Belgian Immunological Society

Annual Autumn Meeting of the Belgian Immunological Society

Monocytes and macrophages in inflammatory diseases and cancer

Friday November 25th 2016

Salle Dupréel, ULB campus du Solbosh, Avenue Jeanne 44, 1050 Brussels

LOCAL ORGANIZERS

Jo Van Ginderachter
Alain Beschin
Geert Raes

Vrije Universiteit Brussel, Research Unit of Cellular and Molecular Immunology
VIB Inflammation Research Center, Laboratory of Myeloid Cell Immunology

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Program

9h00	Registration, morning coffee and display of posters
9h45	Welcome address by Jo Van Ginderachter (Host of the meeting) and Oberdan Leo (President of BIS)
10h00	**Marco Prinz** (University of Freiburg, Germany) "Origin and function of macrophages in the central nervous system"
10h30	**Martin Guilliams** (VIB, UGent) "Cellular Origin and Functional Specialization of Tissue-resident Macrophages"
11h00	**Karin de Visser** (Netherlands Cancer Institute, Amsterdam, The Netherlands) "Cancer-induced systemic inflammation promotes breast cancer metastasis"
11h30	**Jeroen Bogie** (Universiteit Hasselt) "Collectin sub-family member 12 is a novel receptor involved in the uptake of myelin by phagocytes"
11h45	**Aurélie Detavernier** (Université Libre de Bruxelles) "Regulation of interleukin-27 expression in the context of infection"
12h00	**Pieter Goossens** (Centre d'Immunologie de Marseille-Luminy, France) "Depletion of membrane cholesterol in tumor-associated macrophages promotes their M2 polarization"
12h15	Lunch - Poster session - Visit of exhibitor stands
14h15	General Assembly of the Belgian Immunological Society
14h30	**Catherine Sabatel** (Université de Liège) "Bacterial CpG-DNA prevents asthma by expanding lung interstitial regulatory macrophages from local and splenic reservoir monocytes"
14h45	**Sofie Voet** (VIB, UGent) "A20 critically controls microglia activation in CNS inflammation"
15h00	**Kristiaan Wouters** (MUMC, Maastricht, The Netherlands) "Adipose tissue macrophages induce hepatic neutrophil recruitment and macrophage accumulation in mice"
15h15	**Menno de Winther** (Academic Medical Center, Amsterdam, The Netherlands) "Epigenetic enzymes regulating macrophage activation programs: relevance for cardiovascular disease"
15h45	**Guy Boeckxstaens** (KULeuven) "Neuromodulation of the immune system"
16h15	**Jean-Paul Coutelier** – (Université Catholique de Louvain) "Effect of interferon-modulated macrophage and dendritic cell responses in mouse models of diseases concomitant to a viral infection "
16h45	Award of the best two posters

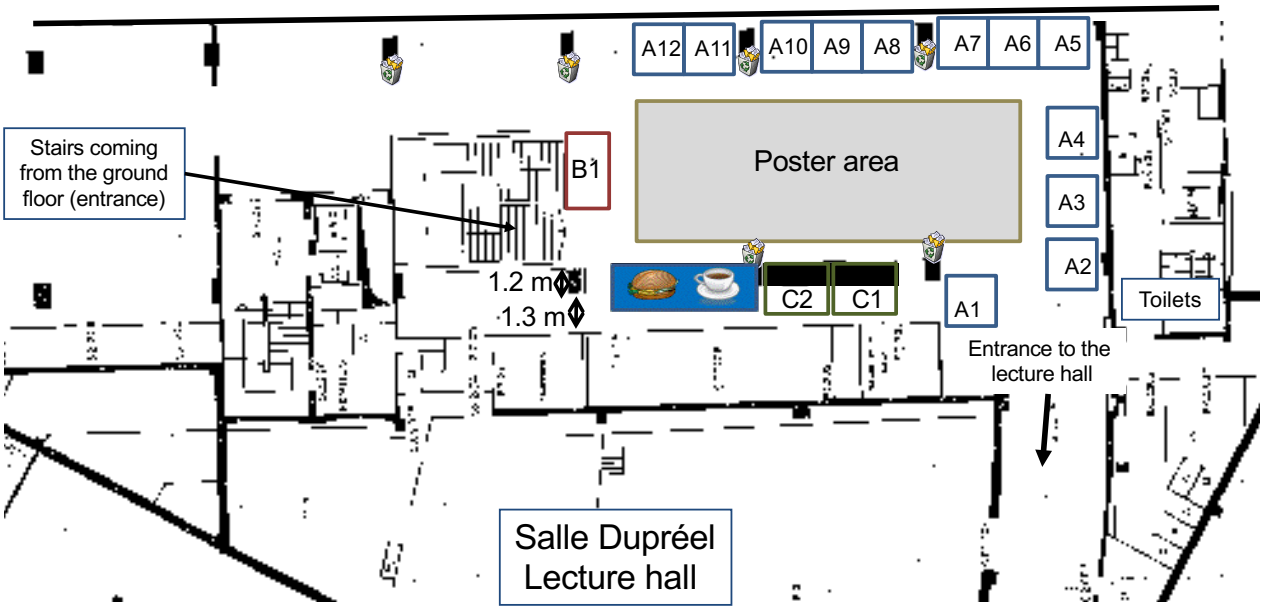
Floor Plan

EXHIBTION STANDS **C1: Bio-Connect** **C2: Merck** B1: Perkin Elmer

A1: Westburg A2: Bio-Techne A3: Becton Dickinson A4: ImTec Diagnostics

A5: Affymetrix eBioscience A6: Bio-Rad Laboratories A7: Filter Service A8: VWR

A9: Stemcell Technologies A10: Analis A11: Enzo Life Sciences A12: BCCM-LMBP



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1 Kupffer cells regulate the onset of immune response against Listeria monocytogenes infection

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In experimental listeriosis, contradictory results were reported on the role of Kupffer cells (KC) due to the inability to correctly distinguish resident embryonic KC from infiltrating monocyte-derived macrophages. In this context, we have identified a Clec4f marker specifically expressed in KC and used this marker to generate a mouse model of diphtheria toxin-mediated depletion of KC. Using these mice, we found a protective role of KC during *L. monocytogenes* infection. KC-depleted mice became highly susceptible to infection. The inability of KC-depleted mice to control the infection was associated with an altered innate cell response and inadequate adaptive type 1 immune response. In the absence of KC, T cells numbers were reduced, and antigen-specific CD8 T cells were impaired in their IFN-g synthesis capacity, coinciding with a drastic reduction in DCs. These results indicate an important interplay between liver-resident KC and other non-parenchymal hepatic antigen-presenting cells. . By addressing the fate of hepatic MFs in infected liver-shielded CD45.1-->CD45.2 chimera, we evidence the existence of 3 MF populations, bone marrow derived CD45.1+ Clec4f- Tim4- moMFs and Clec4f+ Tim4- moKCs, and CD54.2+ Clec4f+ Tim4+ embryonic KCs. Wether these populations fulfil differerent functions is under investigation.

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2 APC-derived IL-6 restricts the expression of GATA3 and IL-4 by follicular helper T cells

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Follicular helper T cells (Tfh) support high affinity antibody production by germinal center B cells through both membrane interactions and secretion of IL-4 and IL-21, two major cytokines implicated in B cell survival and antibody class switch. Tfh-2 cells recently emerged in humans as a strong IL-4 producer Tfh cell subset implicated in both autoimmune and allergic diseases. Although the molecular mechanisms governing Tfh cell differentiation from naive T cells have been widely described, much less is known about the regulation of cytokine secretion by mouse Tfh-2 cells. The purpose of our study was to evaluate the role of dendritic cell (DC)-derived IL-6 in fine-tuning cytokine secretion by Tfh cells.
Our results demonstrate that priming of Th cells by IL-6-deficient antigen presenting DCs preferentially lead to accumulation of a subset of Tfh cells characterized by high expression of GATA3 and IL-4, associated with reduced production of IL-21. STAT3-deficient Tfh cells also overexpress GATA3, suggesting that early IL-6/STAT3 signalling during Tfh cell development inhibits the expression of a set of genes associated with the Th2 differentiation program. Overall, our data indicate that IL-6/STAT3 signalling restrains the expression of Th2-like genes in follicular helper T cells, thus contributing to the control of IgE secretion in vivo.

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3 Identification of a novel mechanism of blood-brain communication during peripheral inflammation via choroid plexus-derived extracellular vesicles

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Aim: Little is known about how the periphery communicates with the central nervous system (CNS), in normal as well as pathophysiological conditions. The choroid plexus epithelium (CPE) is a unique single layer of epithelial cells situated at the unique interface of the blood and cerebro-spinal fluid (CSF) and forms blood-CSF barrier might be equipped to do this function.
Methods: Several high throughput technologies, such as NanoString, advanced mass spectrometry together with *in vitro* and *in vivo* qPCR, western blot and immunohistochemistry analyses.
Results and Conclusion: Here, we found that systemic inflammation induced a fast decrease in miRNA expression levels in the CPE and this was inversely correlated with increased miRNAs levels in the CSF, such as the pro-inflammatory miRNAs miR146, miR155, miR9 and miR1a. This was linked with an increase in the amount of extracellular vesicles (EVs) in the CSF. Using transmission electron microscopy (TEM), we also observed in the CPE cells a time dependent increase in multivesicular bodies (MVBs) filled with EVs, called exosomes, upon inflammatory stimulation *in vivo*. *In vitro* studies revealed that these secreted EVs are taken up by brain parenchymal cells and are able to transfer a message from the blood to the CNS. *In vitro* and *in vivo* pharmacological inhibition of the exosome production reduced the inflammation-induced exosome release and resulted into accumulation of miRNAs in the CPE cells. In conclusion, we identified CPE-derived EVs as new mechanism of blood-CNS communication during peripheral inflammation by transferring pro-inflammatory message from the BCSFB to recipient brain cells.

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4 Collectin sub-family member 12 is a novel receptor involved in the uptake of myelin by phagocytes

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Myelin-containing macrophages and microglia are the most abundant immune cells in active MS lesions. Our recent transcriptomic analysis demonstrated that collectin sub-family member 12 (COLEC12), also known as collectin placenta 1, is one of the most potently induced genes in macrophages after uptake of myelin. COLEC12 is a type II transmembrane protein with both a collagen-like and carbohydrate recognition domain, which plays a key role in host defense. In this study we sought to determine the dynamics of COLEC12 expression on myelin-containing phagocytes and define the role that it plays in MS lesion development. We show that myelin uptake increases the cell surface expression of COLEC12 by mouse and human macrophages, but not by primary mouse microglia *in vitro*. In active demyelinating MS lesions, COLEC12 immunoreactivity was localized to myelin-laden parenchymal and perivascular phagocytes. Finally, we demonstrate that COLEC12 is involved in myelin internalization as knockdown of COLEC12 markedly reduced myelin uptake. Collectively, our data indicate that COLEC12 is a novel receptor involved in myelin uptake by phagocytes and likely plays a role in MS lesion development.

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5 Nanobody-based depletion of protumoral tumor-associated macrophages as novel cancer therapy

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Tumors are considered as organoid structures, which contain not only cancer cells but also non-transformed types of cells, the stromal cells. A bidirectional interplay exists between transformed and non-transformed cells, which results in tumor progression and metastasis. Tumor-associated macrophages (TAMs) are one of the dominant cell types present in murine and human tumors. Clinical and experimental studies have delineated the highly pro-tumorigenic role of TAMs through a variety of mechanisms. For this reason, the depletion of pro-tumorigenic TAMs forms an attractive perspective in the treatment of cancer.

We have previously shown that the Macrophage Mannose Receptor (MMR, CD206) is highly expressed on the surface of pro-tumorigenic TAMs, rendering it an important molecule for targeted TAM depletion. Hence, we intend to deplete the pro-tumorigenic MMR^{high} TAMs by using anti-MMR Nanobody (Nb)-conjugates, whereby Nbs are the antigen-recognition domains of camelid heavy chain-only antibodies. For this purpose, we have generated both anti-mouse and cross-reactive anti-human/mouse MMR Nbs and have selected the lead compounds, cross-reactive Nb3.49 and anti-mouse Nb1, based on their optimal *in vivo* pharmacokinetic properties. [^{99m}Tc]-labeled Nb1 exhibits high tumor penetrance, upon blocking of extra-tumoral binding sites with an excess of unlabeled bivalent Nb1, as visualized via SPECT/microCT imaging. We have currently conjugated the lead anti-MMR Nb1 to a therapeutic radionuclide for radioimmunotherapy. Co-administration of the therapeutic radiolabeled MMRNb1 with an excess of unlabeled bivalent MMRNb1 to TS/A breast tumor-bearing mice, leaded to remarkable retardation of tumor growth compared to non-treated animals.

The present work is granted by Flanders Institute of Innovation and Technology (VLAIO).

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6 TARGETING NEUROPILIN-1 THROUGH SINGLE-DOMAIN ANTIBODIES: A NOVEL STRATEGY TO TACKLE TUMOR-ASSOCIATED MACROPHAGES

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Neuropilin-1 (Nrp-1) is a transmembrane receptor which binds several ligands including VEGF-A, class 3 semaphorin proteins, and PlGF. Recently, Nrp1 expression in the tumor environment has sparked the interest of scientists as a potential target for cancer therapy. In particular, Nrp1 expression in infiltrating immune cells from the host has drawn the attention of immunologists. Nrp1 has shown to be needed by tumor-associated macrophages to migrate to hypoxic regions of the tumor, where they can establish an immunosuppressive and pro-angiogenic environment, which sustain tumor progression. Hence, blocking Nrp1 by means of antibodies or antibody-like molecules holds great therapeutic promise. Given these premises, we have generated a panel of single-domain antibody fragments (also referred to as 'nanobodies') with high affinity for human and mouse Nrp1 which have shown to bind to Nrp1-expressing cell cultures and ex vivo primary cells. Because of their small size and high affinity, we are evaluating the nanobodies' ability to penetrate the tumor tissue and to target tumor-associated macrophages. All together, these studies will establish a novel nanobody-based strategy to tackle the immunosuppressive function of tumor-associated macrophages and, ultimately, will provide a new tool for cancer therapy.

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7 VAGUS NERVE STIMULATION DAMPENS TH2-MEDIATED FOOD ALLERGY

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Background: The cholinergic anti-inflammatory pathway (CAIP) via the vagus nerve has emerged as an important modulator of the intestinal innate immune system. To what extent the CAIP also affects the adaptive immune response remains unclear. Thus, we investigated the effect of vagus nerve stimulation (VNS) in a typical Th2-mediated intestinal disorder such as food allergy.
Methods: Balb/C mice were sensitized with ovalbumin (OVA) (day 0 and 14) followed by intragastric challenges with OVA every other day, from day 28 onwards. Prior to the first challenge, mice received VNS (5min, 5Hz, 1mA) or sham stimulation. Mice were sacrificed 1 hour after the 3rd OVA challenge and cytokine gene expression and lamina propria (LP) immune cells were analyzed by qPCR and flow cytometry, respectively. Mast cell degranulation and antibody response was assessed by measuring mouse mast cell protease 1 (MMCP-1) and OVA-specific IgE in the serum. Data shown as mean ± SEM, $P<0.05$ is considered statistically significant; unpaired t-test or Mann-Whitney U test was performed.
Results: VNS-treated mice showed lower expression in the duodenum of the Th2 cytokines IL4 (Sham 2017 ± 768 fold increase vs. VNS 452 ± 115 fold increase (n=8, p=0.03)) , IL5 (Sham 15.0 ± 4.0 fold increase vs. VNS 2.0 ± 0.5 fold increase (n=8, p=0.001)) and IL13 (Sham 5.8 ± 1.4 fold increase vs. VNS 2.7 ± 0.7 fold increase (n=8, p=0.03)) and the inflammatory cytokine IL-6 (Sham 68 ± 24 fold increase vs. VNS 16 ± 4 fold increase (n=8, p=0.02)) compared to sham-treated mice. Furthermore, VNS significantly reduced serum MMCP-1 after the 2nd (Sham 2934 ± 545 ng/mL vs. VNS 1556 ± 296 (n=8, p=0.04)) and 3rd (Sham 5758 ± 1315 ng/mL vs. VNS 2352 ± 329 (n=8, p=0.03)) intragastric challenge with OVA. VNS was also able to significantly reduce serum levels of OVA-specfic IgE (Sham 85.2 ± 17.2 ng/mL vs. VNS 44.0 ± 9.1 ng/mL (n=8, p=0.05)). Finally, VNS significantly reduced the % and number of neutrophils in the LP (Sham 2.4 ± 0.4% vs. VNS 1.1 ± 0.2% (p=0.008) and Sham $3.3x10^4 \pm 6.4x10^3$ vs. VNS $1.8x10^4 \pm 5.8x10^3$ (p=0.03) (n=8)), but mast cell and eosinophil % and numbers were unaffected.
Conclusion: Our data show that VNS reduces intestinal inflammation and dampens mast cell activation in a murine model of food allergy. This indicates that VNS not only modulates the innate but also the adaptive immune system. Further insight in the underlying mechanism could ultimately lead to new targets and novel therapeutic approaches to treat or improve food allergy.

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8 Role of the CD27/CD70 pathway in regulatory T cell function in vivo

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CD70 and CD27, expressed mainly on antigen presenting cells and T lymphocytes, respectively, are members of the TNF/TNFR family. The CD27/CD70 pathway triggers the differentiation of Th1 lymphocytes and expression of CD70 on immature dendritic cells has been shown to break tolerance and induce immunity, underlying the critical role of this pathway as a switch between tolerance and immunity.
Intriguingly, recent data from our laboratory indicate that CD27 expressed on Tregs is involved in the inhibition of CD70, suggesting that CD27 may display opposite functions, either inducing pro-inflammatory Th1 responses (when expressed on conventional T cells) or inhibiting the inflammatory responses (when expressed on Tregs). Of note, CD27 is expressed at higher rates in Tregs compared to Tconvs suggesting that CD27 may regulate Treg homeostasis/function.
The objective of this project is therefore to investigate the role of CD27 in Treg vs T conv function in vivo.
Our preliminary data suggest that CD27 engagement results in an increased proportion of Tregs among $CD4^+$ T cells, and enhanced proliferative capacity (higher proportion of $Ki67^+$ Tregs). Indeed, hallmarks of Treg activation such as ICOS, GATA-3, Helios, and inhibitory molecules such as CTLA-4, PD-1, were upregulated among the Treg population in mice treated with an agonistic anti- CD27 mAb. Moreover CD27 might potentiate Treg suppressive activity, as assessed by an increased number of $CD62L^-$ $CD44^+$ effector Tregs and Tregs producers of the inhibitory cytokine IL-10.
The role of CD27 in Treg homeostasis and function will be further studied in various in vivo models, including T cell-induced colitis and Graft Versus Host Disease. Interestingly, we have generated a new mouse strain $CD27^{fl/fl}$ which will allow to selectively delete CD27 in Tregs and compare the function of CD27- competent and-deficient Tregs in various disease models.

9 Monocytes of patients with primary ciliary dyskinesia show an inflammatory status

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Primary ciliary dyskinesia (PCD) is a rare, autosomal inherited disorder caused by mutations leading to structural and/or functional defects of motile cilia. Motile cilia are responsible for the movement of extracellular fluid and are generally located on the respiratory epithelium, on the ependymal brain ventricles and in the female reproductive tract. In the respiratory tract, motile cilia are essential in protecting the lungs from injury and infection by transporting the upper layer of the mucus, wherein harmful microorganisms and particles are captured, upward. This process is called mucociliary clearance and its deficiency, due to ineffective movement of the motile cilia, causes recurrent infections of the upper and lower airways in PCD patients. *Situs inversus* and male infertility are also common symptoms in PCD patients. Today, 30 genes are linked with PCD, mostly leading to deficiency or loss of the energy-producing dynein arms. Forty percent of the PCD patients carry a yet unknown gene mutation. The complex structure of motile cilia provokes heterogeneity in terms of symptoms and consequently seriousness of disease and together with its rarity (1/10 000 – 1/20 000 individuals), both diagnosis and treatment of PCD is challenging.
As the recurrent airway infections are the most prominent feature in PCD, our aim is to unravel the role of the innate immune system in PCD pathology and to discover if the chronic inflammatory milieu in the patients affects the activity of blood leukocytes, e.g. monocytes, in their defense against pathogens. Monocytes are essential in detecting pathogens, secreting cytokines and chemokines to activate other blood leukocytes (e.g. neutrophils) and in clearing infection and pathogens by phagocytosis. Chemokines are a group of *chemo*tactic cyto*kines* secreted by resident cells or other blood leukocytes when pathogens are detected. Monocytes recognize these chemokines by their corresponding chemoattractant-receptors and migrate along the chemokine concentration gradient to reach the site of infection. Chemokines are thus indispensable for a proper functioning immune system. Therefore, we first measured the chemoattractant-receptor expression on monocytes of PCD patients and their chemotactic response to CCL2 and CCL3. Further, we measured the expression of inflammatory markers and adhesion molecules on monocytes of PCD patients. Finally, we determined levels of several cytokines released by activated PCD monocytes. We can conclude that monocytes of PCD patients show a primed or inflammatory status compared to healthy controls.
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10 Post-translational modification of GRP78 in human islets of Langerhans: implications for auto-reactive T-cell activation

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Beta-cell destruction in islets of Langerhans, leading to the development of type 1 diabetes (T1D), is mediated by infiltrating T-cells. However, beta-cells also play an active role themselves in this process where loss of tolerance against beta-cell antigens can be induced by the generation of post-translational modifications (PTM). Our group previously showed that inflammatory stress induces citrullination of Glucose-regulated-protein 78 (GRP78) in INS-1E cells and mouse islets. Moreover, NOD mice had autoreactive T-cells and auto-antibodies against citrullinated GRP78.
In this study we aimed to translate previous findings to the human situation. Proteome analysis by 2-dimensional gel electrophoresis (2D-DIGE) of cytokine-exposed (IL-1β/IFNγ/TNFα, 50/1000/1000 U/ml) islets from healthy donors revealed post-translational modification of GRP78 in 3 out of 5 islet preparations, suggestive for citrullination. To investigate the presence of CD4+ and CD8+ T-cell responses in the circulation of T1D patients, a range of citrullinated and native GRP78 peptides were designed, with predicted strong binding for T1D predisposing HLA class-II (DR4/DQ8) and HLA class-I (A*0201). First *in-vitro* tetramer studies showed that one citrullinated GRP78 epitope was recognized by CD4+ T-cells in 3/6 patients and 0/6 healthy controls and GRP78 specific CD4+ T-cells could be visualized directly *ex-vivo* as well. Although these results need further confirmation, they suggest that human islets of Langerhans are prone to post-translational modification upon inflammation and that citrullinated GRP78 may be an autoantigen in human T1D. These findings may open the road for development of new diagnostic or therapeutic applications for patients with T1D.
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11 Serum amyloid A chemoattracts immature dendritic cells and indirectly provokes monocyte chemotaxis by induction of cooperating CC and CXC chemokines

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Serum amyloid A (SAA) is an acute phase protein which is up-regulated in inflammatory diseases and chemoattracts monocytes, lymphocytes and granulocytes via its G protein-coupled receptor FPRL1/FPR2. Here, we demonstrated that the SAA1α isoform also chemoattracts monocyte-derived immature dendritic cells (DCs) in the Boyden and μ-slide chemotaxis assay and that its chemotactic activity for monocytes and DCs was indirectly mediated via rapid chemokine induction. Indeed, SAA1 induced significant amounts (≥5 ng/ml) of MIP-1α/CCL3 and IL-8/CXCL8 in monocytes and DCs in a dose-dependent manner within 3 h. However, SAA1 also directly activated monocytes and DCs for signaling and chemotaxis without chemokine interference. SAA1-induced monocyte migration was nevertheless significantly prevented (60 to 80% inhibition) in the constant presence of desensitizing exogenous MIP-1α/CCL3, neutralizing anti-MIP-1α/CCL3 antibody or a combination of CCR1 and CCR5 antagonists, indicating that this endogenously produced CC chemokine was indirectly contributing to SAA1-mediated chemotaxis. Further, anti-IL-8/CXCL8 antibody neutralized SAA1-induced monocyte migration, suggesting that endogenous IL-8/CXCL8 acted in concert with MIP-1α/CCL3. This explained why SAA1 failed to synergize with exogenously added MIP-1α/CCL3 or SDF-1α/CXCL12 in monocyte and DC chemotaxis. In addition to direct leukocyte activation, SAA1 induces a chemotactic cascade mediated by expression of cooperating chemokines to prolong leukocyte recruitment to the inflammatory site.

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12 Characterization of monoclonal antibody-induced internalization of human and mouse sialoadhesin on macrophages and the effect on phagocytosis

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Sialoadhesin (Siglec-1 or CD169; Sn) is a macrophage-specific receptor. During inflammation and certain diseases, like cancer and rheumatoid arthritis, an upregulation of Sn is observed on macrophages, but also on blood monocytes and dendritic cells. Furthermore, a correlation between Sn and the severity of the disease was observed, for instance during rheumatoid arthritis. Due to the interesting expression pattern during several diseases and the location of Sn-positive macrophages at the border of lymphoid tissues suggest that Sn might be an interesting target for immunotherapy. Moreover, Sn is able to internalize after binding with specific anti-Sn mAbs. Most research on mAb-induced internalization was performed for porcine Sn. However, since the cytoplasmic tail of Sn is not conserved between species and Sn does not contain any known signalization motif, mAb-induced internalization of human (hSn) and mouse Sn (mSn) might be different. Furthermore, the use of novel mAbs, especially cross-reactive mAbs, would aid the development of Sn-specific immunotherapy.
Novel cross-reactive mAbs were developed recognizing both hSn and porcine Sn, but no cross-reactivity was observed with mSn. Also, a new mSn specific mAb was developed. The new developed mAbs recognized different epitopes compared to existing available antibodies, yet all mAbs inhibit sialic acid-dependent binding suggesting that they bind the N-terminal domain of Sn. All mAbs induce Sn internalization, indicating that the recognized epitope is not crucial for internalization. The mAb-induced internalization of hSn and mSn was analyzed in more detail in primary cells and cell lines using two newly developed mAbs. Internalization of both hSn and mSn, in primary cells and cell lines, was time-dependent, dynamin-dependent and clathrin-mediated. Intracellular localization of the Sn-mAb complex is crucial for proper development of Sn-specific immunotherapy, because the cargo linked to the anti-Sn mAb, such as a toxin or a vaccine antigen, must be properly delivered and processed in the cell. In primary cells, internalized Sn-mAb complexes were located mostly in the endosomes and barely in the lysosomes. In contrast, internalized vesicles were also observed in lysosomes in cell lines expressing a recombinant Sn.
An important aspect of immunotherapeutics is their safety. Antibody induced Sn signaling could lead to unwanted side-effects on macrophage functionality. Since phagocytosis is a primary function of macrophages, the effect of targeting Sn with mAb on the phagocytosis capacity of macrophages was studied. Surprisingly, phagocytosis of 1 μm polystyrene beads was significantly reduced and this effect persisted for a long time after targeting. In contrast, Fc-mediated phagocytosis and uptake of bacteria was respectively unaltered or even augmented.

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13 Interferon response factor-3 promotes the pro-Th2 activity of mouse myeloid CD11b+ dendritic cells

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Only few transcription factors have been identified to date that are required for antigen-presenting cells (APCs) to induce T helper type 2 (Th2) responses. Using mouse lung resident conventional CD11b$^+$ dendritic cells (CD11b$^+$ cDCs) in the context of house-dust mite (HDM)-driven allergic airway sensitization as a model, we aimed here to identify transcriptional events regulating the pro-Th2 activity of cDCs. Transcriptomic profiling of lung CD11b$^+$ cDCs exposed to HDM in vivo revealed first that HDM triggered a prominent antiviral defence-like response and second that the majority of HDM-induced transcriptional changes in CD11b$^+$ cDCs depended on the transcription factor Interferon Response Factor-3 (Irf3). Validating the functional relevance of these observations, Irf3-deficient CD11b$^+$ cDCs displayed reduced pro-allergic activity. This effect was not related to impaired antigen uptake or migratory activity of Irf3-deficient CD11b$^+$ cDCs. Instead, following direct contact with naïve T cells, Irf3-deficient CD11b$^+$ cDC induced less Th2, more regulatory T cell, and similar Th1 differentiation compared to their wild-type counterparts. The altered APC activity of Irf3 CD11b$^+$ cDCs was associated with reduced expression of the pro-Th2 costimulatory molecule CD86 and was phenocopied by blocking CD86 activity in wild-type CD11b$^+$ cDCs. Altogether, these results establish Irf3, mostly known for its antiviral activities, as a transcription factor involved in the induction of Th2 responses through the control of pro-Th2 costimulation in CD11b$^+$ DCs.

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14 Regulation of interleukin-27 expression in the context of infection

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The inflammatory response, initiated by pathogens or danger signals, is required for the establishment of the immune response. This process has to be tightly regulated, as it may lead to tissular damages and chronic inflammatory states. Among the Interleukin (IL)-12 family members, IL-27 plays a central role in the control of inflammation. However, little is known about the *in vivo* cellular sources of IL-27. Using IL12-YFP mice, we confirmed that conventional dendritic cells produce primarily IL-12 upon *Toxoplasma gondii* or *Listeria monocytogenes* infection. In sharp contrast, using a novel IL27-GFP reporter mouse, we identified CD11b$^+$ CD64$^+$ LY6C$^+$ inflammatory monocytes as the main source of IL-27. This result suggests that these populations fulfill different functions in the course of infection. By studying the expression profile of inflammatory genes within inflammatory monocytes by single cell-qPCR, we showed that among the most correlated genes with IL27p28, many are associated with IFN (type I/II) signaling. Further work will aim at identifying the epigenetic regulation of IL-27 that may lead to this functional specialization.

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15 Increased proportions of blood tolerogenic monocytes and decreased proportions of blood dendritic cells subpopulations as hallmark of human active tuberculosis

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Tuberculosis (TB) remains a major public health problem, the development of better preventive and therapeutic approaches being hindered by incomplete understanding of its pathogenesis. Effective cellular immune responses are key components for protection against disease, such responses being depressed in patients with active TB (aTB). This is at least partially due to higher T lymphocytes apoptosis in patients with aTB and to the inhibition of CD4+ T lymphocyte responses by regulatory CD4+ T cells (Treg). In view of the major role of antigen-presenting cells (APC) in their induction, we analyzed by flow cytometry the expression of different receptors by circulating monocytes (Mo) and dendritic cells (DC) in 2 independent cohorts each comprising patients with aTB, latently-TB-infected (LTBI) subjects and Mtb-uninfected controls. The proportions of CD14+CD16- and CD14+CD16+ Mo were higher in aTB compared to both LTBI and controls and TB treatment tends to normalize these proportions. The second cohort allowed the identification of lower proportion of mDC2 (CD11c+CD123-CD141+) as hallmark of TB disease. In depth analysis of the expression of several molecules was evaluated on the Mo and DC subsets of the subjects from the second cohort. Specifically, the percentage of PDL-1+CD14+CD16- Mo were higher among aTB patients controls, coinciding with higher Mo PDL-1 MFI ratio (MFI studied molecule-tube/MFI FMO-tube) suggesting a role of T cell apoptosis in the down-regulated T cell responses. Analysis of the expression of ILT-3, expressed by tolerogenic APC indicated higher proportions and higher MFI ratio of ILT-3+CD14+CD16- Mo in patients with aTB compared to controls. We therefore not only report abnormal proportions of circulating Mo and mDC with a particular phenotype in aTB patients, but we identified high proportions of tolerogenic monocytes that could play a major role in the induction of Treg cells during aTB.

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16 Hypoxia and anti-tumor immunity

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Although cancer immunotherapy is demonstrating encouraging clinical results, they are still limited to a minority of patients probably because of mechanisms of tumoral resistance to immune rejection. These mechanisms involve the modulation of the microenvironment of the tumor and may cause local immunosuppression. In particular, an hypoxic environment within the tumor could be a key factor of the immunosuppression. This project will focus on the effect of hypoxia on tumor-infiltrating lymphocytes and evaluate the role of a key protein necessary for adaptation to hypoxic environment, the transcription factor HIF-1, which activates transcription of a number of immunosuppressive proteins. We generated T cell specific HIF-1α-deficient or PHD2-deficient (PHD2 degrades HIF-1α) mice to examine the role of HIF-1α in T cells. Our data show that conditional ablation of PHD2 in T cells leads to rejection of EG7- OVA tumor in 100% of mice. Furthermore, the rejection is dependent on $CD8^+$ T cells and correlates with increased cytotoxic response *in vivo*. These observations suggest that stabilisation of (presumably) the oxygen sensor HIF-1α plays a beneficial effect on anti-tumor immunity. The RNAseq approach will be used to identify the molecular mechanism underlying the control of CD8 function by PHD2 (and the HIF family).

17 Susceptibility to Streptococcus pneumoniae infection is conditioned by the somatotrope GHRH/GH/IGF-1 axis

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Deletion of the growth hormone-releasing hormone gene (*Ghrh*) results in a severe deficiency of the somatotrope GHRH-GH-IGF-1 axis causing dwarf phenotype that can be reversed by GH or GHRH supplementation (Alba & Salvatori, Endocrinology 2004;145:4134). In basal conditions, the immunological phenotype of *Ghrh*$^{-/-}$ mice is not markedly disturbed except for a decrease in B cells, an increase in thymic Treg cells, and slight modifications in stimulated responses (submitted for publication). These data prompted us to investigate immune responses of *Ghrh*$^{-/-}$ mice using vaccination and infection by *S.pneumoniae* as models since the response to both stimuli relies on the innate immune system and B cells.
Ghrh$^{-/-}$ mice were unable to trigger production of specific IgM and IgG against serotype 1 pneumococcal polysaccharide (PPS) after vaccination with either native PPS (Pnx23) or protein-PPS conjugate (Prev-13) vaccines. Pnx 23 induces an immune response in the absence of T helper cells, mainly based on IgM and B1-b and marginal zone are responsible of this production in mice. The Prev-13 vaccine elicits a T-cell dependent response by conjugation of the PPS to highly immunogenic cross-reactive material 197 (CRM197), a non-toxic diphtheria protein. The origin of this response comes from the follicular B cells of the spleen. These 2 vaccines include the serotype 1 (our bacterial strain), and they have been described as providing an effective protection in mice. A short GH supplementation to *Ghrh*$^{-/-}$ mice (1 daily injection of 1 mg/kg GH for 4 weeks) restored IgM and IgG response to Pnx23 vaccine but not to Prev-13. This suggests that GH has different impacts on the spleen areas.
Furthermore, after intranasal instillation of a non-lethal dose (defined by the full clearance by WT C57BL/6 mice after 24h) of serotype 1 *S.pneumoniae*, *Ghrh*$^{-/-}$ mice exhibited a dramatic susceptibility reflected by increased pulmonary bacterial load with time, bacteremia 24h after infection and a survival limit of 72h, a dramatic decrease in lung B and T cells populations and an increased proportion of inflammatory macrophages, while lung immune populations returned to basal proportion after 48h in WT mice.
The possible impact of GH deficiency on components of the innate immune system that play an important role in defense of the respiratory tract against pneumococcal infection is under current investigation.

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18 Depletion of membrane cholesterol in tumor-associated macrophages promotes their M2 polarization

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In the vast majority of cancers the accumulation of tumor-associated macrophages (TAM) is associated with poor prognosis. Despite the intrinsic anti-tumoral capacities of macrophages, TAM rapidly adopt a tumor-promoting phenotype upon contact with the cancer micro-environment, producing growth factors, promoting angiogenesis and suppressing anti-tumor immune responses.
Transcriptomic analysis of TAM derived from a mouse model of stage IV ovarian cancer revealed that the expression of genes involved in cholesterol efflux is upregulated by TAM in established tumors, as compared to naïve tissue resident macrophages or early recruited monocyte-derived macrophages. Both *in vitro* and *ex vivo* studies show that the resulting cholesterol efflux leads to a marked reduction in cholesterol-rich membrane microdomains, also called "lipid rafts". This depletion of membrane cholesterol in TAM coincides with an increased sensitivity to the M2-polarising cytokines IL-4 and IL-13, both at signaling and transcriptional level, while their response to the M1-skewing cytokine IFNγ is strongly reduced. The polarized response to M1 and M2 cytokines can be mimicked using other methods of lipid raft depletion but is alleviated in macrophages lacking the cholesterol efflux transporters ABCA1 and ABCG1. Indeed, macrophage-specific deletion of these transporters results in reduced tumor growth and expression of genes associated with the M2-like polarization of TAM.

These data suggest that depletion of the macrophages' membrane cholesterol, and consequently lipid rafts, in response to tumor cell-produced factors is an important mechanism in their polarization towards a tumor-promoting phenotype.

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19 CXCL4 and CXCL4L1 differentially affect monocyte survival and differentiation

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Under inflammatory conditions, macrophages can differentiate from peripheral blood monocytes under the influence of various growth factors, cytokines or infectious agents. Chemokines, in particular the platelet-derived CXCL4, are also involved in polarization and survival of monocytes. Indeed, CXCL4 induces the polarization into an M4 phenotype with a unique transcriptome. In this study we compared the effect of CXCL4 and its variant CXCL4L1 on the differentiation of monocytes. $CD14^+$ monocytes were cultured in the presence of CXCL4, CXCL4L1 and M-CSF. The expression levels of several genes and surface receptors were analyzed and cytokines released were measured. In contrast to M-CSF and CXCL4, CXCL4L1 did not induce monocyte survival. CXCL4L1-treated $CD14^+$ monocytes showed a different transcriptional profile compared to CXCL4-polarized macrophages. In contrast to CXCL4, CXCL4L1 (10 µg/ml) stimulated the release of CXCL8 and CCL2. On the other hand, CXCL4 enhanced production of CCL22 compared to CXCL4L1- and M-CSF-stimulated $CD14^+$ monocytes. Furthermore, unlike CXCL4, CXCL4L1 increased expression of the inflammatory chemokine receptors CCR2, CCR5 and CXCR3. We can conclude that both CXCL4 and its variant CXCL4L1 exert a direct, but distinct effect on monocytes yielding differently polarized macrophage phenotypes.

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20 Evaluation of cellular immune responses induced by vectored recombinant HVT vaccines using Gumboro disease as a model

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Recombinant Herpesvirus of Turkey (rHVT)-IBD vaccines are successfully used to induce an immune protective response against Infectious Bursal Disease (IBD). Low titers of rHVT-IBD-induced antibodies are detected in vaccinated chickens before 14 days post-vaccination (dpv) (Gelb et al. 2016). In addition, IFN-γ production by splenocytes can be measured after specific stimulation at 3 weeks after rHVT-IBD vaccination (Ingrao et al. 2016, submitted), suggesting that protection may also partially be attributed to cell-mediated immunity. In the present study, by a phenotypic analysis of the peripheral blood and splenic T cells subsets of rHVT-IBD-vaccinated chickens, we observed an increase of CD4-CD8+ and CD4+CD8+ cells populations at 7 dpv. In a second experiment, a boost with a live attenuated IBDV was done to specifically restimulate *in vivo* the rHVT-IBD vaccinated chickens one week after the first immunization. The induction of immune responses was characterized by analyzing the transcriptional levels of several immune-related genes in spleen, bursa and lung at 2-5-7 days post-boost (dpb), and was compared to three control groups of chickens, namely rHVT-IBD alone, live attenuated IBDV and unvaccinated. The expression level of IFN-γ in rHVT-IBD/IBD chickens was significantly higher at 7 dpb in bursa and lung when compared with live attenuated IBD group. In lung, CD8 expression was upregulated in rHVT-IBD/IBD group at all time points tested, suggesting a reactivation of CD8 T cells in response to live IBDV. An increased expression of Granzymes A and K was also observed in lung at 7 dpb for this rHVT-IBD/IBD group, implying a specific activation of cytotoxic response to rHVT-IBD vaccines. In spleen, an upregulated CD8 expression was only observed at 2 dpb in rHVT-IBD/IBD group and was not measurable at any other time points, suggesting a homing of cytotoxic T cells out of this lymphoid organ. As observed in lung, an upregulated CD8 expression is detected in bursa of rHVT-IBD-vaccinated birds.
These data strongly support the involvement of cell-mediated response components in triggering effective rHVT-IBD-induced immunity to IBDV.

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21 The tumor microenvironment harbors ontogenically distinct dendritic cell populations with opposing effects on tumor immunity

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The identification of functionally distinct tumor-associated DC (TADC) subpopulations could prove essential for the understanding of basic TADC biology and for envisaging targeted immunotherapies. We demonstrate that multiple mouse *tumors as well as human tumors* harbor ontogenically discrete TADC subsets. Monocyte-derived TADC are prominent in tumor antigen processing, but lack strong T-cell stimulatory capacity due to NO-mediated immunosuppression. Pre-cDC-derived TADC have lymph node migratory potential, whereby cDC1 efficiently activate $CD8^+$ T cells and cDC2 induce Th17 cells. Mice vaccinated with cDC2 displayed a reduced tumor growth accompanied by a reprogramming of pro-tumoral TAM and a reduction of MDSC, while cDC1 vaccination strongly induces anti-tumor CTL. Our data might prove important for therapeutic interventions targeted at specific TADC subsets or their precursors.

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22 The CCL8/CCR8 axis maintains an immunosuppressive tumor microenvironment

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Tumors are highly infiltrated with tumor-associated macrophages (TAMs), which have been shown to play roles in invasion, angiogenesis, metastasis and immunosuppression. Thus, it seems appealing to target these cells by eliminating them or reprogramming their functions for therapeutic purposes. Previously, two phenotypically and functionally distinct TAM subsets were identified, namely MHC-II^{lo} and MHC-II^{hi} TAMs, with the MHC-II^{lo} TAMs being more angiogenic and immunosuppressive. The importance of chemokine pathways has been studied extensively in cancer, however, a detailed study exploring chemokine production by the different TAM subsets and its functional implication in tumor progression is largely lacking.
In this study, we show that MHC-II^{lo} and MHC-II^{hi} TAM subsets possess distinct chemokine profiles with many similarities among different mouse tumor models. We found that the chemokine CCL8 is produced primarily by MHC-II^{lo} TAMs in 3LL-R lung carcinoma as well as in 4T1 and TS/A breast carcinomas. CCL8 was found to be induced both by hypoxia and the Th2 cytokines IL-4 and IL-10 in mouse macrophages *in vitro.* To examine the importance of CCL8 production *in vivo*, we assessed tumor growth in mice lacking its receptor, CCR8. We found that 3LL-R lung carcinoma growth was attenuated in CCR8-/- mice accompanied by a pro-inflammatory phenotype of TAMs and tumor-infiltrating T cells. In addition, CCR8 deletion decreased the immunosuppressive activity of TAMs. The attenuated tumor growth in CCR8-/- mice could be reverted by depleting tumor-infiltrating $CD4^+$ and $CD8^+$ T cells demonstrating their pivotal role in the enhanced anti-tumoral immune response.
Overall, these results suggest that the CCL8/CCR8 chemokine axis plays a crucial role in maintaining an immunosuppressive tumor microenvironment acting both on macrophages and T cells. Hence this pathway represents a potential target for tumor immunotherapy.

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23 Myeloid sources of TNF-α promote T-cell response against *Listeria monocytogenes* through modulation of neonatal preCD8α/Clec9A+ dendritic cells.

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Infection by Listeria monocytogenes (Lm) causes serious sepsis and meningitis leading to mortality in neonates. We explored the ability of $CD11c^{high}$ lineage DCs to induce $CD8^+$ T-cell immune protection against Lm in mice before 7 days of life, a period symbolized by the absence of murine IL-12p70-producing $CD11c^{high}CD8\alpha^+$ dendritic cells (DCs). We characterized a dominant functional Batf3-dependent precursor of $CD11c^{high}$ DCs that are $Clec9A^+CD205^+CD24^+$ but $CD8\alpha^-$ at 3 days of life. After Lm-OVA infection, these pre-DCs that cross-present Ag, display the unique abilities to produce high levels of IL-12p40 (no IL-12p70 nor IL-23) which enhance OVA-specific $CD8^+$ T cell response and regulatory IL-10 which limit OVA-specific $CD8^+$ T cell response. In parallel, we identified a myeloid source of TNFα upon Lm infection in early life. Using $TNF^{Flox}LysM^{Cre}$, we showed that this myeloid TNFα is involved in neonatal protection against Lm and in the generation of Lm-specific $CD8^+$ T cells responses. This function of TNFα was at least partially due to a reversion of the IL-12p40/IL-10 balance secreted by pre-DCs. Targeting these neonatal pre-DCs for the first time with a single treatment of anti-Clec9A-OVA antibody in combination with DC activating agent such as poly(I:C) increased the protection against later exposure to the Lm-OVA strain. Poly(I:C) has been shown to induce IL-12p40 production but not IL-10 by neonatal pre-DCs.
In conclusion, we characterized in early life a biological active precursor of $Clec9A^+$ DC lineage that represents a valuable target to augment memory immune responses to vaccines that could be optimized by promoting its IL-12p40 production and limiting its IL-10 production. This discovery opens new Clec9A antigen targeting strategies for human vaccine development.
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24 MODULATION OF RESIDENT MACROPHAGES IN THE MUSCULARIS EXTERNA BY STIMULATION OF ENTERIC NEURONS

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Background: Neuroimmune mechanisms contribute to the maintenance of intestinal immune homeostasis. We showed that electrical stimulation (ES) of the vagus nerve and administration of prucalopride reduced muscular inflammation and improved intestinal transit in a murine model of postoperative ileus, most likely by dampening the activation of resident muscular macrophages (MF). As the vagus nerve is not directly interacting with these MF but synapses with the enteric nervous system, we hypothesize that the resident MF are modulated by enteric neurons (EN).
Methods: MF activation was assessed by responses to ATP (100µM) using *in situ* live calcium imaging of the muscularis externa of adult CX3CR1.GFP/wt C57BL/6 mice. Responses are represented as mean difference in ΔF/F0 (ΔΔF/F0) before and after ES (20Hz, 5min) or prucalopride (150µM) application. To study the effect of EN on MF phenotype skewing, bone marrow derived macrophages were cultured overnight in the presence/absence of supernatant (SN) of EN cultures (14div, containing 80±3% $ChAT^+$ neurons) or co-cultured with freshly isolated myenteric plexus (MP) ganglia (n=300). Subsequently, MF were stimulated with lipopolysaccharide (100ng/ml) for 12h and cytokine release in the SN was assessed. Results are represented as mean±SEM. One-way ANOVA with Tukey's multiple comparisons test was applied for statistical analysis. Alpha value<0.05 was considered statistically significant.
Results: Activation of MFs by ATP was significantly reduced by ES (ΔΔF/F0: -1.042±0.168; n=57 MFs/5 mice) and prucalopride treatment (ΔΔF/F0: -1.042±0.1223; n=87/4) compared to Krebs' solution (ΔΔF/F0: -0.0357±0.1925; n=100/7). In the presence of TTX, ES failed to significantly reduce MF activation (ΔΔF/F0: -0.5341±0.1297; n=69/3) compared to Krebs' solution, indicating the involvement of neurons. In addition, MF cultured in the presence of SN of EN or co-cultured with MP ganglia released more IL-10 (835±34pg/ml and 1016±24pg/ml resp.) compared to MF cultured in unconditioned medium (287±23pg/ml and 777±46pg/ml resp.). No difference in TNF-α was detected (EN: 3071±77pg/ml vs unconditioned: 3247±276pg/ml and MP ganglia: 3089±70pg/ml vs 3278±11pg/ml).
Conclusion: Our data show that activation of ENs dampens ATP-induced MF activation and induces a shift towards a more tolerogenic phenotype. These data indicate a neuromodulatory role for the enteric nervous system most likely contributing to the tolerogenic phenotype of resident muscularis MFs.
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25 M-CSF and GM-CSF receptor signaling differentially affect the maturation of monocytes and the polarization of macrophage subsets in the tumor microenvironment

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Tumors contain a heterogeneous myeloid fraction, encompassing discrete MHC-II^{hi} and MHC-II^{lo} tumor-associated macrophage (TAM) subpopulations, which originate from Ly6C^{hi} monocytes. However, the mechanisms regulating the numbers and the phenotype of distinct TAM subsets remain unknown.
Here, we showed that treatment of tumor-bearing mice with a blocking anti-M-CSFR mAb resulted in a reduction of mature TAM in different tumor models, by impairing the recruitment, extravasation, proliferation and maturation of their Ly6C^{hi} monocytic precursors. At the macrophage level, M-CSFR signaling blockade caused a shift in the MHC-II^{lo}/MHC-II^{hi} TAM balance in favor of the latter due to a preferential differentiation of Ly6C^{hi} monocytes towards MHC-II^{hi} TAM. In addition, M-CSFR signaling appeared to be crucial in shaping the MHC-II^{lo} TAM phenotype, since genes, proteins and functions associated with MHC-II^{lo} TAM were downregulated upon M-CSFR blockade. Conversely, GM-CSFR had no role in the recruitment or intratumoral differentiation of Ly6C^{hi} monocytes, since the mononuclear tumor infiltrate and relative abundance of TAM subsets was unaltered in GM-CSFR-deficient mice. However, GM-CSFR signaling played an important role in the fine-tuning of the MHC-II^{hi} phenotype.
Overall, our data uncover the multifaceted and opposing roles of M-CSFR and GM-CSFR signaling in determining the phenotype of macrophage subsets in tumors.

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26 *Toxoplasma gondii* elicits fetal Vγ9Vδ2 T cell responses *in utero*

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The Vγ9Vδ2 T cell subset is the main γδ T cell population in adult peripheral blood. Their semi-invariant T cell receptor (TCR) reacts towards microbe-and host-derived phosphorylated prenyl metabolites ('phosphoantigens'), the most potent being the microbe-derived HMB-PP. We have recently shown that this T cell subset is the main γδ T cell subset in fetal blood and is already programmed at the level of their TCR and their function, suggesting that they could play an important role upon infections *in utero*. Here we explored this role by investigating the response of Vγ9Vδ2 T cells towards congenital infection with the HMB-PP-producing parasite *Toxoplasma gondii*. In congenitally-infected infants (peripheral blood sample within first week after birth), Vγ9Vδ2 T cells, but not other γδ T cell subsets, where highly expanded compared to age-matched controls. This was associated with differentiation of the Vγ9Vδ2 T cells and increased expression of activation marker. Thus congenital infection with a phosphoantigen-producing pathogen induces a clear and specific response in Vγ9Vδ2 T cells *in utero*. Currently we are investigating this response in more detail at the molecular level and we plan to follow these infants after birth in order assess the longer-term effect of congenital infection on the γδ T cell repertoire.

27 A respiratory gammaherpesvirus infection protects against allergic asthma through alveolar recruitment of regulatory monocytes

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The hygiene hypothesis postulates that augmentation of allergic diseases in developed countries could be linked to reduced exposure to infections during childhood. Surprisingly, the potential protective role of herpesvirus infections against allergy development has never been addressed in details. Here, we investigated how a gammaherpesvirus infection affects the subsequent development of allergic asthma. Our results demonstrate that respiratory infection by Murid herpesvirus 4 (MuHV-4) inhibits the development of House Dust Mites (HDM)-induced asthma by modulating functions of lung innate immune cells. Specifically, MuHV-4 infection induced the replacement of alveolar macrophages (AMs) by monocytes educated to display markers associated with regulatory functions. These monocyte-derived AMs blocked the ability of dendritic cells to trigger a Th2 response against HDM. Collectively, our results reveal that replacement of embryonic AMs by regulatory monocytes is a major mechanism underlying the long-term training of the lung immunity after infections.
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28 Beta cell stress and activation of immune cells in models of type 1 diabetes

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Macrophages have a specialized molecular machinery to sense and respond to apoptotic cells, but signals from the dying cell itself are also important to determine whether the outcome will be immunogenic or tolerogenic. We explored whether interactions between dying beta-cells and macrophages in a mouse model of type 1 diabetes (T1D) are different as compared to a homeostatic condition, and also evaluated whether the type of beta-cell stress affects immune responses during T1D.
MIN6 cells were exposed to different stress-induced agents, namely thapsigargin (5 µM); cytokines (hIL-1β/mIFN-γ/mTNF-α, 50/250/1000 U/ml); cycloheximide (5 µg/ml) or staurosporin (5µM). The different treatments resulted in approximately 60% beta-cell apoptosis. Stressed or control (non-treated) beta-cells were co-cultured with peritoneal macrophages from 8-week old C57BL/6 or NOD mice.
NOD macrophages exhibited the same ability to clear apoptotic beta-cells as from the C57BL/6 macrophages (n=15, $p<0.001$) irrespective of the mode of apoptosis. Interestingly, NOD macrophages produced higher levels of TNF-α (n= 14, $p<0.05$) and lower levels of IL-10 (n=14, $p<0.05$) after interacting with beta-cells, indicating a hyperinflammatory phenotype. Staurosporine-induced beta-cell death led to a remarkable lower cytokine production by macrophages. Expression of scavenging receptors CD204, CD36 and TIM4 are higher, while expression of the mannose receptor CD206 is lower expressed on the membrane of NOD macrophages compared to C57BL/6 macrophages (n= 9-12, $p< 0.01$). The inhibitory molecule PDL1 is higher expressed after cytokines or CHX treatment on the membrane of NOD macrophages compared to C57BL/6 macrophages (n= 9-12, $p< 0.01$). In particular, the type of beta cell stress will affect the surface expression profile of the macrophage. The expression of CD204, CD36, SIRPα are lower, but the expression of activating molecules CD80 and CD86 expression are higher after thapsigargin and/or staurosporine treatment, compared to other stress signals.
Clearing of apoptotic cells by C57BL/6 or NOD macrophages (n=3) dampened T-cell activation/proliferation, an effect that was most marked after thapsigargin and staurosporin treatment.
These observations suggest that macrophages obtained from diabetes-prone NOD mice express a more pro-inflammatory profile when confronted with dying beta-cells, as compared with macrophages from control mice. Additionally, the type of beta-cell stress is important for the contribution of immunogenic response of macrophages during T1D.

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29 Cytokine expression in chickens infected with H9N2 influenza viruses with different pathogenicities

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H9N2 is a low pathogenic avian influenza virus (LPAI) which can cause significant economic losses in poultry industry via drop in egg production and increases in mortality among commercial domestic poultry and wild birds.
In chickens, H9N2 viruses display the variable morbidity and mortality. The coinfection or environmental factors can play a role as in the field, H9N2 infection was shown to be exacerbated by the secondary virus or bacteria. In addition to those external conditions, the differences in outcome of the disease might also due to the internal factors: intrinsic replication properties of the viruses; host immune response against H9N2 infection via cytokine and interferon production.
To evaluate these effects on disease outcome, two H9N2 virus models based on H9N2 clones, which were purified either on chicken embryo fibroblast (CEF) or Madin-Darby canine kidney (MDCK) cells, were built up. The replication ability and cytokine induction these clones were tested by RRT-PCR in various organs.
The results suggested the higher pathogenicity of MDCK clone and its stronger induction of proinflammatory cytokines. In contrast, more antiviral cytokines were expressed by CEF clone with less pathogenicity. Some differences in their genomes were also revealed. Taken together, different cell cultures could have impact on H9N2 clone selection and the cytokine expression by the host might associate with the viral pathogenicity.
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30 ROLE OF NEUTROPHILS IN THE LIVER ISCHEMIA REPERFUSION INJURY

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INTRODUCTION: The interruption of the blood flow (ischemia), with consequent lack of oxygen in an inherent phenomenon during surgical procedures, such as during removal of liver tumors and transplantation. Once the blood flow is restored (reperfusion), there is an increased injury initiated by ischemia. This phenomenon is known as liver ischemia-reperfusion (IR). Neutrophils are central players for the pathophysiologic changes during hepatic IR injury. They migrate from the circulation to the liver through a gradient of inflammatory chemokines. These include CXCL1/KC and CXCL2/MIP-2, two murine analogues of human CXCL8 that are recognized by CXCR1 and CXCR2 receptors expressed on neutrophils. Among several neutrophil-related attractants, CXC chemokine receptor (CXCR2)-binding chemokines are studied in different inflammatory contexts.
OBJECTIVES: We wish to investigate whether the blockade of chemokine receptor CXCR2 may have a beneficial role in the liver inflammation and damage following IR.
METHODS: C57/BL6 mice were subjected to 60 minutes of no-flow ischemia followed by reperfusion for up to 24h. The CXCR2 inhibitor Reparexin (30mg/kg) was given 15 minutes before the reperfusion and every two hours later. Considering that few studies have examined the recruitment of neutrophils in real-time *in vivo*, we also evaluated this effect using Lysm-eGFP mice and intravital confocal microscopy (IVM) to observe how neutrophils behave during the injury development.
RESULTS: The most prominent IR-induced liver injury and inflammation was detected 6h hours after reperfusion, as observed by the high levels of ALT in serum and huge neutrophil infiltration in the liver, respectively. The liver injury was followed by high expression of cytokines (TNFα, IL-6) and chemokines (CXCL1 and CXCL2). Reparexin treatment significantly reduced liver injury by reducing ALT and inflammation by preventing neutrophil influx (liver and lungs). The Lysm-eGFP mice subjected to IR showed a huge infiltration of neutrophils into the liver. By evaluating the behavior of these cells, it was observed that, especially 6h after reperfusion, neutrophils move longer distances and faster. In addition, after 6h, the neutrophils are bigger and many clusters of neutrophils are formed. Moreover, they become more elongated cells with an increased axis, which is a signal of cell activation. The Reparexin treatment was able to decrease the neutrophil influx, and changed the behavior of these cells. With Reparexin treatment, the neutrophils move shorter distances, and Reparexin was able to reduce the size of these cells.
CONCLUSIONS: CXC chemokines, acting on CXCR2, have a important role during the livre IR injury. Thus, drugs, such Reparexin, developed to block the function of CXCR2 receptors, may be effective at preventing reperfusion injury in relevant clinical situations.
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31 Programming *in utero* and post-natal acquisition of effector functions of human Vγ9Vδ2 T cells

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γδ T cells are unconventional lymphocytes sharing attributes of both innate and adaptive immunity. The Vγ9Vδ2 T cells, which react towards microbe- and host-derived phosphorylated prenyl metabolites (phosphoantigens), are the main γδ T cell population in adult human peripheral blood. Their main effector functions include IFN-γ production and killing of infected and transformed cells.
The aim of this study is to understand at the molecular level the programming *in utero* versus the post-natal acquisition of a series of effector functions of Vγ9Vδ2 T cells. We analysed Vγ9Vδ2 T cells derived from new-borns (cord blood), 10 week-old infants vaccinated or not at birth with the phosphoantigen-containing tuberculosis vaccine Mycobacterium bovis Bacillus Calmette-Guérin (BCG), and from adult blood.
At birth, Vγ9Vδ2 T cells expressed no perforin or granzyme B (as determined by flow cytometry) whereas at 10 weeks they did greatly. In contrast, IFN-γ production (after polyclonal stimulation) within neonatal Vγ9Vδ2 T cells was already high at birth and did not change significantly at 10 weeks of age while Granzyme A, expressed at birth, was significantly increased at 10 weeks. Surprisingly, BCG vaccination at birth had no eminent effect on the effector molecules investigated.
Overall, these data indicate that environmental exposure and/or aging for 10 weeks has a potent influence on the expression of particular effector molecules within Vγ9Vδ2 T cells. At the same time, other effector functions are already programmed *in utero*. We are currently investigating the molecular mechanisms involved in the programming *in utero* and post-natal acquisition of the effector functions of Vγ9Vδ2 T cells.

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32 Hemozoin is pathogenic in experimental malaria-associated acute respiratory distress syndrome

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Malaria is a major health hazard and causes approximately 650 000 deaths each year. One of the lethal complications is malaria-associated acute respiratory distress syndrome (MA-ARDS), occurring in 2-20% of adult patients with malaria complications. MA-ARDS results in poor prognosis with a lethality rate of 80%, despite antimalarial treatment. Our lab has developed a MA-ARDS mouse model, using *P. berghei* NK65 infections in C57BL/6J mice. Infections in these mice result in similar histological features as seen in MA-ARDS patients, such as leukocyte infiltration, hyaline membrane formation, pulmonary edema, microhemorrhages and hemozoin deposition. Hemozoin, a detoxification product of heme produced during hemoglobin digestion by the parasite, has immune modulating properties. To prove the pathogenic role of Hz in MA-ARDS, we generated transgenic parasites deficient for specific hemoglobinases. Dipeptidyl aminopeptidase 1 (DPAP1) knockout parasites have a slower growth rate but normal hemozoin production and were as virulent as wild type (WT) parasites. In contrast, the pulmonary pathology was significantly attenuated with plasmepsin 4 (PM4) knockout parasites, which produce less hemozoin and have a similar growth rate as DPAP1 knockout parasites. Remarkably, this attenuated pathology was not paralleled by a lower inflammatory response in the lungs, as the pulmonary mRNA expression of TNF, IL10, IP10, MCP1 and MHCII was similarly increased upon infection with PM4 KO, DPAP1 KO and WT parasites. The decrease in edema was paralleled by a lower pulmonary secretion of VEGF, a factor associated with acute lung injury in malaria. These data show unambiguously and for the first time that hemozoin is a crucial pathogenic factor in MA-ARDS. Detailed characterizations of the involved pathways are ongoing.

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33 Role of AMP-activated Kinase, a key metabolic enzyme, in the homeostasis of highly proliferative T helper cells

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CD4-expressing T helper lymphocytes (Th cells) play an important regulatory role during an immune response. After antigen encounter, naïve T cells proliferate, acquire effector functions and eventually differentiate into memory cells. During continuous (chronic) antigen stimulation T cells can also become exhausted (i.e. lose their functions). At each step of these developmental stages, CD4 Th cells display a particular energy metabolism and it has been shown that fine-tuning of T cells metabolism might govern their differentiation.
The AMP-activated kinase (AMPK) is a cellular sensor that protects T cells against metabolic stress and promotes ATP production through fatty acid uptake/oxidation and mitochondrial biogenesis. However, the role of AMPK in T lymphocytes metabolism readjustments is still ill defined. Hence, we propose a role for this enzyme in the control of the metabolic switch occurring during situations of high energy demand.
In order to characterize the role of AMPK in situations of high energy demand we evaluated the consequences of the depletion of AMPK on the homeostasis of T cells during graft versus host disease (GVHD), a model of chronic antigen stimulation. WT or AMPK ΔT cells from C57BL/6 donor mice were injected into irradiated, allogenic BALB/C hosts. Our present results show that expression of AMPK in transferred T cells accelerates the kinetic of the GVHD.
As it has been shown that alloreactive T cells reprogram their metabolism during GVHD and that AMPK play key role in metabolism regulation we decided to further investigate the relationships between AMPK and cell metabolism in an in vitro model allowing us to differentiate naïve $CD4^+$T cells into effector and memory-like Th1 cells; two T cell subsets known to rely on different metabolisms, glycolysis and oxidative phosphorylation respectively. Experiments comparing the differentiation program of WT and AMPK ΔTh cells revealed a role for this enzyme in regulating oxidative metabolism in memory T lymphocytes. Memory-like AMPK ΔTh cells express a reduced mitochondrial potential along with a decrease in the oxygen consumption rate, suggesting that AMPK may influence the metabolic shift observed during in vitro differentiation. Moreover, AMPK Δ memory-like Th cells had a slower secondary proliferative rate in response to alloantigens in vitro, suggesting that AMPK is required for an optimal sustained secondary proliferation.
We conclude that AMPK promotes the mitochondrial activity of in vitro-differentiated memory T cells, optimal sustained secondary proliferation and GVHD in vivo. Our future experiments will be aimed at identifying the molecular mechanisms linking AMPK, mitochondrion, metabolism and T cells homeostasis during chronic stimulation.
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34 IL-4Rα-dependent macrophage responses in the liver during murine schistosomiasis

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Alternatively activated macrophages (aaMφ) accumulate around the eggs of the helminth parasite *Schistosoma mansoni* within the context of a strong Th2 response. IL-4Rα-dependent aaMφ have been implicated in host protection through modulation of inflammation. However, little is known on the dynamic of the macrophage responses in the liver after *S. mansoni* infection and the contribution of IL-4Rα. Here, we used mice specifically deficient for IL-4Rα in myeloid cells ($LysM^{cre}Il4ra^{-/lox}$). First, cross between $LysM^{cre}$ and Rosa-tdRFP mice identified successful cre-mediated excision in neutrophils and macrophages from different origins. Cre-mediated excision in liver MΦ was successful at the steady state but reduced at 6 weeks post-infection (pi). These results supported recent data attesting for ineffective *Il4ra* deletion in liver MΦ of *S. mansoni* infected $LysM^{cre}Il4ra^{-/lox}$ mice. Next, we further investigated the MΦ populations in the liver using flow cytometry at steady-state and after 4, 6 and 8 weeks pi. We observed a significantly increased number of leukocytes at 6 and 8 weeks pi, including eosinophils and $Ly6C^{hi}$ recruited monocytes, which were significantly higher in $LysM^{cre}Il4ra^{-/lox}$ mice. We observed that $MerTK^+CD64^+$ resident Küpffer cells (KCs) were reducing in term of numbers over the course of the infection independently of IL-4Rα signalling. While KCs were reduced, we observed the recruitment of $Ly6C^{hi}$ monocytes in the liver from 6 weeks pi and strong proliferation of this population at week 8. Interestingly, while $Ly6C^{hi}$ acquired CD64 expression, $CD11b^{low}$ KCs seemed to be replaced from 8 weeks pi by a $CD11b^{high}$ Mφ population, probably arising from recruited monocytes. Hepatic Mφ were alternatively activated from week 6 pi as attested by Ym1 overexpression, which was significantly reduced in $LysM^{cre}Il4ra^{-/lox}$ mice. These results suggest that recruited monocytes differentiate into aaMφ at the cost of resident KCs independently of IL-4Rα. Future work should determine whether differentiation of $Ly6C^{hi}$ monocytes into macrophages exert protective functions against *S. mansoni* egg-induced inflammation in the liver.
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35 Bacterial CpG-DNA prevents asthma by expanding lung interstitial regulatory macrophages from local and splenic reservoir monocytes

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Living in a microbe-rich environment reduces the risk of developing asthma. Unmethylated CpG-DNA from bacteria is particularly efficient in reproducing these protective effects when administered to humans or animals, suggesting a major contribution of CpG-DNA to microbe-induced asthma resistance. However, how CpG-DNA confers protection remains elusive. Here, we show, in mice, that CpG-DNA has the unique ability to expand lung interstitial regulatory macrophages (IM). Experiments in clodronate-treated, chimeric, parabiotic and splenectomized wild-type mice and mice lacking CCR2, a receptor required for classical monocyte mobilization from the bone marrow, unexpectedly demonstrated that CpG-DNA-induced IM (IM_{CpG}) developed from monocytes residing in the lung or recruited from the spleen independently of CCR2. Furthermore, IM_{CpG} produced much higher levels of the immunosuppressive cytokine interleukin-10 than their steady-state counterparts and recapitulated the protective effects of CpG-DNA when adoptively transferred to mice before allergen sensitization or challenge. By identifying CCR2-independent pulmonary and splenic reservoir monocytes as CpG-DNA-responsive precursors for hypersuppressive IM, our study reveals a possible mechanism by which exposure to the environmental microbiome, or treatment with synthetic CpG-DNA, protects against asthma.
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36 Scriptaid and SBHA have no effect on functional recovery after spinal cord injury

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After spinal cord injury (SCI), excessive inflammation and glial scar formation are important causes of secondary damage to the healthy tissue surrounding the lesion and thus exacerbate the underlying neurodegenerative events (1). During this inflammatory response monocyte-derived macrophages are major players in modulating functional recovery after SCI (2). Pro-inflammatory M1 macrophages dominate the spinal cord after injury and exert detrimental effects by secreting multiple pro-inflammatory and neurotoxic factors and by stimulating the formation of the inhibitory fibrotic scar. In contrast, anti-inflammatory M2 macrophages may contribute to regeneration after SCI (3). In this context, epigenetic modulation of gene expression is emerging as a new possibility to overcome regenerative failure after SCI (4,5). We hypothesized that the histone deacetylase inhibitors (HDACi) induce a beneficial M2 immune switch, resulting in improved functional recovery after SCI. Our *in vitro* results show that the HDAC inhibitors scriptaid and SBHA are able to reduce IL-6 and NO production in bone marrow derived macrophages upon LPS treatment, which points towards an impaired M1 polarization. However, *in vivo,* scriptaid and SBHA showed no improved functional recovery after SCI.

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37 MACROPHAGE-SPECIFIC ADAM17-DEFICIENCY DOES NOT PROMOTE RECOVERY AFTER SPINAL CORD INJURY

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After spinal cord injury (SCI) monocyte-derived macrophages infiltrate the spinal cord within 3 days and contribute to the excessive secondary injury damage. Previous results from our group indicated that 'A disintegrin and metalloproteinase 17' (ADAM17) substantially modulates SCI outcome. Therefore, we investigated here whether ADAM17 deficiency on macrophages influences the functional outcome after SCI. We show that bone-marrow derived macrophages (BMDM) of hypomorphic ADAM17 mice had impaired capacity to produce soluble TNF-α even after LPS stimulation. Besides, we demonstrate that ADAM17 deficiency did not affect the phenotype of BMDM upon LPS or IL-4 stimulation. Furthermore, we investigated the role of ADAM17 on macrophages in SCI using the ADAM17$^{flox/flox}$-LysM Cre mouse model with a knockout of ADAM17 in cells of myeloid origin. In a preliminary in vivo study, ADAM17$^{flox/flox}$-LysM Cre$^{+/-}$ mice did not show improved functional recovery after T-cut hemisection SCI compared to control mice based on the 9-point Basso Mouse Scale test. These data indicate that ADAM17 on macrophages does not affect functional recovery after SCI.

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38 CCR2-monocytes are essential for the resolution of inflammation and tissue repair in colitis

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Background: Monocyte recruitment in the gut wall via C-C chemokine receptor 2 (CCR2) is a major hallmark in the pathogenesis of inflammatory bowel disease (IBD). Classically, monocytes are considered as the main mediators of tissue damage during colitis. However, monocyte-derived macrophages may be essential for the resolution of inflammation. In the current study, we aim to identify the function of monocytes during colitis and to define whether monocytes play a role in the induction of inflammation and/or are crucial for tissue repair.
Methods: To study the contribution of monocytes, acute colitis was induced in wild type (WT, C57BL/6) and CCR2$^{-/-}$ mice by 2.5% dextran sodium sulfate (DSS) in drinking water for 5 days. Disease progression was assessed via a standardized disease activity index (DAI) including body weight loss, stool consistency and blood in the feces. To study the role of monocytes during chronic colitis, mice were subjected to 3 cycles of 2% DSS. Monocyte and neutrophil recruitment and macrophage differentiation in the colon were assessed by flow cytometry and cell sorting. Histological evaluation of colonic tissues was performed.
Results: During acute DSS colitis (day 5), CCR2$^{-/-}$ mice displayed a reduced DAI and body weight loss as compared to WT mice indicating that inhibiting monocyte recruitment reduces tissue inflammation. On the contrary, during the recovery phase (day 10 to 14), CCR2$^{-/-}$ mice showed an increased DAI and body weight loss compared to WT mice (24.5% vs 8.6%). Interestingly, the induction of chronic colitis confirmed that CCR2^{+} monocytes are crucial for the resolution of inflammation. During the 1st cycle of DSS, body weight loss in WT mice (6.8%) was 10-fold higher than in CCR2$^{-/-}$ animals (0.67%). Interestingly from day 10 onward, CCR2$^{-/-}$ mice continued to lose weight. During the 2nd and 3rd subsequent DSS cycles, CCR2$^{-/-}$ mice failed to recover body weight and presented increased disease severity compared to WT mice. This phenotype in CCR2$^{-/-}$ mice correlated with increased colonic tissue alterations such as epithelial erosion, cell infiltration and fibrosis. Flow cytometry analysis showed increased accumulation of ROS-producing neutrophils in CCR2$^{-/-}$ mice (WT:9.5x10^{5}; CCR2$^{-/-}$:37.6x10^{5}; $p<0.05$), while immune infiltrate in WT mice mainly consisted of differentiated MHCII^{+} macrophages expressing typical M2 markers such as IL-10, CD163, Lyve1 and Stab1.
Conclusion: Our data demonstrate a dual role of monocytes during colitis, inducing the inflammatory response during the first phase, and playing a crucial role in the resolution of inflammation and tissue repair at a later stage. Further understanding of the mechanisms leading to immune-regulation and mucosal repair is vitally important to improve treatment for patients suffering from IBD.

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39 High Density Lipoproteins exert pro-inflammatory effects on macrophages via passive cholesterol depletion and PKC/NF-κB/STAT1 signalling

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Membrane cholesterol is known to modulate a variety of cell signalling pathways and functions. While cholesterol depletion by High-Density-Lipoproteins (HDL) has potent anti-inflammatory effects in various cell types, its effects on inflammatory responses in macrophages remain ill defined.
Pre-incubation of human and murine macrophages *in vitro* with human reconstituted (apolipoproteinA-I/phosphatidylcholine) or native HDL significantly decreased LPS-induced anti-inflammatory IL-10 production, while the opposite was observed for the pro-inflammatory mediators IL-12 and TNF. We show that these effects are mediated by passive cholesterol depletion and lipid raft disruption, without involvement of ABCA1, ABCG1, SR-BI or CD36. These pro-inflammatory effects are confirmed *in vivo* in peritoneal macrophages from ApoA-I transgenic mice, which have high circulating HDL levels. Native and reconstituted HDL enhances Toll-Like-Receptor-induced signalling by activating protein kinase C (PKC), since inhibition of PKC ablated the observed HDL effects. Using macrophages from NF-κB luciferase mice, we observed that HDL induces NF-κB activation. Using specific inhibitors and/or knock-out mice, we show that the observed HDL effects are independent of IKK, NIK and CKII. Furthermore, we observed that STAT1 is involved in the pro-inflammatory HDL effects on IL-10 and IL-12.
HDL exerts pro-inflammatory effects on macrophages via passive cholesterol depletion by activation of PKC/NF-kB/STAT1. These pro-inflammatory activities on macrophages could at least partly underlie the disappointing therapeutic potential of HDL raising therapy in current cardiovascular clinical trials.
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40 Human foetal haematopoietic stem and progenitor cells (HSPC) generate invariant γδ T cells

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Although there are common characteristics among γδ T cells, it is clear that γδ T cells do not represent a homogenous population of cells with a single physiological role. We have shown that γδ T cells expressing (semi)invariant germline-encoded T cell receptors (TCR) dominate the γδ T cell repertoire in human foetal life. We hypothesized that these 'early' γδ T cell subsets are made by specific haematopoietic stem and precursor cells (HSPC) present at this period of life, and thus that human γδ T cell development is layered according to age.
In an *in vitro* T cell development system, human foetal (blood and thymus, gestation range 14-30 weeks) HSPC-derived γδ T cells and post-natal blood/thymus HSPC-derived γδ T cells were enriched for different γ and δ chain combinations as determined by flow cytometry. A more detailed analysis of the complementary determining region-3 (CDR3)γ and CDR3δ repertoire by high-throughput sequencing indicated that foetal HSPC-derived γδ T cells, in contrast to post-natal HSPC-derived γδ T cells, were highly enriched for particular invariant germline-encoded CDR3 sequences. Importantly, some of these sequences were found *in vivo*.
These data indicate that HSPC in different periods during human development can generate different types of γδ T cells. We are currently investigating the function of these foetal HSPC-derived γδ T cells.
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41 IL-15 stimulated NK cells can kill both pancreatic cancer and stellate cells

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) is the 4th leading cause of cancer-related death in Western countries with a 5-year survival rate below 5%. Within the tumour microenvironment, a strong desmoplastic reaction orchestrated by activated pancreatic stellate cells (PSC) occurs and is held responsible for acting both as functional and mechanical shield. Tackling this stromal shield is needed to overcome treatment resistance. Since conventional therapies have limited effects, we investigated innate immunotherapy for PDAC, more specifically the possibility to kill pancreatic cancer cells (PCC) as well as PSC with IL-15 activated natural killer (NK) cells.
Materials and methods: Peripheral blood NK cells were purified from healthy controls and stimulated overnight with recombinant human IL-15. The effect of IL-15 stimulation on the surface expression of several NK cell receptors was evaluated. NK cell-mediated cytotoxicity against three PCC lines and three PSC lines was measured using a 4h flow cytometric annexin V/propidium iodide assay. The impact of cell-to-cell contact and soluble mechanisms on NK cell-mediated killing were assessed using a transwell system and neutralizing antibodies, respectively. Finally, the cytotoxic potential of NK cells towards PSC, both isolated from PDAC patients, was tested in an autologous setting.
Results: IL-15-activated NK cells have the capacity to significantly kill PCC and PSC (9-35% and 20-50%, respectively), as compared to resting peripheral blood NK cells, in a contact-dependent manner. This is also the case in the autologous setting in 3 out of 4 patients. IL-15 induces significant upregulation of TIM-3 and NKG2D. Our results regarding the unravelling of the mechanism of IL-15-activated NK cell cytotoxicity, point towards involvement of these two receptors.
Discussion: We are the first to demonstrate that both PCC and PSC can be killed *in vitro* by IL-15-stimulated NK cells. The fact that this principle has been proven in 3 out of 4 patients stresses the therapeutic potential of IL-15 in the treatment of pancreatic cancer.

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42 Mitochondrial dysfunction prevents repolarization of inflammatory macrophages

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Macrophages are innate immune cells that adopt diverse activation states in response to their microenvironment. Editing macrophage activation is of high interest to dampen inflammatory diseases by promoting the repolarization of inflammatory (M1) macrophages to anti-inflammatory (M2) macrophages. Here, we found that mouse and human M1 macrophages completely failed to convert into M2 cells upon IL-4 exposure *in vitro* and *in vivo*. In sharp contrast, M2 macrophages were more plastic and readily repolarized into an inflammatory M1 state. We identified M1-associated inhibition of mitochondrial oxidative phosphorylation as the factor responsible for preventing M1→M2 repolarization. Inhibiting nitric oxide production, a key effector molecule in M1 cells, dampened the decline in mitochondrial function and improved metabolic and phenotypic reprogramming to M2 macrophages. Thus, inflammatory macrophage activation blunts oxidative phosphorylation, thereby preventing repolarization. Therapeutically restoring mitochondrial function might be useful to improve the reprogramming of inflammatory macrophages into anti-inflammatory cells to control cardiovascular disease.

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43 Endogenous glucocorticoids do not prevent peripheral inflammation but protect from lethal hypoglycaemia

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Malaria is a global health problem with around 500 000 deaths each year caused by complications that still cannot be treated efficiently. Many lethal complications, such as malaria-associated acute respiratory distress syndrome (MA-ARDS), have a clear inflammatory nature. Anti-inflammatory mediators are crucial to prevent severe inflammation while controlling the infection. Endogenous glucocorticoids (GCs) provide potent anti-inflammatory effects amongst having other homeostatic functions. Plasma GC levels are increased in patients with malaria but their precise role and importance in malaria remain unknown. We investigated the role of endogenous GCs in mouse models of malaria by interfering with adrenal production (adrenalectomy) or peripheral reactivation (11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) knockout) of GCs. Deficiency in 11β -HSD1 did not alter malaria-associated pathology. However, in the absence of adrenal GCs, mice became lethally sick early during infection. This lethality was independent of the parasite-mouse strain combination, could not be attributed to a change in parasitemia and was rescued by treatment with dexamethasone, a synthetic GC. Surprisingly, endogenous GCs did not alter lung or liver inflammation during infection. Instead, our findings point out that the primary role of endogenous GCs in malaria is to prevent lethal hypoglycaemia. This hypoglycaemia in the infected adrenalectomised mice was associated with an increased expression of pro-inflammatory cytokines and chemokines in the brain. Overall, we conclude that adrenal production of GCs does not protect against peripheral inflammation in malaria but is instead crucial to avoid lethal hypoglycaemia.

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44 Cell penetrating peptide based nanoparticles : potent cytotoxic T-cell inducing vaccines

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Cytotoxic T cells (CTLs) have a unique potential to destroy viral infected cells and cancer cells. Vaccination strategies that can elicit CTLs are thus actively pursued within cancer and HIV immunotherapy field. Because induction of CTLs requires antigen to be processed in the cellular cytosol, conventional vaccines relying on the injection of recombinant protein antigens generally fail to evoke CTL responses. To acquire access to the cytosolic route of antigen presentation, we chose cell penetrating peptides (CPPs) as carriers for an antigenic cargo. CPPs are small peptide sequences inspired by viral protein which is used to cross cellular membranes. Here, two different approaches were executed. In the first approach, we used cationic, amphipathic CPP to deliver antigenic information to immune system in the format of in-vitro transcribed messenger RNA (mRNA) encoding model antigen ovalbumin. Through its cationic residues, CPP condensed mRNA into nanoparticles that allowed antigen expression inside the cytosol of dendritic cells. These CPP-mRNA nanoparticles were superior in evoking CTLs when compared to conventional lipid based mRNA vaccines. In the next approach, we used CPPs to deliver peptide epitopes to the immune system. Here mRNA used as scaffold for nanoparticle assembly. In this case mRNA did not encode antigen, but activated innate immune responses. Thus, the obtained nanoparticles were extremely potent at inducing CTLs and inhibited tumor growth in pre-clinical murine melanoma model.

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45 A20 critically controls microglia activation in CNS inflammation

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The nuclear factor kappa B (NF-κB) signaling pathway plays a critical role in different cellular processes. Besides the regulation of immune and inflammatory responses, NF-κB is also important in controlling physiological processes, like cell survival, differentiation, and proliferation. Given the central role of NF-κB activation in these processes, a tight regulation of NF-κB-dependent signaling pathways is an absolute requirement. One of the proteins playing a key role in the termination of NF-κB signaling and inflammation is the ubiquitin-editing enzyme A20, as shown by the phenotype of A20 deficient mice which develop multi-organ inflammation leading to premature lethality.
Microglial cells are the resident mononuclear phagocytes of the central nervous system (CNS) and are constantly surveilling the CNS to detect signs of pathogenic invasion or tissue damage. Upon activation they orchestrate an innate immune response and are involved in clearing debris to restore CNS homeostasis. Besides their role as immune sentinels, microglia also support the maintenance of synapses and neurogenesis. Although A20 is only marginally expressed during early stages of microglia development, an increase in expression is seen at later stages, and further raised in adult microglia, suggesting a role for A20 in microglia development. Using a genetic targeting strategy in microglia, we examined the role of A20 in microglia function during CNS development and pathology. Microglia specific deletion of A20 in mice ($A20^{Cx3Cr1KO}$) induces microglial cell proliferation, leading to an increase in the total amount of microglia under physiological conditions. Next, models of neuroinflammation were used to look at the microglia-specific function of A20. Challenging these mice with lipopolysaccharide (LPS), showed that $A20^{Cx3Cr1\text{-}KO}$ mice display severe hypothermia and mortality rate compared to control littermates. Additionally, $A20^{Cx3Cr1\text{-}KO}$ show an exacerbated response upon EAE induction, reflected by faster disease development and increased paralysis. We could show that the increased disease susceptibility of $A20^{Cx3Cr1\text{-}KO}$ mice is caused by the hyperactivation of the Nlrp3 inflammasome specifically in microglia. This hyperactivation leads to enhanced IL-1β secretion and CNS inflammation. Together, these results demonstrate a role for A20 in microglial cells, both in physiological and pathological conditions, and suggest that A20 in microglia is essential to keep inflammatory responses under control.
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46 IMPAIRED INFLAMMATORY SIGNALING IN MACROPHAGES FROM MS PATIENTS

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Multiple sclerosis (MS) is a neurodegenerative autoimmune disease of the central nervous system (CNS) in which macrophages play a crucial role. Apart from being important mediators of CNS inflammation, demyelination, and axonal degeneration, monocyte-derived macrophages (MDMs) are indispensable for CNS repair in MS. In particular, we and others found that myelin uptake skews macrophages towards a less-inflammatory, reparative phenotype by activation of lipid sensing nuclear receptors, such as liver X receptors (LXRs) and peroxisome proliferator-activated receptors (PPARs). In this study we set out to define if and how the inflammatory environment in MS patients changes the phenotype of myelin-phagocytosing MDMs. We show that MDMs from MS patients display a reduced ability to suppress an inflammatory phenotype upon myelin internalization compared to MDMs of healthy individuals. This inflammatory phenotype of myelin-phagocytosing MDMs of MS patients is accompanied by a decreased expression of the nuclear receptor PPARγ. In contrast, a decrease in PPARα, PPARδ, LXRα or LXRβ expression was not observed. By using qPCR and a luciferase ligand binding reporter assay, we further show that myelin potently ligates and activates PPARγ. Interestingly, the decline in PPARγ expression could be mimicked by exposing MDMs of healthy individuals to inflammatory stimuli typically associated with MS. Taken together these findings indicate that in MS the protective phenotype of myelin-phagocytosing macrophages is impaired, and that a reduced expression of PPARγ may underlie this compromised phenotype.
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47 Adipose tissue macrophages induce hepatic neutrophil recruitment and macrophage accumulation in mice

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Background & aims
Obesity is a risk factor for non-alcoholic steatohepatitis (NASH). This risk has been attributed to visceral adipose tissue (vAT) expansion associated with increased pro-inflammatory mediators. Accumulation of $CD11C^+$ pro-inflammatory adipose tissue macrophages (ATMs) is an important driver of vAT inflammation. We investigated the role of ATMs in hepatic inflammation during NASH development.
Methods
vAT from lean, obese, or ATM-depleted (using clodronate liposomes) obese mice was transplanted to lean $ldlr^{-/-}$ acceptor mice. Systemic and hepatic inflammation was assessed either after 2 weeks on standard chow or after 8 weeks on high cholesterol diet (HCD) to induce NASH.
Results
Transplanting donor vAT from obese mice increased HCD-induced hepatic macrophage content compared to lean-transplanted mice without affecting other parameters of NASH. ATM depletion prior to vAT transplantation reduced this increased hepatic macrophage accumulation. On chow, vAT transplantation induced a more pronounced increase in circulating and hepatic neutrophil numbers in obese-transplanted than lean-transplanted mice, while ATM depletion prior to vAT transplantation reversed this effect. Microarray analysis of FACS-sorted $CD11C^+$ and $CD11C^-$ macrophages isolated from donor AT showed that obesity resulted in enhanced expression of neutrophil chemotaxis genes specifically in $CD11C^+$ ATMs. Lastly, CD11C expression in vAT of severely obese individuals correlated with vAT expression of neutrophil chemotactic genes and with hepatic expression of neutrophil and macrophage marker genes.
Conclusions
ATMs from obese vAT induce hepatic macrophage accumulation during NASH development, possibly by enhancing neutrophil recruitment.
Keywords
NASH; ATM; Obesity; Neutrophils; CD11C

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48 Malaria parasites in combination with interferon-γ induce endothelial glucocorticoid resistance in malaria-associated acute respiratory distress syndrome

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Malaria-associated acute respiratory distress syndrome (MA-ARDS) is a severe complication of malaria characterized by overwhelming inflammation and edema in the lungs due to endothelial activation and endothelial barrier damage. Glucocorticoids (GCs) are potent anti-inflammatory drugs with endothelium-protective properties. However, in an *in vivo* model of MA-ARDS with *Plasmodium berghei* NK65-infected mice, GC resistance impaired the therapeutic effects. To further study molecular mechanisms of this phenomenon, we established an *in vitro* model with murine microvascular lung endothelial cells. Stimulation with *Plasmodium berghei* NK65 extract and IFN-γ induced GC resistance. Protein levels of GC receptor and its nuclear translocation remained unaffected by the stimulation. Since little data exist on GC-sensitivity of endothelial cells, we performed RNA-Seq after stimulation with IFN-γ and dexamethasone. With this information, we are currently further investigating molecular mechanisms of malaria-induced GC-resistance in lung endothelium.

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49 Cyclophosphamide treatment reprograms selected anti-tumor CD8+ T cells into effector memory cells

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There is increasing evidence that the effect of chemotherapy on tumor rejection is not cell autonomous but relies on the immune system. Indeed, several reports have shown that human and murine tumors respond to chemotherapeutic agents more efficiently when the host immune system is intact. In particular, we have shown that cyclophosphamide treatment of DBA/2 mice bearing P815 mastocytoma induces rejection and long term protection in a CD4- and CD8-dependent manner. We used this tumor model, as it is poorly immunogenic, allows the identification of some tumor-specific CD8 T cells and expresses tumor-associated P1A and tumor-specific P1E antigens, encoded by germline and mutated genes, respectively.
We have previously reported that tumor regression correlates with selective infiltration of $CD8^+$ T cells specific for P1E/H-$2K^d$ antigen in tumor bed upon cyclophosphamide treatment. Unexpectedly, the proportion of $CD8^+$ T cells specific for the tumor-associated antigen P1A in the context of H-$2L^d$ decreases concomitantly, indicating that cyclophosphamide alters the repertoire of $CD8^+$ T cells recognizing tumor antigens. Using P1A KO mice, we found that preferential activation of CD8 T cells to P1E is not solely due to thymic negative selection. The major role of mutated antigens in tumor resistance has been recently highlighted in humans and raises an interesting question about the immune mechanisms of tumor rejection.
Additionally to its effect on the specific immune response, cyclophosphamide promotes tumor infiltration by terminal effector (P1E/H-$2K^d$)$^+$ $CD8^+$ T cells which are characterized by higher expression of KLRG-1 and Eomes. Our data point to a role of IL-15 and type 1 IFN for their development, as increased levels of IL-15 and IRF7 were measured in tumor after cyclophosphamide. The neutralization of type 1 IFN signalling prevents tumor rejection in 50% of mice and decreases the (P1E/H-$2K^d$)$^+$ CD8 T cell infiltration induced by cyclophosphamide, suggesting a role of this cytokine in the induction and/or recruitment of (P1E/H-$2K^d$)$^+$ CD8 T cells *in vivo*.
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50 Utilizing zebrafish for new insights into the roles of chemerin in cancer biology

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Chemerin is a chemoattractant agent identified at the IRIBHM that appears to regulate leukocyte trafficking and inflammatory responses, and also displays anti-tumoral properties in several models of cancer, including melanoma. The mechanisms by which chemerin acts on tumor initiation and/or progression are however mostly unknown and have yet to be addressed *in vivo*. Preliminary observations in our laboratory have shown the chemerin system is relatively well conserved from zebrafish to mammals. This is atypical among inflammatory chemokine-associated genes and could reflect important functions. This also suggests the zebrafish model may substantially contribute to improving our knowledge on chemerin biology and functions *in vivo*. Indeed, several models of melanoma have been established in zebrafish that may provide for unprecedented opportunities for determining chemerin functions in disease condition, in ways not possible in other vertebrates.
Due to the well-established whole-genome duplication in the evolution of teleosts, and/or local duplication events, it appears that zebrafish possess more chemerin and receptor genes than mammalian species. While this could make the analysis more complex, we show here that these chemerin paralogs exhibit distinct pharmacological and expression properties. We identify chemerin1a and 1b as the only paralogs being expressed in the skin, starting from embryonic development and throughout adulthood. To investigate their biological functions *in vivo*, we have generated several mutant alleles of each paralog by transcription activator-like effector nuclease (TALEN)-mediated mutagenesis. These novel mutant lines have been instrumental to address whether chemerin anti-tumoral properties are conserved in zebrafish. To do so, we used the MiniCoopR system, a specialized transposon vector engineered to rescue pigmentation and melanomas in the melanocyte-deficient Tg(*mitfa*$^{-/-}$) zebrafish line, while driving the concomitant expression of the NRAS oncogene gene in these rescued tissues. Our preliminary observations, presented here, suggest that *chemerin* disruption does modify tumoral development in a statistically significant way, by accelerating melanoma formation. Based on these exciting results, additional experiments are currently being conducted that combine the unique strengths of the zebrafish model system with state-of-the-art techniques, with the aim to provide mechanistic insights into the roles of chemerin in regulating tumorigenesis *in vivo*.

51 TGF-β signaling promotes microglia development in the zebrafish embryo

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Because neurodegenerative diseases are often associated with neuroinflammation, microglia, the resident macrophages in the central nervous system, represent a potential target for the development of novel therapeutic approaches. Our research aims at characterizing microglia ontogeny, an important aspect of microglia biology that remains poorly understood. Because the first steps of microglia ontogeny occur early during development, transparent transgenic zebrafish embryos offer great opportunities to characterize these processes in a non-invasive way. In an effort to characterize the molecular signature of zebrafish microglia, we have generated novel transgenic fluorescent reporter lines allowing for the prospective isolation of a pure population of microglial cells from the adult brain. Through microarray and whole transcriptome high-throughput sequencing (RNA-seq), we identified, among others, TGF-ß receptor I as a candidate for further investigations into microglia biology. The results we present here demonstrate that, as previously shown in mice, TGF-ß receptor signaling acts as an essential positive regulator of microglia ontogeny in the zebrafish embryo. Exploiting the strengths of the zebrafish model, we further demonstrated that this signaling pathway seems to be specifically required during the last steps of microglia differentiation. These preliminary observations have opened the door to future investigations aiming at characterizing the precise molecular mechanisms underlying TGF-ß receptor-mediated activity. Furthermore, these results validate the feasibility of our approach for providing novel insights into the genetic control of microglia ontogeny and differentiation. This could ultimately open new directions for the generation of microglia cells *in vitro,* a goal that has been largely unmet so far.

52 Characterization of the chemerin axis in the zebrafish model

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Zebrafish became over the recent years a major animal model in many research fields, including inflammation, regeneration and cancer. Here, we propose to utilize the unique strengths of this model system to obtain new insights into key functional aspects of chemerin, an extracellular mediator that was identified in our lab as the natural ligand of CMKLR1, and was initially showed to display potent chemotactic activity for macrophages, dendritic and natural killer cells. Work performed at the Institute and by others suggest that chemerin displays complex activities, that are either pro- or anti-inflammatory, depending on the disease model used. These investigations have also highlighted the possible tumor-suppressive properties of this chemoattractant in cancer biology, as well as a possible function in metabolism. However, these studies rely mostly on experiments performed *in vitro* or on cell lines, and their results are often conflicting. The multifunctional nature of chemerin *in vivo* remains therefore an open question.
Sequence and phylogenetic analyses indicate the chemerin system is relatively well conserved between zebrafish and mammalian species. Due to a whole-genome duplication event in the evolution of teleosts, it appears however that zebrafish possess more chemerin and receptor genes than their mammalian counterparts. These observations strongly suggest that subfunctionalization contributed to the evolution of the chemerin family in zebrafish, which may provide an advantage for the *in vivo* functional dissection of chemerin activities in physiology and disease.
As a first step towards using the zebrafish to gain insights into chemerin biology, we have performed a thorough analysis of the chemerin system in this animal model. Interestingly, our findings demonstrate that the different chemerin paralogs exhibit distinct pharmacological and expression properties. Using an approach that relies on protein fractionation of whole fish homogenates by affinity chromatography, receptor functional testing and mass spectrometry analyses, we were able to pair each chemerin paralog to its functional receptor. This allowed us to show that the ligands and receptors are not redundant in term of pharmacology, as zchemerin1 and zchemerin2 paralogs activate respectively zCMKLR1a and zCMKLR1b. Through the use of C-terminal-derived synthetic peptides, we also demonstrate that, as in human and mouse, accurate and specific C-terminal processing of chemerin is required for bioactivity in zebrafish. Finally, determination of the expression profiles of the different chemerin paralogs also demonstrated specificity. The validation of a functional conservation of the chemerin system in teleosts has now opened new avenues in utilizing zebrafish to complement studies performed on mouse models, and should ultimately lead to uncovering important new functions of this evolutionary conserved immune factor.