection of Liposomal Clodronate. Subsequent studies employed adoptive transfer of syngeneic bone marrow derived macrophages (BMDM), in vitro polarized to M1, M2 and model-specific TAM phenotypes.
Lymphoma growth was assessed by measuring lymph node weight, and cross-sectional tumour area in tissue sections, as well as by flow cytometry. Gene expression changes in whole lymph nodes with and without lymphoma, and following interventions to manipulate macrophage populations, were determined by real-time PCR. Changes to the cellular composition of the immune microenvironment were assessed by FACS analysis of single-cell suspensions of lymph nodes, and by immunohistochemistry.
Intravenous Liposomal Clodronate successfully depleted macrophages in the bone marrow, lymph nodes, and spleen, and significantly reduced lymphoma mass compared to vehicle controls. Adoptive transfer of M1-polarized BMDM attenuated lymphoma growth, and transfer of TAM augmented lymphoma growth.
Our studies support a relationship between macrophage numbers/phenotype, and lymphoma progression. Targeting TAM may provide an attractive therapeutic opportunity in human B-lymphomas.

D08 The Role of Macrophages in a Mouse Model of Pancreatic Cancer

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Macrophages are important regulators of many key functions under physiological and pathological conditions. In the tumour microenvironment macrophages are abundant in several types of cancer. Their abundance is negatively correlated with prognosis and overall survival.
The recruitment of macrophages into tumours is mediated by cytokines and chemokines, such as colony-stimulating factor-1 (CSF-1), a key component in macrophage function, proliferation, maturation and survival that is regulated through the receptor tyrosine kinase CSF-1R. The importance of the CSF-1/CSF-1R pathway has been shown in experimental animal models of tumours. Therefore blockade of macrophage recruitment using CSF-1R antagonists may represent an effective therapeutic strategy in tumours where macrophages are abundant.
Here, I describe the effects of a potent and selective inhibitor of CSF-1R, AZD7507, using an *in vivo* mouse model of pancreatic cancer. In a genetic mouse model of pancreatic adenocarcinoma, the administration of AZD7507 reduced the numbers of macrophages in the tumour-bearing pancreas, suggesting that the recruitment of macrophages during tumour growth is CSF-1 dependent.
Inhibition of CSF-1/CSF-1R signalling could provide a therapeutic tool, in particular after chemotherapy with gemcitabine, to block macrophage recruitment in pancreatic cancer. This could possibly be used as an additive therapy in future applications.

D09 Tlr-4 dependent inflammation in the tumor microenvironment drives metastatic progression of melanoma

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Metastatic disease is responsible for most cancer-related deaths. Recently, evidence has accumulated that the activation of innate immunity in the tumor microenvironment can promote migration, invasion and metastatic spread of malignant cells. Here we tested this hypothesis in the genetically engineered Hgf-Cdk4^{R24C} mouse model where primary melanomas can be induced by a single epicutaneous application of DMBA on the back skin. Cohorts of DMBA-exposed mice were either treated epicutaneously with TPA to promote a chronic inflammatory response in the skin or received vehicle only. All mice developed multiple primary cutaneous melanomas 59±4 days after initiation with DMBA and had to be sacrificed on day 108±13 due to large tumor burden. Surprisingly, TPA-induced chronic inflammation did not affect incidence, multiplicity and growth kinetics of primary melanomas in the skin but significantly promoted metastatic spread of melanoma cells into the draining lymph nodes and lungs. To understand how tumor-associated inflammation promotes tumor progression, we treated subcutaneous Hgf-Cdk4^{R24C} melanoma transplants repetitively with TPA. Again, TPA-induced inflammation significantly enhanced the regional and systemic metastatic spread of melanoma cells. This effect was largely abolished in Tlr4-deficient mice, indicating that TPA did not act directly on melanoma cells but rather indirectly via the induction of endogenous Tlr4-ligands in the tumor microenvironment. TPA-treated Tlr4-deficient mice showed significantly lower levels of proinflammatory mediators and decreased numbers of local and systemic Gr-1^{+}CD11b^{+} myeloid immune cells compared to wildtype mice. Taken together these results suggest that a Tlr-4 driven inflammatory response in the tumor microenvironment drives melanoma metastases.

D10 Intratumoral monocyte differentiation to M1-like versus M2-like tumor-associated macrophage subsets correlates with tumor malignancy

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Tumors are often highly infiltrated with pro-tumoral inflammatory cells, such as tumor-associated macrophages (TAMs). We recently reported the co-existence of M1- and M2-like TAM subsets derived from Ly6C^{high} monocytes (Movahedi et al, Cancer Res, 2010), but to which extent intratumoral monocyte differentiation is skewed by the malignancy of the tumor is unknown.
Here we show that tumors from both high- and low-malignant 3LL lung carcinoma and T241 fibrosarcoma variants were infiltrated with a large heterogeneous mononuclear fraction, encompassing two discrete TAM subpopulations (Ly6C^{low}MHCII^{high} and Ly6C^{low}MHCII^{low}) and tumor-associated DCs (TADCs). Monocyte tracking and BrdU kinetics suggested that both TAM subsets as well as the TADCs originated from tumor-infiltrating Ly6C^{high}inflammatory monocytes. However, while Ly6C^{high}monocytes preferentially differentiate to Ly6C^{int}MHCII^{low}immature TAM and subsequently to Ly6C^{low}MHCII^{low}TAM in high-malignant 3LL-R and T241 tumors, mainly Ly6C^{low}MHCII^{high}TAM were present in low-malignant 3LL-S and T241/HRG tumors.
In 3LL tumors, gene and protein analyses confirmed that the different TAM subsets had a distinct molecular profile and activation state, with the MHCII^{high}TAM being more M1-like(MMR^{low}Stab-1^{low}IL-4Ra^{low}CD11c^{+}) and the MHCII^{low}TAM being M2-like(MMR^{high}Stab-1^{high}IL-4Ra^{high}CD11c^{-}). While both TAM subsets are equally angiogenic, they differed in antigen-presenting capacity, T-cell suppressive activity and responsiveness to environmental stimuli.
Consequently, the proportion of MHCII^{low} (M2-like) versus MHCII^{high} (M1-like) TAM appears to correlate with the malignancy of a tumor.

D11 Study of THP-1 macrophage M2 polarization by MDA-MB-231 breast cancer cells and their involvement in chemotherapy resistance

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Tumor associated macrophages (TAM) are a major tumor stroma component. Once recruited from the blood flow, monocytes differentiate into macrophages and adopt a M2 polarization. M2 macrophages promote, unlike M1 macrophages, tumor progression and invasion. Their protective effect on the effectiveness of chemotherapeutic drugs has been also already described. Another tumor microenvironment component responsible for chemoresistance is hypoxia.
We aim to study the influence of M2 macrophages on MDA-MB-231 cell response to chemotherapy both under normoxia and hypoxia. For this purpose, we used THP-1 monocytes differentiated into macrophages by using PMA. To obtain TAM, we then incubated these macrophages with MDA-MB-231 cell conditioned medium during 72 hours. A cocktail of IL-4 and IL-13 was used as a positive control to induce M2 polarization.
M2 polarization induced by MDA-MB-231 cell conditioned medium was verified by studying the expression of known markers: CD206 receptor and IL-10. CD206 mRNA expression was highly induced by incubation in the presence of MDA-MB-231 cell conditioned medium. Change in CD206 expression was confirmed at the protein level by FACS analysis. IL-10 expression was studied at the mRNA and protein level by RT-qPCR and ELISA respectively. Both approaches showed an increase in IL-10 expression after THP-1 derived macrophages incubation with MDA-MB-231 cell conditioned medium. These results showed that MDA-MB-231 cell conditioned medium was able to polarize THP-1 cells into M2-like macrophages. In the future, we shall study the impact of these M2-like TAM on the MDA-MB-231 cell response to chemotherapy both under normoxia and hypoxia.

D12 Interaction between human monocyte-derived macrophages and tumor cell lines *in vitro*

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Macrophages, derived from monocytes recruited at the tumor site, can either suppress (so-called M1) or promote (so-called M2) tumorigenesis depending on the nature of the microenvironment cues.
In order to understand the interactions between macrophages and tumor cells, we studied the effect of 22 tumor cell line conditioned media (TCM) on freshly-isolated human monocytes. The phenotype of the subsequent monocyte-derived macrophages (MDM) was characterized with regards to the expression of 14 surface receptors as well as 42 cytokines/chemokines, and compared to the phenotype of monocytes treated with M1, M2a or M2c phenotype inducing cytokines.
We identified 8 TCM supporting monocyte survival and differentiation into either M1-like or M2-like macrophages. Five tumor cell lines (Hs578T, KPL-4, MDA-MB-468, SKOV-3 and PC-3) expressed sufficient amount of M-CSF to maintain monocyte survival and support differentiation towards an M2-like phenotype ($CD163^+$, $CD80^-$, absence of pro-inflammatory cytokines expression) whereas 3 tumor cell lines (MDA-MB-231, Du-145 and SKHep-1), expressing both M-CSF and GM-CSF, sustained a differentiation into a mixed M1/M2 like phenotype ($CD163^+$, $CD206^+$, $CD80^{+or-}$, pro and anti-inflammatory cytokines release).
To further understand the cross-talk between macrophages and tumor cells, the culture media from 6-day monocyte/TCM culture (Mφ-TCM) was used to culture the 8 tumor lines identified above. Interestingly, we observed an increase in proliferation of KPL-4 and MDA-MB-468 in these conditions compared to TCM alone indicating the presence of soluble factors in Mφ-TCM, responsible for the increase in tumor cell proliferation. The factors and their role in tumor-macrophage interaction are under investigation.

D13 Human CLEC4C/BDCA-2/CD303 is a Receptor for Asialo Galactosyl Oligosaccharides

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Plasmacytoid dendritic cells (pDCs) are specialized in the production of type I interferon (IFN-I), which promotes antiviral and anti-tumor responses, as well as autoimmune disorders. Activation of IFN-I secretion depends on TLR7 and TLR9, which sense microbial RNA and DNA respectively. IFN-I production is modulated by several receptors, including the type II C-type lectin CLEC4C. The natural ligand of CLEC4C is unknown. To identify it, here we probed a glycan array with a soluble form of the CLEC4C ectodomain. We found that CLEC4C recognizes complex type sugars with terminal galactose. Importantly, soluble CLEC4C bound peripheral blood tumor cells that express glycans with galactose residues at the non reducing ends. The positive and negative modulation of galactose residues expression on cell membrane was paralleled by the regulation of IFN-I secretion by pDCs in vitro in cell co-culture experiments. These results suggest that the modulation of terminal galactose sugar moieties by invading pathogens or transformed cells may result in the regulation of IFN-I response and immune surveillance.

D14 A role for CD32B and humoral immunity in the polarization of monocytes and macrophage in human cancer

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Several studies in mice tumor models have shown myelomonocytic cells to be polarized to a tumor promoting phenotype during tumor progression. However, evidence for such an observation in human cancers and the molecules which direct this process is still not well known. In this context, we investigated the role of human monocytes/macrophages in the progression of human Renal Cell Carcinoma (RCC). Microarray study of blood monocytes from RCC patients as compared to monocytes from healthy subjects showed a distinct gene expression profile. Transcriptomics as well as qPCR analysis showed RCC-Mo to be refractory to major inflammatory stimuli such as Lipid A (LPA), TNFα and IL-1β. RCC-Mo exposed to Lipid A (LPA) stimulation showed a failure to upregulate many inflammatory genes (such as TNFA, IL6, IL12p40 and IL1B) linked to M1 macrophage activation. Signaling studies revealed this profile to be linked to a defective activation of MAPK, NF-κB and AP-1. The refractoriness could be reproduced at the gene expression and signaling level *in vitro* by incubating normal monocytes with patient sera but not sera from healthy donors, suggesting the involvement of a serum-associated soluble factor. Interestingly, data from transcriptome, qPCR and flow cytometric studies showed RCC-Mo to consistently upregulated the expression of CD32b. Subsequent studies revealed the role of CD32b and humoral immunity in polarizing monocytes and tumor associated macrophages during renal carcinoma progression.

D15 Reduced influx of immunosuppressive myeloid cells upon intratumoral galectin-1 knockdown in the brain of glioma-bearing mice

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Background: Galectin-1 is a glycan-binding protein of which the expression is altered in several tumors and metastatic lesions including high-grade glioma. Given the plethora of immunosuppressive functions exerted by this lectin, we hypothesize that galectin-1 is an important contributor to glioma-mediated immune escape and thereby counteracts the efficacy of immunotherapeutic strategies.
Methodology: We investigated how intratumoral galectin-1 knockdown (KD) influences tumor progression and changes innate and adaptive antitumoral immune responses in a syngeneic GL261 orthotopic murine glioma model. Stable Gal-1 knockdown was achieved via transduction of GL261 cells with a lentiviral vector encoding a Gal-1-targeting miRNA.
Results: Intracranial challenge with Gal-1 KD GL261 cells significantly improved median survival compared to Mock-bearing mice. Prolonged survival required an intact $CD8^+$ T cell response as survival was significantly shortened in Gal-1 KD bearing mice in which $CD8^+$ T cells were depleted. Flow-cytometric analysis of the brain-infiltrating immune cell population revealed a strong decrease in the percentage of tumor-infiltrating macrophages ($CD11b^+$ $F4/80^+$) and myeloid-derived suppressor cells ($CD11b^+$ $Ly6C^+$) in Gal-1 KD bearing mice compared to Mock-bearing mice. Moreover a decreased PD-L1 expression was found on CD11b+ F4/80+ myeloid cells isolated from Gal-1 KD bearing mice.
These recent findings suggest that galectin-1, when present in the tumor microenvironment, will not only interfere with the T-cell compartment, but can also counteract innate antitumor immune responses via the recruitment of macrophages with a pro-tumoral phenotype and myeloid-derived suppressor cells. Whether glioma-derived galectin-1 is also involved in the polarization of macrophages is currently under investigation.

D16 Induction of anti tumor responses against malignant melanoma via antigen targeting *in vivo*

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Dendritic cells (DCs) are very important antigen presenting cells in the immune system. They are essential for the initiation of immune responses as well as for maintaining central and peripheral tolerance. By using chimeric antigen carrying antibodies directed against the DC-subset specific C-type lectin and endocytosis receptors DCIR2 (33D1) and DEC205, we are able to target antigens to $CD11c^+CD8^-$ or $CD11c^+CD8^+$ DCs *in vivo*, respectively. We have demonstrated that the type of T cell response generated is dependent on the DC subset that presents the antigen *in vivo*. Here, we wanted to investigate if we can induce a protective anti-melanoma response by targeting DCs in naïve animals *in vivo*. For inducing an efficient immune response antigen carrying antibodies 33D1 or DEC205 were applied under immunizing conditions. In the used murine melanoma mouse model and immunization protocol mice showed a mixed T_H1/T_H2 mediated antibody response and a strongly prolonged survival with a diminished tumor growth. Moreover, antigen targeting to both DC subsets induced an even better anti tumor response. Antigen targeting in a therapeutic setting induced a delayed tumor growth and prolonged survival. Our results show that antigen targeting of DCs might be a future option for the induction of protective anti-tumor responses.
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D17 Macrophage contribution to gastric cancer cell invasion and angiogenesis

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Invasion is the hallmark of malignancy and one of the most appealing targets for anti-cancer therapy. To design efficient therapeutic tools it is relevant to dissect the molecular crosstalk established between cancer cells and elements of the tumour ecosystem. Macrophages are critical for breast cancer cell migration, invasion and metastasis but their role in gastric cancer is still poorly documented.
Envisaging therapeutic applications, we investigated the role of macrophages in gastric cancer cell invasion and angiogenesis, exploring the underlying molecular mechanisms. Therefore, primary human monocytes isolated from healthy blood donors were differentiated into distinct macrophage populations (naïve, pro-inflammatory/M1 and anti-inflammatory/M2), and confronted with gastric cancer cells in Matrigel-invasion assays and in the angiogenesis chorioallantoic-membrane (CAM) assay. Our results indicate that the distinct macrophage populations differently affect gastric cancer cell invasion and angiogenesis, being the anti-inflammatory/M2 the most efficient in such stimulation. Interestingly, invasion and angiogenesis did not require direct contact but the release into the medium of soluble pro-invasive factors. Gelatin-zymogram analysis and invasion assays with siRNA or inhibitors targeting matrix metalloprotease (MMP) activity revealed that MMP-9 is required for macrophage-mediated cancer invasion. By time-lapse-confocal-microscopy and RhoGTPases pull-down assays, the effect of the distinct macrophage populations on cancer cell motility was evaluated. Additionally, our results revealed that EGFR-phosphorylation plays a key role in macrophage-mediated cancer invasion, and siRNA and immunoprecipitation experiments identified relevant EGFR-tyrosine phosphorylation sites and downstream-interacting partners. These results bring novel insights on the role of tumour ecosystem on cancer invasion opening new perspectives for therapeutic intervention.

D18 Re-programming macrophages to enhance antibody immunotherapy

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Monoclonal antibodies (mAb) have become established in the treatment of a variety of malignancies - transforming patient outcomes. Despite this undoubted impact, responses remain variable and their mechanisms of action and of tumour resistance are controversial. Several strategies are being employed in an attempt to improve responses and one area in which there is growing interest is re-programming the tumour microenvironment to augment effector cell recruitment and function.
Antibody immunotherapy relies predominantly on activatory Fc-gamma-Receptors (FcγR) expressing macrophages for effector function. However, tumour associated macrophages have a pro-tumour, anti-inflammatory phenotype associated with a reduction in the activatory:inhibitory FcγR balance which we hypothesise reduces the potency of antibody therapy. The understanding of how macrophages are manipulated by tumours in vivo and how they may be re-polarised to augment mAb immunotherapy is a critical area of study where data is currently lacking.
Although previous studies have shown that macrophage polarity can be manipulated in vitro with characteristic phenotypic outcomes little has been done to correlate phenotypic changes with effector cell activity. Here we demonstrate using Toll-like Receptor agonists and other stimuli that we can efficiently polarise macrophage to an activatory FcγR phenotype both in vitro and in vivo. Further we show using our recently developed in vitro phagocytosis assay that these phenotypic changes lead to an enhancement of antibody mediated uptake of B cells. Finally, we demonstrate using an adoptive transfer model that we are able to use these clinically relevant reagents to enhance mAb mediated depletion of B cells in vivo.

D19 Functional comparison of regulatory T-cells isolated by different magnetic bead separation techniques – recommendation of the use of "untouched" cells for *in vitro* co-culture experiments

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Tregs are elevated in blood and pancreatic tumour tissue of patients. To analyse the mechanisms responsible for their enrichment and how they interact with tumour microenvironment, co-culture experiments with highly pure Tregs have to be performed. Tregs were usually isolated by magnetic based CD25-positive selection from pre-enriched CD4+ T-cells. These preparations can contain activated T-effector and γδ-T-cells which might falsify the results. Thus, we isolated Tregs by negative separation depleting contaminating cells by anti-CD127 and -CD49d antibodies [Kleinewietfeld, *Blood*, 2009]. When comparing positively selected and "untouched" Tregs they showed differences in their proliferation activity and inhibitory capacity for CD4+ T-effector cell s: CD4+CD25+CD127+49d+ T-cells exhibited higher activation and proliferation with/without additional stimulation than "untouched" CD4+25+127-49d- Tregs, but the latter were more potent inhibitors for CD4+ T-cell proliferation. Furthermore, CD4+ T-cells treated with anti-CD25-beads alone considerably affected T-cell proliferation indicating that labelling per se influences T-cell function. Since these effects might overlay with effects mediated by epithelial/tumour or stroma cells during co-cultures, the use of "untouched" Tregs was more suitable. In our first studies on the interplay of human pancreatic ductal epithelial (HPDE) cells and Tregs or control CD4+ T-cells, mock- and L1CAM-transfected HPDE cells were directly co-cultured with T-cells. L1CAM is highly expressed in pancreatic tumours and supposed to promote pancreatic tumour genesis. Preliminary results demonstrate that HPDE-mock cells induce Treg and CD4+ T-cells proliferation, whereas HPDE-L1CAM cells inhibited CD4+ T-cells but not Tregs. However, additional stimulation could restore CD4+ T-cell proliferation. Overall, these data point to a role for L1CAM in the modulation of T-cell proliferation.

D20 Antigen presenting and cytotoxic activities of monocytes generated from haematopoietic CD34$^+$ stem cells of colon cancer patients

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Monocytes exhibit direct and indirect antitumour activity and may be potentially useful for various forms of adoptive cellular immunotherapy of cancer. However, blood is a limited source of them. This study explored whether monocytes can be obtained from bone marrow haematopoietic CD34$^+$ stem cells of colon cancer patients, using previously described protocol of expansion and differentiation to monocytes of cord blood-derived CD34$^+$ haematopoietic progenitors. Data show that in two step cultures the yield of cells was increased approximately 200 fold and among these cells up to 60% of CD14$^+$ monocytes were found. They consisted of two subpopulations: CD14^{++}CD16$^+$ and CD14$^+$CD16$^-$, at approximately 1:1 ratio, that differed in HLA-DR expression, being higher on the former. No differences in expression of costimulatory molecules were observed, as CD80 was not detected while CD86 expression was comparable. Monocytes showed the ability to present recall antigens (PPD, *Candida*) and neoantigens expressed on tumour cells and tumour-derived microvesicles (TMV) to autologous CD3$^+$ T cells isolated from the blood. Like blood monocytes, they exhibited cytotoxicity towards tumour cells *in vitro* and CD14^{++}CD16$^+$ subset showed an enhanced activity. These small scale preliminary observations indicate that generation of monocytes from CD34$^+$ stem cells of cancer patients is feasible. To our knowledge it is the first demonstration of such approach that may open a way to obtain monocytes in large numbers for alternative forms of adaptive and adoptive cellular immunotherapy of cancer.

D21 NANOBODY MEDIATED TARGETING OF LENTIVIRAL VECTORS TO DENDRITIC CELLS: IMPLICATIONS FOR CANCER IMMUNOTHERAPY

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Lentiviral vectors (LVs) encoding tumour antigens are ideal candidates for anti-cancer vaccination, since they deliver the transgene as well as activation stimuli to dendritic cells (DCs) *in situ*. A critical step to improve LV safety while retaining its efficacy is to avoid off-target transduction.
We developed the nanobody display technology to target LVs to DCs (and macrophages). This innovative approach exploits the budding mechanism of LVs to incorporate a nanobody and a binding-defective but fusion-competent form of VSV.G in the viral envelope. Four nanobodies were used: BCII10, DC2.1, DC1.8 and R3_13. These were shown to bind to β-lactamase, DCs and macrophages of mouse and human origin, mouse DCs and human DCs respectively.
We first generated producer cell lines stably expressing a membrane bound form of these nanobodies to produce high titer LVs. Next, selective transduction *in situ* of conventional DCs (DC1.8) or the latter, plasmacytoid DCs and macrophages (DC2.1) was shown upon intranodal administration of targeted LVs in C57BL/6 mice. Importantly, selective transduction of human lymph node-derived myeloid DCs (R3_13), or these, plasmacytoid DCs and macrophages (DC2.1) was demonstrated *in vitro*. Using the mouse model, we further demonstrated *in vitro* that targeted LVs induce less TNF-α production by DCs than broad tropism LVs. Consequently the transgene-specific CD8$^+$ T cell response was less pronounced. This can be partially explained by the lack of TLR2 activation by targeted LVs. Nevertheless, targeted LVs induced a robust transgene-specific CD8$^+$ T cell response *in vivo,* demonstrating their potential as an off-the-shelf vaccine.

SUBMITTED ABSTRACTS TOPIC E: INFLAMMATORY SIGNALING IN MACROPHAGES AND DCS

E01 Microenvironment dictates plasmacytoid dendritic cells to favor inflammation or tolerance induction, how control to it?

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Plasmacytoid dendritic cells (pDC) are involved in innate immune response to viral infection. They can mount a strong anti-viral response with the release of high amount of type I IFN after recognition of pathogen-derived material via their specific receptors (TLR…). Since pDC demonstrate potent antigen (Ag) presentation capacity and marked ability to stimulate Ag-specific T cell responses, pDC have been involved in many adaptive mechanisms. Indeed, well-known in Th1 immune response to pathogen, pDC have been recently implicated in Th17 commitment as well as in Treg polarization and expansion. Effector T cell polarization is highly dictates by the microenvironment created by the Ag presenting cell (APC) giving TCR signaling, APC itself affected by the microenvironment shaped by the infection or pathogen elimination. In our hand, we demonstrated that the microenvironment created by apoptotic cell elimination –that can be relevant at the end of the immune response- will favor pDC to induce Treg polarization, *in vitro* and *in vivo*; such effect being dependent on TGF-β produced by phagocytes eliminating apoptotic cells. However, we also demonstrated that a TGF-β-rich microenvironment will dictate pDC to induce Th17 commitment. These data suggest that environmental factor(s), in addition to TGF-β, are able to either block or favor IL-6 secretion by pDC. Such factor(s) will thus represent a critical target to either promote pDC immunity or tolerance induction in response to virus or self-antigen, respectively.

E02 The ubiquitin-editing protein A20 prevents dendritic cell activation, recognition of apoptotic cells and systemic autoimmunity

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Dendritic cells (DC) are known as the prime inducers of effector immune responses, they also play an essential role in the maintenance of tolerance. DCs can be activated by Toll like receptors (TLRs, which recognize microbial products. Stimulation ultimately leads to activation of the transcription factor NF-κB. The signaling events downstream of TLR activation are closely regulated to allow proper threshold setting and avoid self-perpetuating activation that could potentially lead to autoimmunity. The most important negative regulator of NF-κB activation known to date is the ubiquitin-modifying enzyme A20/*Tnfaip3*.
We have established an *in vivo* mouse model, which carry DC-specific A20 gene ablation (using the Cre/loxP system). We have observed that A20/*Tnfaip3* determines the activation threshold of DCs, via control of canonical NF-κB activation. *Tnfaip3*$^{fl/fl}$*Cd11c-cre*$^{+}$ mice demonstrated spontaneous proliferation of conventional and double negative T cells, their conversion to interferon-□ (IFN-□□) producing effector cells, and expansion of plasma cells. They developed ds-DNA antibodies, nephritis, the anti-phospholipid syndrome and lymphosplenomegaly -features of systemic lupus erythematosus- and extramedullary hematopoiesis. A20-deficient DCs were resistant to apoptosis, caused by increased sensitivity to CD40L and RANKL pro-survival signals and upregulation of anti-apoptotic proteins Bcl-2 and Bcl-x. They captured injected apoptotic cells more efficiently, resisted the inhibitory effects of apoptotic cells, and induced self-reactive effector lymphocytes.
As genetic polymorphisms in *TNFAIP3* are associated with human autoimmune disorders, our findings put A20-mediated control of DC activation, survival and apoptotic cell handling as a crucial checkpoint in tolerance to self.

E03 Differential transcription factor activation by CLR and TLR ligands in macrophages: dissecting gene expression induced by the mycobacterial cord factor

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The mycobacterial cord factor trehalose-6,6-mycolate (TDM), a major virulence factor of *M. tuberculosis*, is recognized by macrophages in a Syk-Card9 dependent manner. Recently, we identified the C-type lectin receptor (CLR) Mincle as the receptor for TDM and its synthetic analogue trehalose-6,6-dibehenate (TDB). These two glycolipids are potent adjuvants that elicit mixed Th1/Th17 responses, whereas the Toll-like receptor TLR9 ligand CpG DNA induces exclusively Th1 responses**.** CLR and TLR ligands trigger distinct profiles of cytokine gene expression in APCs. How these unique transcriptional responses are generated, and which role they play for Th1 and Th17 induction is incompletely understood. TLR and CLR signaling activates the MAP-Kinase pathway and the transcription factor (TF) NF-kappaB. In contrast, the latent TF NF-AT is activated upon CLR but not upon TLR stimulation. However, little is known about the TF profile induced downstream of these constitutively expressed TFs. Here we assessed the expression kinetics of inducible TFs in macrophages after TDB and CpG stimulation to understand similarities and differences between expression profiles elicited by the two types of PRRs. TDB rapidly and directly induced “early growth response genes” Egr1, Egr2 and Egr3 expression dependent on Mincle. Cebpbeta and Hif1alpha were upregulated at the protein level after stimulation with TDB or CpG. Hif1alpha deficient macrophages produce less NO after TDB but not CpG stimulation and ChIP experiments confirmed Hif1alpha recruitment to the iNOS promoter. Taken together, our data shed new light on the regulatory network controlling transcriptional activation induced by the CLR Mincle in response to microbial Mincle ligands.

E04 Cellular and molecular basis of Interleukin-12 family members expression

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Interleukin(IL)-12 and the related heterodimeric cytokines IL-23 and IL-27 play crucial and distinct roles in shaping the innate and adaptive immune responses against invading pathogens. Imbalanced expression of these three cytokines can lead to auto-immune or inflammatory disorders. We previously demonstrated the critical role of Interferon Regulatory Factors (IRFs) in controlling their transcriptional activation. Herein, we show that DCs and macrophages subpopulations do not display the same capacity to express each of these cytokines. We observed that bone marrow-derived macrophages (BMMs) display a high capacity to produce IL-27p28 while dendritic cells (BMDCs) are prone to produce high levels of IL12/23p40. Furthermore, within BMDCs culture, these cytokines tend to be produced by distinct subpopulations. This situation is plastic as the pattern of cytokine production can be modulated by exogenous cytokines such as IFN-γ or IL-10. We are currently assessing the role of lineage-specific IRF members such as IRF8 in this functional specialization. Finally, we intend to use this model to understand how distinct gene expression patterns can be established in APC subpopulations by assessing constitutive and inducible histone marks at selected loci.

E05 MCS-18, a novel natural plant product prevents autoimmune diabetes

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There is still a vital need for new therapies in order to prevent or treat type I diabetes. In this respect, we report that MCS-18 a novel natural product isolated from the plant *Helleborus purpurascens* (i.e. Christmas rose) is able to increase diabetes free survival using the NOD-mouse model, which is accompanied with a diminished IFN-γ secretion of splenocytes. In the animal group which has been treated with MCS-18 during week 8 and week 12 of age 70% of the animals showed a diabetes free survival at week 30, whereas in contrast in the untreated animals less than 10% were free of diabetes. MCS-18 treatment significantly reduced islet T-cell infiltrates as well as the rate of T-cell proliferation. Periinsular infiltrates in the MCS-18 treated animals showed a significantly enhanced number of $Foxp3^+$ $CD25^+$ T cells, indicating the increased presence of regulatory T cells. These studies show that MCS-18 exerts an efficient immunosuppressive activity with remarkable potential for the therapy of diseases characterized by pathological over-activation of the immune system.

E06 Myeloid-specific A20 deletion induces expansion of Myeloid-Derived Suppressor Cells (MDSCs) protecting mice from experimental colitis.

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Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of undifferentiated myeloid cells with distinct immunoregulatory capacities. MDSCs represent a minor cell population in homeostatic conditions, but are expanded in response to inflammatory conditions and cancer. We generated mice with specific ablation of the anti-inflammatory protein A20 in myeloid cells. A20-deficient macrophages have hyperactive TNF receptor- and Toll-like receptor-induced NF-κB responses *in vitro*, and myeloid A20 knockout mice spontaneously produce high levels of pro-inflammatory cytokines. This pro-inflammatory environment induces the spontaneous expansion and activation of MDSCs, suppressing T-cell and innate immune responses. Consequently, myeloid A20 knockout mice are protected from DSS-induced colitis in an MDSC-dependent manner. Although A20 defects are associated with multiple human inflammatory and auto-immune pathologies, myeloid-specific A20 deficiency results in immunosuppression and protection from colitis.

E07 A Pellino3 knockdown inhibits TLR4–signaling in macrophages

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In macrophages the Toll–like receptor (TLR) 4 is activated in response to lipopolysaccharide (LPS), an integral component of the outer membranes of gram negative bacteria. Following extracellular binding of LPS to TLR4 an intracellular signal transduction cascade is initiated, provoking activation of the transcription factors NFκB and AP1, consequently inducing pro–inflammatory gene expression. While the TLR4 adaptor protein MyD88, as well as the kinases IRAK1/4 and TRAF6, are well defined, the IRAK E3 ligase Pellino3 has only been identified quite recently. As its exact role in TLR4–signaling remains obscure, we were interested in elucidating its impact on TLR4–dependent activation of NFκB and AP1.
We hypothesized that Pellino3 is a key regulator in the TLR4–signaling cascade and designed *in vivo* and *in vitro* experiments in mouse macrophages.
In a CLP (Cecal Ligation and Puncture) mouse model causing polymicrobial sepsis, we demonstrated upregulation of Pellino3 mRNA in primary $CD11b^+$ splenocytes. Based on these data we investigated the impact of Pellino3 on TLR4–signaling *in vitro*. Therefore, we performed a stable Pellino3 knockdown in RAW264.7 macrophages and stimulated cells with 1µg/ml LPS for different times. Our results point to a reduced NFκB and AP-1 reporter activity as well as a mRNA decline of the pro–inflammatory cytokine TNFα.
Our observations highlight a regulatory impact of Pellino3 on the TLR4–signaling pathway. Thus, antagonism of Pellino3 may provide a new strategy for the development of a therapy approach in sepsis.

E08 IRF3-dependent activation of inflammatory dendritic cells by extracellular host DNA mediates the adjuvant activity of alum on T_H2 responses

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Aluminum-based adjuvants (aluminum salts or alum) are widely used in human vaccination, although their mechanisms of action are poorly understood. We observed that, in mice, alum causes cell death and the release of host cell DNA, which acts as a potent endogenous immunostimulatory signal mediating the adjuvant activity of alum. Indeed, we observed that host DNA in quantities similar to those detected at alum injections sites is as potent as alum itself in boosting T and B cell responses. Furthermore, digestion of extracellular DNA at sites of alum injection decreased both cellular and humoral responses. Mechanistically, we observed that, following alum immunization, host DNA activates $CD11c^+$ $CD11b^+$ $Ly6C^+$ $Ly6G^-$ inflammatory dendritic cells (iDCs) through TANK-binding kinase-1 and Interferon response factor (Irf)3-dependent mechanisms, a process that implicates autocrine signaling by IL12p40 homodimers. These iDCs in turn stimulate 'canonical' T helper type 2 (T_H2) responses, associated with IgE isotype switching and peripheral effector responses. Furthermore, we propose that host DNA release also boosts IgG1 production through the induction of T follicular helper responses via iDC- and Irf3-independent mechanisms. The finding that host cell DNA released from dying cells mediates alum adjuvant activity may increase our understanding of the mechanisms of action of current vaccines and help in the design of new adjuvants.

E09 Binding of angiotensin IV on the insulin-regulated aminopeptidase in macrophages: more than enzymatic inhibition?

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The peptide angiotensin IV (Ang IV), a bioactive fragment of angiotensin II, is described to play a role in inflammation and in the progression of atherosclerosis. These Ang IV effects are mediated through high affinity binding to the AT4-receptor, which is identified as the insulin regulated aminopeptidase (IRAP). IRAP is a membrane-bound zinc-dependent aminopeptidase found in diverse tissues (heart, muscle, fat, …).
Via radioligand binding experiments with the synthetic Ang IV-analogue [^{3}H]AL-11 we have demonstrated the presence of IRAP on mouse and human *ex vivo* macrophages. Also, we have shown that, under basal conditions, only a fraction (10 %) of the total IRAP is exposed on the cell surface of macrophages, and that the internalization of IRAP is a dynamic process.
At the gene expression level, IRAP is induced by IFN-γ and LPS and is hence preferentially expressed by pro-inflammatory M1-activated macrophages, suggesting a potential role in inflammation.
The functional impact of the interaction between Ang IV and IRAP on inflammation is currently under investigation. Stimulation of macrophages by Ang IV or AL-11 alone had no effect on inflammatory gene expression. However, in the case of an inflammatory setting (IFN-γ, LPS stimulation), Ang IV and AL-11 partly neutralized the induction of inflammatory genes (e.g. NF-κB regulated genes such as iNOS, COX-2, PAI-1 and TNFα in mouse peritoneal macrophages) in wild-type but not IRAP-deficient macrophages. Similar effects have been observed both in mouse and human macrophages, thereby proposing Ang IV/IRAP as a novel anti-inflammatory ligand/receptor pair.

E10 IRF5 AND RELA IN SETTING UP PRO-INFLAMMATORY MACROPHAGE PHENOTYPE

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Macrophages are immune cells that produce inflammatory mediators and are of central importance in the pathogenesis of chronic inflammatory diseases. The state of macrophage activation depends on the environmental factors and can change from pro- (M1) to anti-inflammatory (M2). M1 macrophages mediate resistance to pathogens and tissue destruction, whereas M2 macrophages promote tissue repair and remodelling as well as tumour progression. The molecular mechanisms underlying macrophage polarization remained elusive until now.
We have recently discovered that the transcription factor IRF5 is a major factor defining the pro-inflammatory M1 macrophage polarization. It is highly expressed in M1 macrophages, directly regulates the secretion of specific inflammatory mediators, characteristic of M1 macrophages (e.g. IL-12, IL-23, TNF, IL-1), that set up the environment for a potent Th1/Th17 response (Nature Immunology 2011). We have also begun to map molecular mechanisms of IRF5 function and identified two modes of action, such as direct binding to DNA and indirect recruitment via the formation of a protein complex with NF-κB RelA (Blood 2010). The latter mode of action provides an attractive possibility for designing inhibitory molecules, capable of breaking these interactions. The results of the RelA-IRF5 interaction interface mapping will be presented.
We will also discuss our genomic strategy and new data in identifying genes important for M1 macrophage polarization and co-dependent on RelA-IRF5 interactions with the aim of controlling the pro-inflammatory macrophage phenotype by interfering molecules to the IRF5-RelA interaction interface.

E11 Caspase-11 in inflammasome signaling

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Inflammasomes exert critical roles in innate and adaptive immune signaling by maturing the pro-inflammatory cytokines interleukin (IL)-1β and IL-18, by regulating the unconventional protein secretion of leaderless cytokines and growth factors, and by inducing pyroptosis, a pro-inflammatory cell death mode of macrophages and dendritic cells. Pattern-recognition receptors of NOD-like receptor (NLR) and HIN-200 family interact with the adaptor protein ASC to recruit and activate the cysteine protease caspase-1 (Casp1) in inflammasome complexes. However, the role of the related protease caspase-11 (Casp11) in inflammasome signaling is not clear. To this aim, we generated a new line of caspase-11-deficient mice by gene targeting in C57BL/6 ES cells. Interestingly, *Casp11*$^{-/-}$ mice were resistant to LPS-induced lethality *in vivo*, and they failed to produce mature (IL)-1β and IL-18 in circulation. In agreement, Casp1 was not activated in splenocytes from these mice. *In vitro* challenged *Casp11*$^{-/-}$ macrophages and dendritic cells required caspase-11 for a number of inflammasome-activating stimuli, but not for canonical activators of the Nlrp3 inflammasome (such as ATP, nigericin and silica) or the Nlrc4 inflammasome (bacterial flagellin and Salmonella typhimurium), suggesting a focused role for Casp11 in inflammasome signaling. Given that macrophages derived from the previously published Casp1-deficient mice failed to express Casp11 as well, the immunological phenotypes of Casp1-deficient mice may be (at least partially) due to defective Casp11 expression.

E12 Control of p38 MAP kinase pathway by NAD in LPS-induced monocytes/macrophages

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It has been known for several years that the expression of the cytokine TNF-alpha is critical for the development and the coordination of the immune response. The existence of pathologies, such as septic shock, highlights the relevance of a tight regulation of TNF-alpha mRNA translation and stability in monocytes/macrophages and others specific myeloid cells. Beside transcription, TNF-alpha gene expression is regulated at a post-transcriptional level by a specific sequence (AU-Rich Element, or ARE) present in its 3' untranslated region. The dynamic network of proteins binding to this sequence (ARE-BPs) during the inflammatory process plays a crucial role in this mechanism. We recently investigated the effect of nicotinamide (Nam) – an anti-inflammatory molecule – on ARE-BP expression and post-translational modifications. Our work demonstrates that nicotinamide markedly down-regulates the expression and phosphorylation of tristetraprolin (TTP), a major ARE-BP controlling TNF-alpha mRNA decay in monocytes/macrophages induced by lipopolysaccharides (LPS). TTP is a major target of the p38 MAP Kinase signaling pathway induced by LPS. We thus analyzed p38 MAPK phosphorylation status in macrophages stimulated by LPS in the presence or the absence of Nam. These experiments revealed that Nam inhibits LPS-induced p38 phosphorylation. Using a pharmacological approach, we also demonstrated that LPS-induced p38 phosphorylation is controlled by the intracellular concentration of NAD. Altogether, these results indicate that LPS signaling by the p38 MAPK pathway is controlled by NAD metabolism. We are currently investigating the enzyme(s) consuming NAD and inhibited by Nam which would control p38 MAPK signaling in LPS-induced monocytes/macrophages.

E13 Human dendritic cells reprogramming by hypoxia: induction of a proinflammatory phenotype and identification of TREM-1 as a novel hypoxic marker

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Myeloid dendritic cells (DCs) are professional APCs critical for the induction of protective immunity to microbial invasion and maintainance of self-tolerance. DC functions are tightly regulated by a network of inhibitory and activating signals present in the local microenvironment, and dysregulated DC responses may result in amplification of inflammation, loss of tolerance, or establishment of immune escape mechanisms. Pathological conditions such as infections, autoimmune disorders, and cancer are characterized by low partial oxygen pressure (pO_2). Understanding the biology of DCs in low O_2 environments may, thus, open new therapeutic opportunities for these diseases. We present data showing that hypoxia can profoundly impact on human mature DC (mDC) functions promoting the onset of a proinflammatory phenotype characterized by increased expression of inflammatory cytokines/chemokines and immunoregulatory receptors. Within the immunoregulatory receptor gene cluster, the triggering receptor expressed on myeloid cells (TREM)-1, a member of the Ig-like receptor family and a strong amplifier of inflammation, was selectively expressed *in vitro* by H-mDCs, but not by the normoxic counterpart, and *in vivo* by mDCs infiltrating the inflamed hypoxic joints of Juvenile Idiopathic Arthritis patients. TREM-1 engagement elicited DAP12-linked signaling resulting in further increase of inflammatory cytokine/chemokine production. These findings indicate that reduced O_2 availability critically contributes to the persistence and amplification of inflammation by regulating mDC capacity to promote leukocyte trafficking in diseased tissues and identify TREM-1 as a novel marker of hypoxic mDCs endowed with pro-inflammatory properties. The potential implications of mDC functional reprogramming by pathologic hypoxia for disease progression will be discussed.

E14 The Effect of Vitamin D on Monocyte Biology: A Physiological Perspective

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The active metabolite of vitamin D, $1,25(OH)_2D_3$ and activation of its cognate receptor (VDR), have been shown to have wide-ranging effects within the immune system, spanning both innate and adaptive responses. These include important roles in differentiation of immune cells, particularly mononuclear phagocytes; induction of antimicrobial peptides; homeostatic regulation of immune responses through modulation of innate immune signalling pathways and dendritic cell (DC) interactions with T cells. However, the physiological significance of these reports requires further investigation. Vitamin D deficiency is associated with numerous infectious and autoimmune diseases, and defined by serum levels of the precursor of the active metabolite- $25(OH)D_3$. We have established that conventional cell culture conditions are analogous to severe $25(OH)D_3$ deficiency and we have tested the effect of $25(OH)D_3$ supplementation on macrophage and DC biology *in vitro,* in comparison to stimulation with $1,25(OH)_2D_3$. We present data on the capacity of each of these cells to convert $25(OH)D_3$ to the active metabolite, the effect of vitamin D supplementation on the host cell transcriptome, and functional effects on innate immune activation pathways and down-stream responses. We show differential capacity for each of these cell types to activate $25(OH)D_3$, and diverse gene expression changes to stimulation with $1,25(OH)_2D_3$ that suggest a major contribution by alternative VDR signalling pathways. We also test the effect of **in vivo** vitamin D supplementation on innate immune responses by circulating monocytes and DC. Our findings extend previous understanding of the effects of vitamin D deficiency and physiologically relevant supplementation on macrophages and dendritic cells.

E15 Liver X receptors in central nervous system inflammation

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Multiple Sclerosis (MS) is a chronic inflammatory disease of the central nervous system in which macrophages play a pivotal role. Initially, macrophages where thought to be only detrimental in MS. However, recent evidence suggests that macrophages can also have anti-inflammatory effects. Nonetheless, underlying mechanisms inducing a protective phenotype in macrophages remain to be clarified. Liver X receptors (LXRs) are ligand dependent transcription factors that regulate the expression of genes involved in cholesterol metabolism. In addition, LXRs have been described to repress the expression of certain inflammatory genes in macrophages. Since myelin contains cholesterol, which is the natural ligand for LXRs, these receptors may play a role in the induction of a protective phenotype in macrophages. We hypothesize that LXRs are activated after myelin phagocytosis and induce a protective, anti-inflammatory phenotype in macrophages. The goal of this study is to unravel the role of LXRs in the macrophage response after myelin phagocytosis.
Real-time PCR data show that LXR response genes are upregulated in macrophages after both myelin and T09 incubation. Furthermore, macrophage nitric oxide production decreases after myelin and T09 incubation. Finally DHR assays show that macrophage ROS production increases after both myelin and T09 incubation.
These results indicate that LXRs are activated after myelin phagocytosis and multiple pathways, probably including LXR signalling, are responsible for the myelin-induced protective phenotype in macrophages. During demyelination, macrophages with a protective phenotype may be induced that limit lesion progression. Targeting LXRs may improve disease outcome in MS.

E16 Interleukin (IL)-36γ is a product of human dendritic cells: role in activation of keratinocytes

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The newly identified IL-36γ is an atypical cytokine that signals via the IL-1Rrp2 and IL-1RacP receptors and acts mainly on epithelial tissues. IL-36γ belongs to the IL-1 cytokine family and apparently exerts proinflammatory activities similar to the related family members IL-1α and IL-1β. In the present study we demonstrate that proinflammatotory cytokines, in particular IL-18 and IFNγ, are able to induce IL-36γ mRNA and protein in the AML-derived predendritic cell line KG1. Mutational analysis of the IL-36γ promoter as well as pharmacological studies identified NFκB as being one major pillar of IL-36γ induction. Data generated by using KG1 cells translated well into the experimental system of monocyte-derived dendritic cells. Here, IFNγ alone or in combination with IL-1β, TNFα likewise mediated induction of IL-36γ. Since keratinocytes obviously play a key role in the pathogenesis of skin inflammation and have been connected to IL-36γ biology, human primary keratinocytes were further characterized regarding IL-36γ function. In concert with IL-1β, TNFα and IFNγ, IL-36γ enhanced the expression of the defensin S100A7, of iNOS and of IL-36γ itself. Interestingly, keratinocyte expression of the T-cell chemoattractant chemokine MIG (CXCL9) was inducible by IL-36γ as sole stimulus. Data presented indicate that IL-36γ may play an important role in dendritic cell biology. This should in particular be relevant in the context of Th1-associated inflammation. Moreover, IL-36γ derived from dendritic cells appears to efficiently amplify keratinocyte activation, a function obviously relevant for immunoactivation in the skin compartment.

E17 Role of MEK/ERK and p38MAPK in maturation of monocyte derived dendritic cells: Identification of specific gene expression profiles.

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Dendritic cells are highly specialized antigen-presenting cells that undergo profound alterations in their gene expression program ("maturation") upon activation by inflammatory stimuli or infectious agents. Previous works of our group and others have reported differential roles for MAP Kinases in dendritic cell maturation.
In the present study, we dissect human monocyte derived dendritic cells (MDDC) LPS-induced maturation, and evaluate in depth the effect of MEK/ERK and p38MAPK signalling pathways in the maturation process. For this purpose, we analyze the gene expression profile of MDDC at different time points after LPS treatment, and in the presence or absence of specific inhibitors of each MAP kinase signalling pathway.
Gene expression clustering of maturation-induced alterations revealed the existence of 25 different sets of genes involved in specific cellular and molecular functions and whose expression is controlled by distinct transcription factors, according to consensus sequences enrichment in their proximal regulatory regions.
On the other hand, p38MAPK inhibition along maturation led to greater genomic alterations than MEK1/2 blockade, since the expression of up to 60% of the LPS-dependent genes was significatively modified 24 hours after SB203580 treatment, opposite to 24% genes modified when maturing in the presence of U0126. In addition, p38 inhibition had a predominantly inhibitory action on LPS-triggered effects, thus preventing MDDC maturation, while U0126 equally enhanced and diminished LPS-induced alterations. The biological functions controlled by ERK and p38MAPK-regulated maturation-dependent genes will be presented, as a mean to further disclose the specific role of both MAP Kinases in the MDDC maturation process.

E18 The TLR4 ligand MPL and QS-21 saponin combined in the Adjuvant System AS01 synergise at the innate level to enhance vaccine responses.

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The design of vaccine adjuvants by combining immunostimulants is an attractive avenue to improve or develop new vaccines. The Adjuvant System AS01 that contains both monophosphoryl lipid A (MPL) and the saponin QS-21 has been selected for the development of the RTS,S malaria vaccine candidate (currently in phase III). RTS,S/ AS01 induces $CD4^+$ T-cell and antibody responses and has been shown to provide substantial, although not complete, protection against malaria in humans. AS01 is also used in HIV and TB candidate vaccines.
In mice, intramuscular injection of AS01 led to a transient cytokine production and successive waves of innate cell recruitment at the injection site, including granulocytes and inflammatory monocytes. The combination of MPL and QS-21 synergistically induced cytokines. In the draining lymph node (dLN) there was a concomitant increase in the number of activated $CD11c^+$ $MHCII^{high}$ dendritic cells (DC) and $Ly6C^+$ monocytes carrying the antigen. In contrast to DCs, $Ly6C^+$ monocyte-derived cells were not able to present antigen to specific T cells, although they became $MHCII^+$ $CD11c^+$ with time. MPL was the main activator of DCs whereas QS-21 preferentially activated monocytes but not DCs. The combined impact of MPL and QS-21 on cytokines and monocytes/DC was associated with a synergistic induction of antigen-specific IFN-γ producing $CD4^+$ T cells and increased $CD8^+$ T cell and antibody response. In addition to unraveling key steps in AS01's mechanism of action, this study suggests that synergitic response at the innate level by combining specific immunostimulants in adjuvants is useful to improve vaccine responses.

E19 NLRC4-triggered Cell Death Releases HMGB1 Independent of IL-1ß Production

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The cytoplasmic, "Nod-like" innate immune receptors (NLR) NLRP3 (Nalp3) and NLRC4 (Ipaf), activate the cystine protease Caspase-1 to induce cell death and process the pro-form of the inflammatory cytokine IL-1ß. They are triggered by stimuli of microbial origin, inorganic 'irritants', metabolic dysfunction, and molecules resulting from cell stress and death.
Macrophage NLRC4 is activated by the C-terminal 34 amino-acids of bacterial flagellin (C34) by infection with flagellated bacteria, transduction with retroviral vectors expressing flagellin, or flagellin protein transfection. Responses induced by these methods may affect other signaling pathways influencing NLRC4 responses. Additionally, the responses of macrophages activated by NLRC4 alone is unknown.
To study NLRC4 activation independent of these issues we established a system to inducibly express EGFP-C34 in macrophages. Expression of EGFP-C34 in the cell cytoplasm triggers rapid cell death (starting at 2hr) and HMGB1 released compared to controls. LPS priming does not affect cell death but allows for IL-1ß release. Overexpression of the Caspase-1/8 inhibitor CrmA in activated C34 cells inhibits death but the Caspase-8-specific inhibitor cFLIP or anti-apoptotic mitochondrial outer membrane permebalization inhibitor Bcl-X_L do not.
We observe a direct correlation between the amount of EGFP-C34 produced, cell death, and IL-1ß release suggesting that cell death may be necessary for IL-1ß release. Additionally, cell death is Caspase-1 dependent but independent of NF-kB/IRF activation, Caspase-8, and mitochondrial depolarization. The development of this system should allow for the detailed study of many cellular processes which affect, and are affected by, NLR activation.

E20 Abrogating myeloid IL-10R signaling reduces atherosclerosis development

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IL-10 has been shown to be a potent anti-inflammatory cytokine that affects vascular (immune) cells and thereby dampens atherogenesis. To what extent specific cell types contribute to the atheroprotective properties of IL-10 is yet to be elucidated. Here we studied the contribution of myeloid IL-10 receptor signalling to atherosclerosis development.
Using bone marrow derived macrophages from mice that were either wildtype (IL-10R1^{wt}) or deficient (IL-10R1^{del}) for the IL-10R in their myeloid cells, we could show that macrophages lacking Il-10R signalling are hyperresponsive to LPS and fail to repress pro-inflammatory cytokines in response to IL-10.
To investigate the role of myeloid IL-10R signaling in atherosclerosis, we reconstituted lethally irradiated LDLR$^{-/-}$ mice with bone marrow from IL-10R1^{wt} and IL-10R1^{del} donors. Interestingly, subsequent histological assessment of aortic roots in IL-10R1^{del}-tp LDLR$^{-/-}$ mice revealed a 70% reduction in atherosclerotic lesion area ($57.3x10^3$ µm^2 ± 10.1 vs. $192.5x10^3$ µm^2 ± 16.7, p<0.0001) and a less advanced plaque phenotype as compared to controls. This finding was accompanied by, yet not correlated to substantially lower cholesterol concentrations in both liver (5.8 µg/µg protein ± 0.2 vs. 8.3 µg/µg protein ± 0.4, p<0.0001) and plasma (24.9 mM ± 1.2 vs. 37.1 mM ± 2.8, p=0.0004) of IL-10R1^{del}-tp recipients. Fractionated plasma samples showed decreased VLDL and LDL levels in IL-10R1^{del}-tp mice, whereas HDL levels and triglyceride content in liver and plasma was unaffected.
In conclusion, our data show an unexpected pro-atherogenic role for IL-10R signaling in myeloid cells. Our current efforts focus on identifying the mechanisms underlying this phenotype.

E21 Phenotypical characterization of TLR induced tolerogenic APCs

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The TLR-4 ligand LPS and the TLR-7/8 ligand R848 interfere with GM-CSF and IL-4 (G4) driven differentiation of immature dendritic cells (iDC, G4) from CD14$^+$ monocytes. An antigen presenting cell (TLR-APC) is generated that is still CD14 positive, CD1a negative and highly PD-L1 positive. We have shown recently that those TLR-APCs are able to induce CD4$^+$CD25$^+$FoxP3$^+$ regulatory T cells. Furthermore we could show that PD-L1 is involved in the suppressive activity and that its expression is regulated via a cytokine/MAPK/STAT-3 dependent pathway. Here we analyzed different TLR-stimuli (LTA, S-FSL, poly(I:C), flagellin) for their ability to induce the TLR-APC phenotype. Besides LPS and R848 the TLR-3 ligand poly(I:C) is able to induce the TLR-APC phenotype but less pronounced than LPS and R848. LPS- and R848-induced TLR-APCs have similar cytokine- , STAT- and MAPK-activation profiles whereas poly(I:C) generated TLR-APCs show an aberrant profile. To get more information about the mode of action of the TLR stimuli and the TLR-APC phenotype in general, we started to investigate the proteome of TLR-APCs. To this we compared R848 generated TLR-APCs with immature dendritic cells via Differential In-Gel-Electrophoresis (DIGE). We identified proteins relating to ROS function and regulation, intracellular trafficking and small GTPases.

E22 Dusp16-deficient mice selectively over-produce IL-12 after TLR4 stimulation

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The recognition of microbial danger signal by innate immune cells leads to activation of MAPK pathway controlling the production of cytokines. Dual specificity phosphatases (Dusp) control MAPK activity by dephosphorylating threonine and tyrosine residues. Differential expression and inducibility of Dusp gene expression suggest that these phosphatases specify the outcome of MAPK activation in terms of cytokine production. We have investigated the role of Dusp16 in the immune system. Dusp16 expression in macrophages and dendritic cells was inducible by TLR stimuli in vitro. In vivo, Dusp16 expression was constitutive in some organs and in B lymphocytes; in contrast, LPS challenge up-regulated Dusp16 mRNA in the spleen. A gene trap ES cell clone, bearing an insertion in the Dusp16 locus that abrogates the expression of full length Dusp16 mRNA, was used to generate mice lacking Dusp16. Matings of heterozygous Dusp16trap/+ mice failed to yield homozygous Dusp16trap/trap mice at weaning. Analysis of newborn mice revealed decreased weight and significant mortality of Dusp16 deficient mice on the day of birth. Fetal liver cells were used to generate macrophages and dendritic cells in vitro and to reconstitute lethally irradiated mice for in vivo analysis. Dusp16trap/trap macrophages responded normally to TLR4 stimulation for most cytokines analyzed, but showed a significantly higher production of IL-12p40. In vivo, Dusp16trap/trap fetal liver cells reconstituted T and B cell compartments similar to WT. Following injection of LPS, Dusp16trap/trap-reconstituted mice significantly over-produced IL-12p40, consistent with the phenotype of Dusp16-deficient macrophages in vitro.

SUBMITTED ABSTRACTS TOPIC F: MACROPHAGE ACTIVATION STATES

F01 Polyfunctional Monocyte Responses Following TLR-4 Ligation

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Background: Monocytes have long been considered a heterogeneous group of cells both in terms of morphology and function. In the present study, we have investigated LPS-induced cytokine secretion by monocytes using the newly developed FluoroSpot assay. This method measures the accumulated number of cytokine secreting cells on the single cell level and uses fluorescent detection, allowing for the simultaneous analysis of two cytokines from the same population of isolated cells.
Materials & Methods: Peripheral blood monocytes from healthy donors were isolated using negative selection (RosetteSep) to an average purity of 80%. Enriched monocytes (1000 or 3000 cells/well) were incubated for 20h with or without LPS (50 ng/ml) and the secretion of IL-1β, IL-6, MIP-1β, TNF-α, GM-CSF, IL-10 and IL-12, alone or in combinations, was investigated in the FluoroSpot assay.
Results: By this approach, human monocytes could be divided into several subgroups as IL-1β, IL-6, TNF-α and MIP-1β were secreted by larger populations of responding cells (30.6-40.9%) compared to the smaller populations of GM-CSF (8.3%), IL-10 (1.6%) and IL-12p40 (1.8%). Furthermore, when studying co-secretion in FluoroSpot, an intricate relationship between the monocytes secreting IL-1β and/or IL-6 and those secreting TNF-α, MIP-1β, GM-CSF, IL-10 and IL-12p40 was revealed.
Conclusion: Our results demonstrate that monocytes can be divided into several subpopulations based on their secretion of cytokines in response to LPS. The proportions of these subsets were similar between different donors suggesting a level of predisposition in the functional characteristics of peripheral blood monocytes.

F02 A novel protective therapy for Type 1 Diabetes in NOD mice using cytokine-induced immunomodulatory M2r macrophages

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Macrophages are important immune cells that may either drive or modulate disease pathogenesis depending on their activation phenotype. The autoimmune disease Type 1 diabetes is a chronic pro-inflammatory condition characterized by unresolved destruction of pancreatic islets. Adoptive transfer of macrophages with immunosuppressive properties represents a novel immunotherapeutic opportunity for treatment of such chronic autoimmune diseases. We investigated a panel of cytokines and other stimuli, either alone or in combination, in order to discern the optimal regime for *in vitro* induction of an immunosuppressive macrophage phenotype. Properties included surface receptor expression, cytokine release, gene expression, and T cell suppressive activities. We determined a combination of IL-4, IL-10 and TGF-β to yield the optimal suppressive macrophage phenotype (M2r), each of these cytokines contributing specific properties. M2r cells were characterized by expression of PD-L2, FCγRIIb. IL-10 and TGF-β, having a potent deactivating effect on pre-stimulated pro-inflammatory LPS/IFN-γ stimulated macrophages and significantly suppressed T cell proliferation *in vitro*. Clinical therapeutic efficacy was assessed following adoptive transfer in the spontaneous type 1 diabetes model in NOD mice. Following a single transfer of M2r macrophages more than 80% of treated NOD mice were protected against spontaneous type 1 diabetes for at least 3 months, even when transfer was conducted just prior to clinical onset. Fluorescent imaging analyses revealed that adoptively transferred M2r macrophages specifically homed to the inflamed pancreas, promoting β-cell survival despite not affecting pancreatic T cell numbers. We suggest that M2r macrophage therapy represents a novel intervention that stops ongoing autoimmune Type 1 diabetes.

F03 p16^{INK4a}-deficiency promotes IL-4-induced polarization and inhibits pro-inflammatory signaling in macrophages

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The *CDKN2A* locus, which contains the tumor suppressor gene p16^{INK4a}, is associated with an increased risk of age-related inflammatory diseases, such as cardiovascular disease and type 2 diabetes, in which macrophages play a crucial role. Monocytes can polarize towards classically (CAMϕ) or alternatively (AAMϕ) activated macrophages. However, the molecular mechanisms underlying the acquisition of these phenotypes are not well defined.
We investigated the phenotype of p16^{INK4a}-deficient (p16$^{-/-}$) macrophages. Transcriptome analysis revealed that p16$^{-/-}$ bone marrow-derived macrophages (BMDM) exhibit a phenotype resembling interleukin (IL)-4-induced macrophage polarization. In line with this observation, p16$^{-/-}$ BMDM displayed a decreased response to classically polarizing IFNγ and LPS and an increased sensitivity to alternative polarization by IL-4. Furthermore, mice transplanted with p16$^{-/-}$ bone marrow displayed higher hepatic AAMϕ marker expression levels upon *Schistosoma mansoni* infection, an *in vivo* model of AAMϕ phenotype-skewing. Surprisingly, p16$^{-/-}$ BMDM did not display increased IL-4-induced STAT6 signaling, but decreased IFNγ-induced STAT1 and LPS-induced IKKα,β phosphorylation. This decrease correlated with decreased JAK2 phosphorylation and with higher levels of inhibitory acetylation of STAT1 and IKKα,β. These findings identify p16^{INK4a} as a modulator of macrophage activation and polarization via the JAK2-STAT1 pathway with possible roles in inflammatory diseases.

F04 Macrophages programmed by apoptotic cells promote angiogenesis through prostaglandin E_2

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Macrophages contribute to tissue homeostasis in the developing as well as the adult organism. Recent studies using genetic macrophage depletion models in the mouse have, for example, highlighted that macrophages are critical for successful wound healing. This involves pathogen clearance as well as damage repair. In a similar manner, macrophages are involved in virtually each step of tumor progression, reaching from possible eradication of tumors to promoting invasiveness and metastatic spread. A prominent macrophage-dependent feature of tissue remodelling both in wounds and tumors is neo-angiogenesis. Signaling pathways activating an angiogenic program in macrophages are still poorly defined. We report that apoptotic cells (AC), which are abundant in stressed/damaged tissues, can induce angiogenic properties in primary human macrophages. The signal originating from AC is the lipid mediator sphingosine-1-phosphate (S1P), which activates S1P1/3 on macrophages to up-regulate cyclooxygenase-2. The formation and liberation of prostaglandin E_2 (PGE_2) then stimulates migration of endothelial cells. *In vivo*, neutralization of PGE_2 from pro-angiogenic macrophage supernatants blocked vessel formation into Matrigel plugs. In particular apoptotic cancer cells shifted prostanoid formation in macrophages selectively towards PGE_2, by up-regulating cyclooxygenase-2 as well as microsomal prostaglandin E synthase-1 (mPGES-1), while down-regulating the PGE_2-degrading enzyme 15-hydroxyprostaglandin dehydrogenase (15-PGDH) or prostaglandin-D synthase (PGDS). Angiogenic programming of macrophages by AC therefore may control responses to tissue stress such as in tumors, where macrophages support cancer progression.

F05 Akt2 kinase is a key determinant of M2 macrophage polarization and Tumor Associated Macrophage formation

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Macrophages are prominent in the tumour microenvironment and several reports have shown that they contribute to tumour growth. Tumour associated macrophages (TAMs) exhibit characteristics of Alternative (or M2) macrophages that promote angiogenesis and suppression of Th1 responses. Little information is available on the molecular signalling determinants of TAM and M2 differentiation and function. Akt is a family of serine/threonine protein kinases that are activated by PI3K. Preliminary evidence from our laboratory have indicated that genetic ablation of Akt2 primes macrophages to M2-type of responses, while genetic ablation of Akt1 results in M1-type of macrophage responses. Since epidemiologic studies have revealed that chronic inflammation strongly predisposes to tumor development and progression of different forms of cancer, we investigated the role of Akt2 in the development of inflammation-related intestinal tumorigenesis using the murine model of AOM/DSS carcinogenesis. Our results indicated that ablation of Akt2 exacerbated the effects of the disease, in terms of percent of survival, weight lose, visual evidence of rectal and intestinal bleeding and diarrhea. Histological analysis confirmed these results since Akt2 deficient mice showed a superior number of proliferative lesion as carcinomas, increased intestinal inflammation, with severe damage of intestinal mucosa, edema and inflammatory_cell recruitment. Molecular analysis of the potential involvement of Akt2 in M2/TAMs revealed that absence of Akt2 resulted in increased M2/TAM markers. Overall, our results indicated that Akt2 controls intestinal tumorigenesis via promoting M2/TAM generation.
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F06 ALTERED MICRO-RNA EXPRESSION IN MONOCYTES OF POSTPARTUM PSYCHOSIS PATIENTS

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In support of the concept that an altered inflammatory set point of macrophages (including microglia) contributes to the pathogenesis of major mood disorders, we previously detected an altered gene expression set point in monocytes of patients. The signature contained important transcription factors (TFs), such as ATF3, EGR3, MXD1 and MAFF. MicroRNAs are, besides Tfs, important regulators of gene expression.
Research Design: We profiled the expression of 377 human microRNAs in monocytes of 8 patients with post partum psychosis (considered to be the first episode of a mood disorder typically occurring after delivery), 8 post partum healthy controls and 8 age-matched normal females using TaqMan Array Human MicroRNA cards. We assessed the correlation of the expression of these microRNAs to the expression of the TFs ATF3, EGR3, MXD1 and MAFF in the same samples.
Results: Hsa-miR-139-3p and hsa-miR-124 showed the highest increase in expression in monocytes of patients compared to their controls. The expression of both microRNAs correlated significantly to the expression of ATF3 and MAFF.
Conclusions: MicroRNA-124 is known as a negative modulator of macrophage and microglia activation and its correlation to ATF3 supports this notion, since ATF3 is induced early during immune responses to prevent an uncontrolled inflammatory response. MicroRNA-139 supresses metastasis and carcinoma progression and its correlation to MAFF, a crucial factor in differentiation and oncogenesis, is also logical. Our findings thus indicate that a systems biology approach of macrophages/microglia in major mood disorders may lead to the identification of molecular networks controlling their altered set points.

F07 Expression of the inhibitory CD200 receptor is associated with alternative macrophage activation

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Macrophages are involved in the neutralization of pathogens and harmful endogenous products, in immune regulation, and in wound healing. Although macrophage activation generates a wide spectrum of subpopulations with overlapping activities, it is useful to identify functionally divergent cells since growing evidence demonstrates that these are involved in various pathologies. The receptor for CD200 (CD200R) inhibits effector functions, triggered by classical macrophage activation. To determine the expression of CD200R on polarized macrophages, we subjected purified human monocytes to a panel of pro- and anti-inflammatory stimuli for 3 to 7 days. We found that CD200R mRNA and protein expression was specifically induced in cells of the alternatively activated M2a subtype, generated by incubation with IL-4 or IL-13. In mice, peritoneal M2 macrophages, elicited during infection with the parasites *Taenia crassiceps* or *Trypanosoma b. brucei*, expressed increased CD200R levels compared to those derived from uninfected mice. Interestingly, CD200R expression in PLC-/- *T. b. brucei*-infected mice was only upregulated during the chronic phase of the infection that is characterized by a type II cytokine milieu. However, *in vitro* stimulation of mouse peritoneal macrophages and *T. crassiceps* infection in IL-4-/- and IL-4R-/- mice showed that, in contrast to human, induction of CD200R in mice was not IL-4 or IL-13 dependent. Our data identify CD200R as a suitable marker for alternatively activated macrophages in human and corroborate observations of distinct species- and/or site-specific mechanisms regulating macrophage polarization in mouse and man.

F08 CCL2 AND CX3CR1 DOUBLE KNOCKOUT CHANGES MACROPHAGE FUNCTIONALITY

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Age-related macular degeneration (AMD) is the biggest cause of blindness in developed countries. Inflammation is believed to play an important role in the pathogenesis of the disease, although the detailed pathways involved remain poorly defined. Mice deficient in CCL2 and CX_3CR1 develop AMD-like lesions as early as 6-week old, and the disease progresses with age. This evidence suggests monocyte/macrophage malfunction may play a key role in retinal lesion development in AMD. The aim of this study is to understand which functions of macrophage may be altered in ccl2/cx3cr1 DKO mice and whether these functional alterations are related to retinal lesions. Naive bone marrow-derived macrophages (BMDMs) from DKO mice expressed higher levels of arginase-1 and VEGF genes, but have reduced phagocytic activity compared to cells from wild type (WT) mice. When BMDMs were polarized into the classically activated M1 macrophages (by LPS and IFN-γ), or the alternatively activated M2 macrophages (by IL-4), cells from DKO mice expressed significantly less iNOS in M1 and arginase-1 in M2 macrophage compared to their counterpart cells from WT mice. The level of VEGF expression in M1 and M2 macrophage was also significantly lower in DKO mice than that in WT mice. Our results suggest that deletion of CCL2 and CX_3CR1 results in a low grade spontaneous activation of BMDMs towards the M2-like phenotype. These cells have reduced phagocytic activity and are more angiogenic than WT counterparts. In the absence of CCL2 and CX_3CR1, BMDMs are less capable of further differentiating into M1 or M2 phenotype under inflammatory conditions. These altered macrophage functions may play a critical role in retinal lesion formation in these mice.

F09 Polyamines stimulate the expression of IL-4-induced alternatively activated macrophage markers while inhibiting LPS-induced expression of inflammatory cytokines

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Polyamines are polycationic molecules which regulate various cellular functions. In macrophages, basal polyamine levels are relatively low, but are increased upon IL-4 stimulation. This Th2 cytokine induces arginase-1 activity which converts arginine into ornithine. Next, ornithine is decarboxylated by ornithine decarboxylase (ODC) into putrescine which is further converted into spermidine and spermine. Recently, we proposed polyamines as novel agents in IL-4-dependent E-cadherin regulation in alternatively activated macrophages.
Here we demonstrate that several, but not all, markers for alternatively activated macrophages rely on polyamines for their IL-4-induced expression. Depletion of polyamines resulted in decreased IL-4-mediated expression of Ym, Fizz, PDL2, MMR, CCL17, and E-cadherin, but did not affect arginase-1, CD71, Mgl1 nor Mgl2 expression. Remarkably, arginase-1-deficient macrophages were still able to produce normal amounts of polyamines upon IL-4 treatment suggesting that an arginase-1/ODC independent polyamine synthesis pathway exists in macrophages. Consequently, IL-4-induced gene expression in arginase-1-deficient macrophages was similar to their wt counterparts.
Interestingly, LPS-induced expression of the pro-inflammatory mediators TNF, IL-6 and NO was significantly increased in polyamine-depleted macrophages.
Overall, we propose polyamines as novel regulators of the inflammatory status of the macrophage. Indeed, while polyamines are needed for IL-4-induced expression of anti-inflammatory alternatively activated macrophage markers, they inhibit the LPS-mediated expression of pro-inflammatory mediators in classically activated macrophages.

F10 Modulation of Macrophage Activity in LDV-exacerbated Anemia

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Autoimmune hemolytic anemia (AIHA) is characterized by production of autoantibodies causing anemia as a result of immune destruction of opsonized red blood cells. AIHA typically follows a viral illness and macrophages are the essential effector cells for the development of anemia. Indeed, virus-mediated increase in autoantibody pathogenicity is linked to an enhancement of macrophage phagocytic activity.
LDV (Lactate Dehydrogenase-elevating Virus) is a mouse arterivirus, that causes no harm to the normal host. LDV infection strongly enhances phagocytic activity of macrophages. We use LDV to study how viral infection can exacerbate the development of AIHA. FcγRs and CR3 expression was measured in peritoneal macrophages and splenocytes. Anti-FcγR III mAb and Fc γR III KO mice were used to study Fc γR III function in LDV-exacerbated anemia. The effect of over-secreted cytokines (IFN-γ, M-CSF and type I IFNs) on macrophage activation induced by infection was analyzed. We found that:

1. LDV infection up-regulated FcγR I and FcγR III , down-regulated FcγR III and CR3 expression on peritoneal macrophages and splenocytes.
2. Type I IFNs was responsible to the up-regulation of FcγR I, and down-regulation of FcγR III, CR3 after infection. It provided a protective effect on LDV-exacerbated anemia.
3. FcγR III played an important role in LDV-exacerbated anemia. Anti-FcγR III treatment protected mice from the lethality mediated by anti-red blood cell antibody. These results were confirmed on FcγR III KO mice
4. Over-production of IFN-γ and M-CSF after LDV infection had no effect on the modulation of FcγRs and CR3 expression. IFN-γ was shown to control the severity of FcγR III-mediated phagocytosis.

F11 Stabilin-1/CLEVER-1 on human placental macrophage is involved in trafficking and scavenging function.

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Stabilin-1/Common Lymphatic Endothelial and Vascular Endothelial Receptor-1 (CLEVER-1) is a multidomain protein present in lymphatic and vascular endothelial cells and type 2 immunosuppressive macrophages. The role of Stabilin-1/CLEVER-1 as a scavenging and endocytosis receptor, its role as an adhesion and transmigration in lymphatic and vascular endothelium has been reported, but role of this during development is unknown. Here, we studied stabilin-1/CLEVER-1 expression and functions in human placental macrophages and during human ontogeny. We found that stabilin-1/CLEVER-1 was expressed on almost all macrophages in term placenta by using newly generated mAbs. Stabilin-1/CLEVER-1 in placental macrophages was involved in the uptake of fluorescently labeled model antigen OVA and in scavenging of Ac-LDL (acetylated low density lipoprotein). Cytokine profile produced by placental macrophages was altered by siRNA mediated suppression of Stabilin-1/CLEVER-1.
Stabilin-1/CLEVER-1 on placental macrophages mediated their adhesion to placental vessels and supported their transmigration through vascular endothelium. Finally, we found that stabilin-1/CLEVER-1 is induced very early in fetal macrophages, high endothelial venules, and lymphatic vessels in multiple lymphatic organs. In summary, these data suggest that macrophage Stabilin-1/CLEVER-1 can play a role in scavenging and leukocyte trafficking during the development of the placenta and fetus.

F12 Innate imprinting of murine resident alveolar macrophages by allergic bronchial inflammation causes a switch from hypo- to hyperinflammatory reactivity

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Resident alveolar macrophages residing in the bronchoalveolar lumen of the airways are known to play an important role in limiting excessive inflammatory responses in the respiratory tract. High phagocytic activity along with hyporesponsiveness to inflammatory insults and lack of autonomous IFN-β production are crucial assets in this regulatory function. Using both mouse models of asthma, we now analyzed the fate of rAM during and after allergic bronchial inflammation. Although phenotypically nearly indistinguishable from naïve rAM, post-inflammation rAM exhibited a strongly reduced basal phagocytosis accompanied by a markedly increased inflammatory reactivity to TLR-3 (poly I:C), TLR-4 (LPS) and TLR-7 (imiquimod). Importantly, post-inflammation rAM in addition exhibited a switch from an IFN-β defective to an IFN-β competent phenotype, thus indicating the occurrence of a new, 'inflammatory-released' rAM-population in the post-allergic lung. Analysis of rAM-turnover revealed a rapid disappearance of naïve rAM after the onset of inflammation. This inflammation-induced rAM turnover is critical for the development of the hyperinflammatory rAM-phenotype observed after clearance of the bronchial inflammation. These data document a novel mechanism of innate imprinting in which non-infectious bronchial inflammation causes the alveolar macrophage to acquire a highly modified innate reactivity. The resulting increment in secretion of inflammatory mediators upon TLR-stimulation implies a role of this phenomenon of innate imprinting in the increased sensitivity of post-allergic lungs to inflammatory insults.

F13 RNA-sequencing reveals novel splicing mechanisms in differentially activated human macrophages

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Macrophages react to numerous stimuli within their microenvironment. Hundreds to even thousands of genes are altered in there expression levels upon activation. While transcriptional regulation of known genes can be sufficiently assessed by array-based techniques it has been suggested that next generation sequencing will significantly extend our knowledge about the overall magnitude of transcriptional changes as well as alternative and novel splicing events. Using M1 and M2 macrophage activation as the model we reveal novel and unexpected insights into transcriptional regulation in human macrophages using RNA-sequencing (RNA-seq). First of all, RNA-seq uncovers a significantly larger dynamic range of transcriptional changes in macrophages in response to interferon-gamma (M1) and IL-4 (M2) when directly comparing to current array-based transcriptome data. Furthermore, the amount of novel stimulus-specific transcripts not yet identified by array techniques was surprisingly high. In addition to the detection of new and stimulus-specific splice variants of annotated genes, we also found evidence for mechanisms such as trans-splicing in response to activation. Validation of newly identified transcripts also lead to the identification of novel and more specific protein markers for classical (M1) and alternatively (M2) activated macrophages. In conclusion, RNA-seq of activated macrophages reveals a much more diverse transcriptional program than previously thought. This involves both quantitative transcriptional changes as well as distinct qualitative changes in transcription that are made accessible by next generation sequencing.

F14 NADPH oxidase Is Internalized by Clathrin-Coated Pits and Localizes to a Rab27A/B-Regulated Secretory Storage Compartment in Mature Macrophages

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Here we report that functional maturation of different types of tissue macrophages, including microglia, by lipopolysaccharide (LPS) or GM-CSF stimulation correlates with the quantitative redistribution of NADPH oxidase (cyt b_{558}) from the plasma membrane to an intracellular stimulus-responsive storage compartment. Cryo-immunogold labeling of gp91phox and $CeCl_3$ cytochemistry showed the presence of gp91phox and oxidant production in a numerous population of small (<100 nm) vesicles. Cell homogenization and sucrose gradient centrifugation in combination with transferrin-HRP/DAB ablation showed that more than half of cyt b_{558} is present in fractions free of endosomal markers, which is supported by morphological evidence to show that the cyt b_{558}-containing compartment is distinct from endosomes or biosynthetic organelles. SLO-mediated GTP-g-S loading of microglia caused exocytosis of cyt b_{558} under conditions where lysosomes or endosomes were not mobilized. We establish phagocytic particles and pro-inflammatory agents ATP, TNFa and CD40L as physiological inducers of cyt b_{558} exocytosis of to the cell surface, and by shRNA knock-down we identify Rab27A/B as regulators of vesicular mobilization to the phagosome and the cell surface. Exocytosis was followed by clathrin-dependent internalization of cyt b_{558}, which could be blocked by a dominant negative mutant of the clathrin-coated pit-associated protein Eps15. Re-internalized cyt b_{558} was not delivered to lysosomes, but associated with recycling endosomes and undefined vesicular elements. In conclusion, cyt b_{558} depends on clathrin for internalization, and in mature macrophages NADPH oxidase occupies an Rab27A/B-regulated secretory storage compartment, which allows rapid agonist-induced redistribution of superoxide production in the cell.

F15 miR-378 is a hallmark of alternative macrophage activation *in vitro* and *in vivo* acting as a feedbackinhibitor blocking the PI3K/Akt-pathway

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Depending on their environment macrophages (MΦ) adopt various activation phenotypes. Classical (or M1-) and alternative (or M2-) activation represent the two most divergent phenotypes with the former important for the clearance of microbial pathogens whereas the later are associated with wound-healing and immuno-suppression. In either case MΦ-activation must be tightly controlled, as excessive activation can lead to tissue destruction or fibrosis, respectively. One mechanism to control MΦ activation, which has gained significant interest recently, are microRNAs (miRNAs). miRNAs are short non-coding RNAs that influence gene expression by binding the 3'UTR of specific gene-transcripts inducing translational repression. In MΦs miRNAs have so far been mainly studied during classical activation where they have been found to be important regulators of activation. However, the miRNA-profile associated with alternative activation of MΦs has yet to be described. We therefore aimed to identify miRNAs differentially expressed in an *in vivo* model of alternative activation and dissect their functional roles. Microarray-analysis identified miR-378 as one of the most robustly regulated miRNAs in this context, and further *in vitro* experiments demonstrated that miR-378 is induced in a feedbackloop upon IL-4 stimulation. I*n silico* analysis and overexpression studies identified potential miR-378-targets in the PI3K/Akt-signalling cascade, downstream of the IL-4 receptor. Thus, it seems likely that miRNAs will prove to be a general mechanism for limiting macrophage activation in both alternative and classical activation settings.

F16 The serotonin receptor HT2B, a novel marker for anti-inflammatory M2 (M-CSF) macrophages.

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Introduction: Macrophage activation comprises a continuum of functional states ultimately determined by the cytokine microenvironment. Thus, macrophages are functionally grouped according to their response to pro-Th1/proinflammatory stimuli (M1 macrophages) or pro-Th2/anti-inflammatory stimuli (M2 macrophages). Our group and others have identified M1- and M2-specific markers, some of which have an impact on the macrophage functional activities.
Serotonin (5HT) is a neurotransmitter and vascular active molecule that has been recently shown to exert protumoral activity by inducing angiogenesis and tumor cell growth. Although previous studies have suggested that 5HT acts as a neuro-inmuno modulatory agent, how serotonin connects both systems remains largerly unexplored.
Results and conclusions: We report here that serotonin receptor 2b (HT2B), encoded by the *HTR2B* gene, is expressed specifically by M2 macrophages generated in the presence of M-CSF. In fact, M-CSF promotes HT2B expression, whereas GM-CSF, IL-10 and proinflamatory stimuli like LPS impair its expression, thus suggesting that *HTR2B* transcription is restricted to M2-like homeostatic macrophages. Immunohistochemistry and RT-PCR studies confirmed that this is the case, since HTR2B mRNA and protein were detected in human liver Kuppfer cells and human alveolar macrophages. Preliminary data indicate that HT2B activates ERK-dependent signalling pathways in macrophages, and that HT2B-initiated signaling influences both macrophage function and the expression of macrophage polarization markers. Therefore, our findings identify the serotonin receptor 2b as a novel functionally-relevant marker for anti-inflammatory/homeostatic M2 macrophages, and whose activity might contribute to the pro-tumoral action of M2 macrophages.

F17 Protection of macrophages against apoptosis by bioactive lipids

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Lipoxins (LXs) are endogenous eicosanoids which are released during the resolution phase of inflammation, in the nanomolar range. LXs are mainly generated by transcellular metabolism from arachidonic acid depending on the cellular context. In mammals, lipoxygenase enzymes (LOX) generate two main native products, lipoxin A_4 (LXA_4) and B_4 (LXB_4) being the most studied LXA_4, which exerts potent anti-inflammatory actions modulating leukocyte trafficking and promoting phagocytic clearance of apoptotic cells. The effects of lipoxins as lipid mediators with potent anti-inflammatory actions are well documented, but their role in apoptosis remains controversial. The targets of LXA_4 on neutrophils contribute to attenuate inflammation. However, the effects of lipoxins on macrophage are less known, in particular the action of LXA_4 on the regulation of apoptosis of these cells. Our data show that pre-treatment of human or murine macrophages with LXA_4 at the concentrations prevailing in the course of resolution of inflammation inhibit apoptosis induced by different stimuli. The release of mitochondrial mediators of apoptosis as well as the activation of caspases was abrogated in the presence of LXA_4. In addition to this, the anti-apoptotic proteins of the Bcl-2 family accumulated in the presence of lipoxin. Analysis of the targets of LXA_4 identified an early activation of the PI3K/Akt and ERK/Nrf-2 pathways that was required for the observation of the antiapoptotic effects of LXA_4. These data suggest that the LXA_4 released after recruitment of neutrophils to sites of inflammation exerts a protective effect on macrophage viability that might contribute to a better resolution of inflammation.

F18 CD180/RP105 is a marker of human alternative macrophage activation

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Alternative activation of macrophages (AAM) induced by Th2 type cytokines IL-4 and IL-13 results in cells with anti-inflammatory properties as opposed to Th1 type cytokines mediated classical activation of macrophages (CAM). AAM plays an important role in some physiological and pathological processes including post-inflammatory tissue repair, modulation of metabolism as well as regulation of tumor cell proliferation and migration.
The aim of the current work was to identify novel markers of human alternatively activated macrophages using global gene expression analysis in primary cell models. We differentiated human peripheral blood derived monocytes into macrophages in the absence or presence of IL-4 or INFγ+TNFα. We found a large number of genes differentially expressed in the IL-4 or INFγ+TNFα treated macrophage subtypes. Based on gene ontology analysis several IL-4 regulated genes were overrepresented in functional categories such as immune response, acyl-CoA metabolism and mitochondrial electron transport processes. Among the immune response genes we found CD180/RP105 which is a member of the Toll-like receptor (TLR) family up-regulated by IL-4 at both the mRNA and cell surface protein levels. CD180/RP105 is a negative regulator of TLR-4/LPS signaling in antigen presenting cells. Currently, we are seeking to find out whether the IL-4 induced up-regulation of this molecule can, at least partially, explain the diminished LPS response of alternatively activated macrophages.
Ultimately we hope to identify novel IL-4 induced AAM markers and to better understand the mechanisms by which IL-4 induces a blunted response to inflammatory stimuli including LPS in macrophages.

F19 Suppressive macrophages in chronic rhinosinusitis with nasal polyps

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Classically (M1) and alternatively (M2) activated macrophages are characterized by expression of specific surface receptors, chemokine production and have different functions in control of infections and development of human diseases. In the current study we have analyzed macrophage activation phenotypes in chronic inflammatory disease of the upper airways, which is also known as chronic rhinosinusitis (CRS). The macrophage polarization states were studied in 28 healthy and chronic rhinosinusitis patients by immunohistochemistry, FACS analysis and their phagocytosis of *S. aureus*.
We observed significantly more M2 macrophages ($CD163^+$, MMR^+) in the CRS with nasal polyps compared to CRS without nasal polyps. Expression of these M2 markers was positively correlated to increased levels of IL-5, ECP and total local IgE. The group of patients with high numbers of M2 macrophages also had low levels of IL-6, IL-1β and IFN-γ. FACS analysis on dissociated nasal tissue showed that the number of M2 macrophages ($CD206^+HLADR^+CD14^+CD11c^{low}CD16^-CD20^-$) was significantly higher in patients with nasal polyps as compared to controls (3.5% vs 0.7%), while the number of M1 macrophages ($CD206^-HLADR^+CD14^+CD11c^{low}CD16^{+/-}CD20^-$) was not different between the groups of patients. Phagocytosis of *S. aureus* by human tissue derived macrophages was reduced in CRS with nasal polyps as compared to macrophages from the inferior turbinates of control patients.
Next, M1 and M2 macrophages from nasal tissue were sorted out by FACS and the expression levels of TLRs, NOD1 and NOD2 on these subpopulations of macrophages as well as Foxp3 transcription factor will be discussed.

SUBMITTED ABSTRACTS TOPIC G: IMAGING THE BEHAVIOUR OF MONOCYTES, MACROPHAGES AND DCS

G01 5,6-Secosterol affects macrophage diversity and induces pro-inflammatory macrophage activation

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Introduction: Macrophages play a central role in immunity and homeostasis. The classically activated macrophages (M1) and the alternative ones (M2) coexist and contribute to the pathogenesis of atherosclerosis through their accumulation of cholesterol and the production of inflammatory mediators and cytokines. The oxidised cholesterol product, 3β-hydroxy-5-oxo-5,6-secocholestan-6-al (5,6-secosterol) has been shown to be present in atherosclerotic plaques but its effects on macrophage remain to be elucidated. **Aim:** To investigate whether 5,6 secosterol affects human macrophage diversity and polarizes the two different macrophage subsets M1 and M2 towards a pro- or anti-atherogenic phenotype. **Methods:** Human monocytes obtained from healthy blood donors were induced to differentiate for 6 days in the presence of either GM-CSF, to obtain activated macrophages (M1), or M-CSF, to obtain alternative macrophages (M2). By the use of immunochemical and cytofluorimetric analyses, we investigated surface molecule expression, caspase activation, phagocytosis and pro- and anti-inflammatory cytokine production. The effects of 5,6-secosterol on macrophage migration were evaluated using Boyden chemotaxis chambers. **Results:** Our findings indicate that 5,6-secosterol was able to enhance a pro-inflammatory phenotype in M1 and to increase macrophage functions such as phagocytosis and migration. Moreover, this compound prevented the phenotypical and functional activation of M2. **Conclusion:** 5,6 secosterol may exacerbate pro-inflammatory macrophage activation and polarization subverting the balance between M1 and M2 activation profiles in atherosclerotic arteries.
5,6-secosterol affects macrophage diversity and induces pro-inflammatory macrophage activation.

G02 Classical or alternative activation influences motility of macrophages in the CNS

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Macrophages are considered to play an ambiguous role in the central nervous system (CNS). The subsets include classically activated, pro-inflammatory macrophages (M1), and alternatively activated, anti-inflammatory macrophages (M2).
The aim of this study is to characterize these two subsets of macrophages in terms of morphology, motility, adhesion to extracellular matrix molecules and migration towards cytokines present in the CNS. Differences in chemokine receptor expression as well as chemokine production is also investigated.
Peripheral blood derived monocytes (PBMC) were isolated from healthy volunteers and cultured for seven days. Macrophages were skewed either to become M1 by classical activation with interferon-gamma (IFN-g) and lipopolysaccharide (LPS) or into M2 by alternative activation with interleukin-4 (IL-4) for 48 hours. To assess the morphology, the cytoskeleton was stained with rhodamine. Spontaneous motility was measured one hour after harvesting with a time-lapse microscope. Adhesion to extracellular matrix molecules was assessed using plates coated with fibronectin and collagen. After two hours culturing the percentage of adherent cells was measured using a fluorometer. The migration towards different chemokines was determined using a TaxiScan migration chamber.
The cytoskeleton of M1 macrophages was elongated in contrast to the spherical M2 cells. The adherence assays revealed that M2 macrophages adhere more firmly to the extracellular matrix molecules. The motility and migration assays showed higher levels of motility in the M2 macrophages. Further studies are required to establish the mechanisms involved in these different migratory properties, in particular to investigate different expression of chemokine receptors and differences in cytoskeletal properties.

G03 Generation of Macrophage-EGFP Reporter Rats

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Differentiation of BM progenitors into macrophages requires Csf1 which signals via its receptor Csf1r (whose expression is limited to trophoblasts and cells of the macrophage lineage). MacGreen mice were created previously by placing EGFP expression under the control of the Csf1r proximal promoter. These mice have consistent expression of EGFP in the same locations as the endogenous gene and provide a valuable tool for those interested in macrophage biology. Our laboratory is taking advantage of the recent breakthroughs in rat transgenesis to generate macrophage-EGFP reporter rats. In order to achieve this we are taking two main approaches. The first is to create a transgenic rat via lentiviral injection. As the successful use of lentivirus' are dependent on the size of the construct (i.e.: max 6-7kb between the two LTRs), we have produced a 'cut down' version of the Csf1r-EGFP construct used to produce the MacGreen mice. This lentivirus has been used to successfully transduce both mouse and rat macrophages and the specificity is tested by transducing rat ESC and then differentiating these cells into macrophages. Our second approach is to use gene targeting in rat ES cells to replace the endogenous Csf1r gene product with EGFP to produce a knock-out-knock-in MacGreen rat. The progress on the delivery of these approaches will be presented and discussed.

G04 Targeting DC-SIGN neck region leads to prolonged antigen residence in early endosomes, delayed lysosomal degradation and efficient cross-presentation.

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Targeting antigens to dendritic cell (DC) specific receptors, such as DC-SIGN, induces potent T cell-mediated immune responses. DC-SIGN is a trans-membrane C-type lectin receptor with a long extracellular neck region and a carbohydrate recognition domain (CRD). Thus far, only antibodies or sugar ligands binding the CRD have been employed to target antigens to DC-SIGN. We previously demonstrated that ligand binding to DC-SIGN CRD results in clathrin-mediated internalization of the receptor and routing to late endosomes. Recently, antibodies recognizing the neck domain of DC-SIGN have been shown to display an enhanced ability to trigger receptor internalization compared to CRD-binding antibodies. We therefore used microscopy and immunological approaches to determine whether targeting distinct receptor epitopes affects DC-SIGN internalization, intracellular trafficking and ability to induce $CD8^+$ T cell activation. In contrast to anti-CRD antibodies, anti-neck antibodies induced a clathrin-independent mode of DC-SIGN internalization. Interestingly, we observed that anti-neck and anti-CRD antibodies were differentially routed within DCs. Whereas anti-CRD antibodies were mainly routed to late endosomal compartments, anti-neck antibodies remained associated with early endosomal compartments positive for EEA1 and MHC class I for up to several hours following internalization. Finally, in hDC-SIGN transgenic mice, cross-presentation of protein antigen conjugated to anti-neck antibodies was ~1000 fold more effective than non-conjugated antigen. Our studies demonstrate that anti-neck antibodies trigger a distinct mode of DC-SIGN internalization and reveal the neck domain as a promising target to reach the appropriate intracellular compartment, allowing $CD8^+$ T cell activation and provision of T cell help without completely blocking receptor function.

G05 Antigen stored in Dendritic Cells after macropinocytosis is released unprocessed from late endosomes to target B cells.

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How B lymphocytes can be triggered in lymph nodes by non opsonized antigens (Ag), potentially in their native form is still unclear. We show here that antigens are detected in B cells in the draining lymph nodes of mice injected with live, but not fixed, dendritic cells (DCs) loaded with antigens. This highlights active processes in DCs to promote transfer to B lymphocytes. Using three different model Ag, we then show that immature human DCs efficiently take up Ag by macropinocytosis and store the internalized material in late endocytic compartments. We find that DCs have a unique ability to release antigens from these late compartments in the extracellular medium. B cells then take up the regurgitated Ag, which is not in an exosomal fraction. The regurgitation process is controlled by Rab27 and the chemokine BLC/CXCL13, essential to attract B cells in lymph nodes, enhances the release of Ag and their transfer from DCs to B cells. Our results reveal a novel and unique property of DCs to regurgitate unprocessed Ag that could play an important role in B cell activation.

G07 Macrophage mannose receptor as a molecular marker for *in vivo* imaging of rheumatic joints in mice with collagen-induced arthritis

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Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune disease that affects 0.5-1.0% of the worldwide population. The primary affected target organ is the joint, where inflammatory cells invade the synovium and cause severe damage including bone destruction. Our goal was to provide a method to visualize and quantify joint inflammation by the use of an animal model of RA, namely collagen-induced arthritis (CIA). We focussed on the macrophage mannose receptor (MMR), since this protein is a well described marker for macrophages that are numerously present in the inflamed synovium.
Materials and Methods: CIA was induced in DBA/1 mice by injection of collagen type II in Freund's adjuvant. Flow cytometry and qRT-PCR were used to study the expression of MMR *in vivo* in CIA and *in vitro* in macrophages and osteoclasts. SPECT/CT imaging with nanobodies generated against MMR was performed to visualize and quantify MMR expression in the joints of mice.
Results: MMR expression was observed on $CD11b^{+}F4/80^{+}$ macrophages isolated from inflamed synovium of CIA mice. MMR was also expressed on bone marrow derived macrophages *in vitro* and, intriguingly, was highly upregulated during the formation of multinucleated osteoclasts. SPECT-CT imaging with nanobodies against MMR visualized the inflammation at the level of the joints. The signal from SPECT imaging was significantly higher in mice with arthritic symptoms compared to naïve animals or immunized mice without clinical symptoms.
Conclusions: The use of MMR nanobodies in SPECT/CT imaging generates the possibility to track and quantify inflammatory macrophages and osteoclasts *in vivo* in arthritic joints.

G08 A critical requirement of the actin capping activity of Eps8 in dendritic cell migration

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Dendritic cells (DCs) are essential for the initiation of the acquired immune responses, during which they capture and present antigens, undergo maturation and migrate from peripheral tissues to nearby lymph nodes to activate naive T cells. To perform these functions, the DCs plastically adapt their adhesive, actin-based structures and migratory properties. In this study (accepted for publication in Immunity, 2011), we identified EPS8 as the essential actin capping protein specifically required for DCs migration, but not for other fundamental properties of DCs, such as antigen uptake, processing and presentation. Interestingly, the DCs from *Eps8*-deficient mice were unable to polarize and to form elongated migratory protrusions. In addition, they displayed an impaired ability in directional and chemotactic migration in three-dimensional *in vitro* assays, and were significantly delayed in reaching the draining lymph node *in vivo* after inflammatory challenge. Therefore, *Eps8*-deficient mice were unable to mount a contact hypersensitivity response. This migratory dysfunction is cell autonomous as adoptive transfer of *Eps8*-deficient DCs into wild type animals did not restore their migratory properties. Moreover, the loss of the actin capping activity of EPS8 was responsible for the migratory defect *in vitro*. Finally, we showed that EPS8 is required for the maintenance of architectural organization of the actin meshwork and dynamics of cell protrusions in DCs. Thus, we identified EPS8 as a unique actin capping protein specifically required for DC migration.

G09 Macrophage mannose receptor-specific nanobody-based targeting and in vivo imaging of tumor-associated macrophages

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Tumor-associated macrophages (TAMs) are an important component of the tumor stroma and can exert several tumor-promoting activities. We have previously documented that TAMs in various pre-clinical tumor models contain molecularly and phenotypically distinct subsets differing in angiogenic properties and intra-tumoral localization, with Macrophage Mannose Receptor (MMR) being highly expressed on strongly pro-angiogenic, MHC II^{low} TAMs residing in hypoxic tumor areas. In the current study, we set out to specifically target MMR-positive cells using nanobodies: single-domain antigen-binding fragments derived from *Camelidae* heavy-chain antibodies.
Nanobodies generated against MMR were found to stain TAMs, and more intensely the MHC II^{low} subset, on ex vivo single-cell suspensions of subcutaneous tumors. Upon intravenous injection of ^{99m}Tc-labeled nanobodies in wild-type and MMR-deficient mice, total-body scans acquired using Pinhole SPECT and micro-CT revealed that these probes specifically targeted several tissues. The nanobodies were detected predominantly in liver and spleen and to a lesser extent in cardiac muscle, lymph nodes and bone marrow. In subcutaneous tumor-bearing mice, receptor-specific uptake of anti-MMR nanobodies was observed in the tumor. Interestingly, coinjection of excess unlabeled anti-MMR nanobodies strongly reduced the accumulation of ^{99m}Tc-labeled anti-MMR nanobodies in extra-tumoral organs such as spleen and liver, but not in the tumor. Coinjection of unlabeled bivalent anti-MMR nanobodies further reduced the signal in extra-tumoral organs to background levels, while uptake of ^{99m}Tc-labeled anti-MMR nanobodies in the tumor was only slightly diminished, rendering the tumor the site with the highest MMR-specific nanobody uptake.
These results offer diagnostic and therapeutic perspectives of using anti-MMR nanobodies for selective in vivo targeting of TAM subpopulations.

G10 Afferent lymph-borne dendritic cells and T cells use different CCR7-dependent routes for lymph node entry and intranodal migration

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The continuous delivery of lymph-borne antigens and immune cells into draining lymph nodes (LN) via afferent lymphatics is regarded as a key element of immune surveillance and homeostasis. Performing, for the first time, micromanipulator-guided injections into afferent lymph vessels of the popliteal LN (popLN) in living mice, we characterized the intranodal migratory fate of afferent lymph-derived dendritic cells (DC) and T cells by *ex vivo* and *in vivo* two-photon microscopy.
We found that (1) T cells, but not DC, reached LNs downstream of the primary draining popLN after intralymphatic (i.l.) injection (2) DC entered the LN parenchyma through the floor of the afferent side subcapsular sinus (SCS), while T cells primarily populated the parenchyma by transmigrating from peripheral medullary sinuses (3) transmigrating DC induced local changes of the SCS floor morphology that allowed the concomitant entry of i.l. injected T cells at these sites (4) both DC and T cells absolutely required signals mediated via the chemokine receptor CCR7 for directional migration into the paracortical T cell zone (TCZ) (5) parenchymal entry of T cells and DC, as well as DC dendrite probing occurred independent of CCR7 function (6) i.l. injected CCR7-deficient DC efficiently induced a state of 'LN shutdown' while initiating reduced antigen-specific T cell proliferation responses.
In summary, we report the first characterization of the different routes used by afferent lymph-derived DC and T cells for parenchymal entry and CCR7-dependent directional migration into the paracortical TCZ.

G11 Macrophages.com: an on-line community resource for innate immunity research.

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Macrophages play a major role in tissue remodelling during development, wound healing and tissue homeostasis, and are central to innate immunity and to the pathology of tissue injury and inflammation. With the escalation of genome-scale data derived from macrophages and related haematopoietic cell types, there is a growing need for an integrated resource that seeks to compile, organise and analyse our collective knowledge of macrophage biology. Here we describe a community-driven web-based resource, macrophages.com, that aims to provide a portal onto various types of 'omics data to facilitate comparative genomic studies, promoter and transcriptional network analyses, models of macrophage pathways together with other information on these cells. To this end, the website combines public and in-house analyses of expression data with pre-analysed views of co-expressed genes as supported by the network analysis tool BioLayout Express^{3D}, as well as providing access to maps of pathways active in macrophages. Macrophages.com also provides access to an extensive image library of macrophages in adult/embryonic tissue sections prepared from normal and transgenic mice. Finally, an integrated gene-centric portal provides the tools for rapid promoter analysis studies based on a comprehensive set of CAGE-derived transcription start site sequences in human and mouse genomes from FANTOM projects initiated by the RIKEN Omics Science Center. Our aim is to continue to grow the macrophages.com resource using publicly available data, as well as in-house generated knowledge. In so doing we aim to provide a user-friendly community website and a community portal for access to comprehensive sets of macrophage-related data.

G12 Myeloid Derived Suppressor Cells Suppress Chronic Autoimmune Diseases by the Interaction between PD-L1 and PD-1

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The activation of immature myeloid cells (IMC) into myeloid derived suppressor cells (MDSC) plays an important role in blocking cellular responses through various mechanisms, such as cell-cell contact and production of superoxide, oxygen radicals and nitric oxide. In Th1 and CD8 T cell-driven infection and tumor models, a combination of LPS and IFNγ can activate MDSC to suppress T cell responses. To date, the role of MDSC during autoimmune diseases has not been addressed. We aim to elucidate the role of MDSC in Th17-driven autoimmune diseases. In vitro, Th1 and Th17-like stimuli activate MDSC suppressive activity via nitric oxide production and increased PD-L1 expression. During chronic situations, PD-1 is upregulated on activated memory T cells, which allows them to be susceptible to MDSC suppression via PD-L1 interaction. MDSC production of nitric oxide decreases cytokine production (IFNγ, IL-17, IL-4, and IL-10) in a non-specific manner. Furthermore, the injection of BM-IMC into mice decreases the severity of experimental autoimmune encephalomyelitis (EAE), by decreasing proinflammatory cellular infiltrates into the CNS and lowering cytokine production. Using bioluminescence imaging, we observed the in vivo migration of intravenously injected IMC. During steady state conditions, IMC actively migrated to the spleen, peaking by day 4, suggesting MDSC may play a suppressive role during T cell activation. Alternatively, under inflammatory conditions, IMC home differently. Specifically, when injected post EAE induction, the IMC were found in the cervical lymph nodes. Understanding how MDSC suppress and where they suppress will be important in therapeutical efforts under all pathological conditions.

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