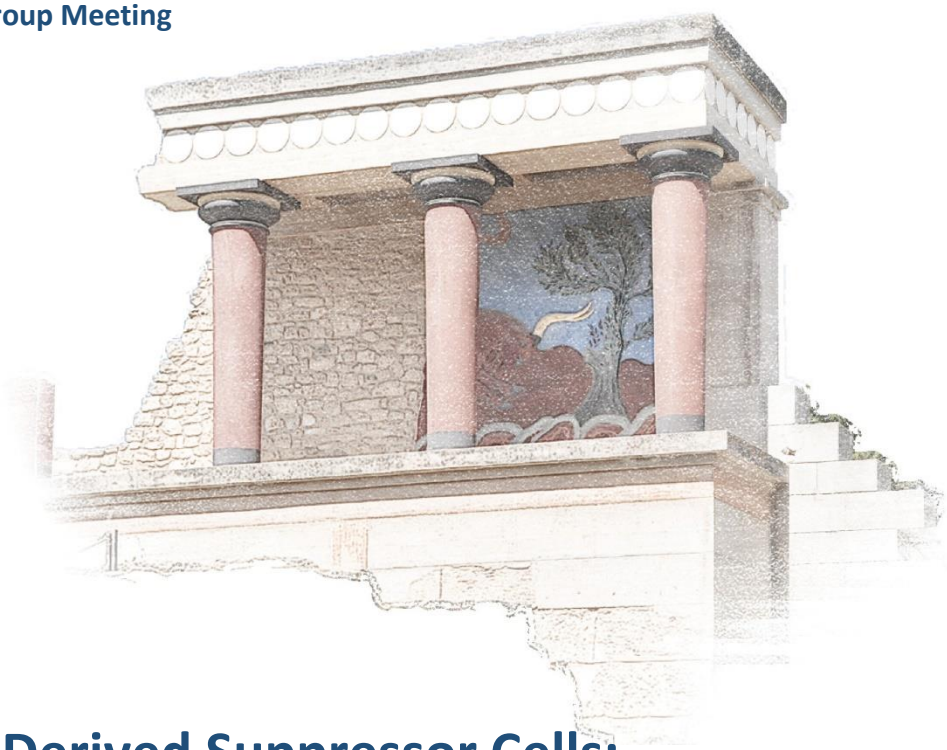
Mye-EUNITER

5th Management Committee & Working Group Meeting
5th Early Career Investigators' Workshop

16-18 April 2018, Heraklion, Crete, Greece



Myeloid Derived Suppressor Cells:

Identification and implications in Human Diseases

Program & Abstract book

Haemopoiesis Research Laboratory
University of Crete

Mye-EUNITER

Welcome message

Dear Colleagues,

It is a great pleasure and honor to welcome you to the 5th Annual Meeting of COST Action Mye-EUNITER and the European Workshop for Young Myeloid Researchers, in Heraklion, Crete, April 16-18, 2018.

Situated close to Knossos, the heart of the Minoan civilization, with a remaining Venetian and Byzantine atmosphere, the modern city of Heraklion is an exceptional location for the Mye-EUNITER Meeting. The Meeting will provide a wonderful forum for Early Career Investigators (ECIs) to advance their knowledge, explore the innovations in the field of Myeloid Regulatory Cells (MRCs), present and discuss their work. The ECIs will also have the opportunity to meet and interact with the leading scientists in the field of MRCs but also with other colleagues for future collaboration and networking. We hope that all members of the Meeting will join us for a symphony of outstanding science, discussions and future plans.

The Meeting is supported by the Mye-EUNITER COST Action BM1404 which primarily aims to harmonize the protocols and guidelines for the analysis and clinical monitoring of MRCs through fruitful meetings and exchange of tools, technologies and ideas. This year, we have prepared a scientific program for both clinicians and basic scientists, in keeping with the true interdisciplinary nature of COST and we have also highlighted novel fields for future research on MRCs.

We would like to thank all participants and we look forward to sharing with you all the latest exciting developments in the field of basic and biomedical research on MRCs. We also hope you enjoy your stay in Heraklion and take a little extra time to get a taste of this spectacular city that combines history, tradition and modern qualities.

Helen Papadaki

Sven Brandau

Delia Cosgrove

Jaroslaw Baran

Participating Organizations:

Cost Association
Cost Association BM1404 (Mye-EUNITER)
Avenue Luise 149
1050 Brussels
Belgium
http://www.mye-euniter.eu/

Haemopoiesis Research Laboratory
Medical School, University of Crete
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University of Crete
Voutes University Campus
Heralion, Crete,
70013, Greece
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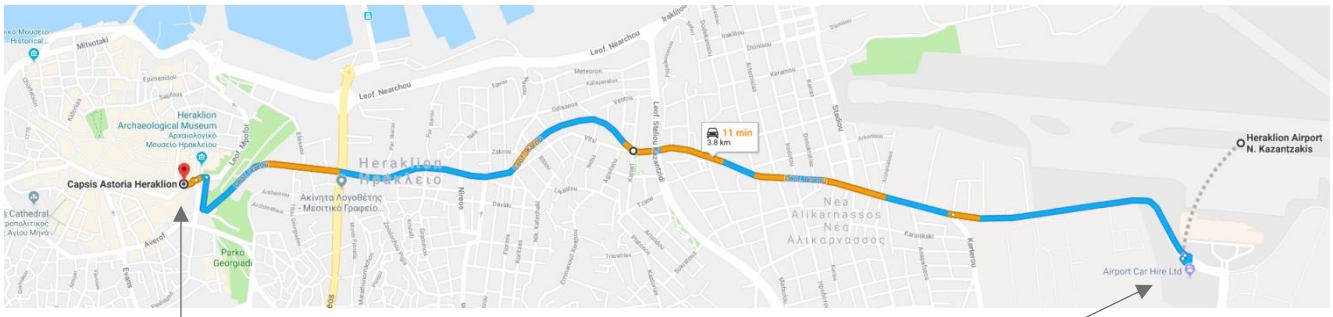
General Information

Meeting Venue

Hotel Capsis Astoria, Plateia Eleftherias 11, 71201 Heraklion, Crete
e-mail: info@astoriacapsis.gr
Phone: +30 2810343080-2
Fax: +30 2810229078
http://www.capsishotels.gr/el/astoria

General info for the city of Heraklion: https://www.heraklion.gr/en



Hotel Capsis Astoria: Plateia Eleftherias Bus Stop.

Airport: Bus Station – Taxi Ramp

From the Main Bus Station outside Heraklion Airport N. Kazantzakis many buses (no.10, no.11, no.12, no.31) stop to "Plateia Eleftherias" bus stop, right outside Hotel Capsis Astoria. The ticket price is € 1.70. Alternatively use a taxi from the taxi ramp. The taxi price is € 10-15. *

Transportation:

- **Bus:** City buses (blue) leave from the airport and the harbor every few minutes. A Bus station is located in front of the Hotel Capsis Astoria. Live viewing of the bus routes is available via telematics tools (http://astiko-irakleiou.gr/en/#main). The ticket price is for B' zone (blue colour) **€ 1.70/route** when bought outside the bus & **€ 2.50/route** inside the bus, while for A' zone (orange) **€ 1.20/route** outside the bus & **€ 2/route** inside the bus. Free of charge mini buses from the Municipality of Heraklion are also available (https://www.heraklion.gr/en).
- **Taxi:** Taxi ramps are placed in various places around the city including the airport, harbour and Hotel Astoria. Price from the airport to the city center (Hotel Capsis Astoria) is approximately 10 - 15 €. ** Please note: COST will only reimburse taxis used before 7 am or after 10 pm.*
- The city center is easily accessible on foot from Hotel Capsis Astoria.
- **Important information can be found in**: https://www.heraklion.gr/en/visitor/travel-guide.html

Parking:

- In the City of Heraklion you can find in this link Municipal Parking Areas

Program

Monday, April 16 2018

09:00-9:30
Arrival of Early Carrier Investigators (ECIs)
Registration of ECIs
Poster hanging

9:30-13:00
Group of participants: ECIs

- Welcome by Prof. Helen Papadaki (Meeting host), practical Information and overview of the meeting.
- Speed dating round of ECIs: *Who are they? What is the focus of their work? Which MDSC type are they working with? In which disease type? Which methods are used in their lab? Which methods are they interested in learning/establishing?*
- Campus tour - Location: Medical School of the University of Crete

13:00-14:00 - Lunch (Location: Hotel Astoria)

Parallel sessions:

14:00-16:00 - ECI Workshop Part I
Group of participants: ECIs, non-MC WG facilitators, non-MC members of the COST Action

- Key note lecture: MDSCs reserves and function in normal states and human diseases.
  Speaker: Viktor Umansky
- Separate pre-defined groups of ECIs will try to solve preselected questions/queries on MDSCs under the supervision of facilitators/tutors (WG members). Findings and proposals will be presented and discussed with the WG members in 17-4-2018.
  Chairs: Panos Verginis, Nikoleta Bizymi.
  1. **Question/Query: Early stage MDSCs: Are these cells a third MDSC-subset?**
  Start by giving the recent recommendations on the nomenclature and identification of M-MDSCs and PMN-MDSCs. Then, try to answer the question based on evidence from the literature. Suggest experimental ways to prove your answer.
  Facilitator/Tutor: Anca Dorhoi
  2. **Question/Query: Epigenetic studies for MDSCs.**
  Discuss their usage. Provide literature.
  Facilitator/Tutor: Panos Verginis
  3. **Question/Query: MDSCs as biomarkers: Is it possible in the clinical setting?**
  Discuss their usage. Where is the field now? What is feasible to investigate? Suggest experimental ways to investigate them as biomarkers (molecular signatures, functional assays).
  Facilitator/Tutor: Estela Paz-Artal
  4. **Question/Query: Animal models for MDSCs: Pros and Cons**
  Give the main animal models. Explain the pros and cons. How to translate the results into the human setting. Give your own experience based on your research and how you deal with it.
  Facilitator/Tutor: Gennadiy Zelinskyy

5. Question/Query: Can MDSCs work as therapeutic targets? Identify 5 (at least) agents (new or old) that are known to affect their number or function.
Provide papers supporting your answer. Explain the pathways affected by the evidence and how this can facilitate the therapeutic strategy. Focus on older agents now found to affect also MDSCs.
Facilitator/Tutor: Katarzyna Barczyk-Kahlert

14:00-16:00 - COST Management Committee Meeting
Group of participants: MC members of the COST Action

- Welcome to participants.
- Verification of the presence of two-thirds of the Participating COST Countries or, if applicable, a quorum.
- Adoption of agenda.
- Approval of minutes and matters arising of last meeting.
- Update from the Action Chair
  a) Status of Action.
  b) Short Term Scientific Missions (STSM) and Training Schools.
- Update from the Grant Holder: Action budget status.
- Update from the COST Association (if a representative is present).
- Monitoring of the Action.
- Implementation of COST policies on:
  a) Promotion of gender balance and ECIs.
  b) Inclusiveness and Excellence.
- Follow-up of MoU objectives: progress report of WGs.
- Scientific planning.
- Scientific strategy (MoU objectives, GP Goals, WG tasks and deliverables).
- Action Budget Planning.
- Long-term planning (including anticipated locations and dates of future activities).
- Dissemination planning (Publications and outreach activities).
- Requests to join the Action.
- AOB.
- Location and date of next meeting.
- Summary of MC decisions.
- Closing.

16:00-16:30 - Coffee Break

16:30-17:30 - Joint Session

Group of participants: All members of COST Action, ECIs

- Welcome to all participants by Sven Brandau. Information about the Meeting.
- Mye-EUNITER related issues.
- Opening Lecture: Chandax - Candia - Heraklion. A short trip in time.
  Liana Starida, Archeologist.

18:00-20:00 - Heraklion: Historic Downtown – Guided Walking Tour

Group of participants: all members of COST Action, ECIs.

20:30 - Dinner

Tuesday, April 17 2018

9:00-10:00 - "Meet the expert" session

Group of participants: mandatory for ECIs; optional for PIs

- Parallel groups, chaired by experts. Registration in advance.

  Group 1. MDSCs: Ontogeny, nomenclature, characterization, intrinsic pathways.
  Experts: Gosse Adema, Jarek Baran

  Group 2. MDSCs: Animal models.
  Experts: Pierre Bruhns, Karin Loré

  Group 3. MDSCs: Their role in cancer.
  Experts: Sven Brandau, Zvi Fridlender

  Group 4. MDSCs: Haematologic diseases (malignant and immune-mediated).
  Experts: Helen Papadaki, Mikael Roussel

  Group 5. MDSCs: Beyond cancer (inflammation, autoimmunity).
  Experts: Joanna Cichy, Annabel Valledor

10:00-10:30 - Coffee Break

10:30-12:30 - ECI Workshop Part II joined with WG/MC members

Group of participants: ECIs, all members of COST Action

- Presentations of selected abstracts by the ECIs (max.10 min + discussion).

  Research Group 1: Cancer group.
  Chair: Karine Serre

  Research Group 2: Haematology group
  Chair: Juan Francisco Santibañez

  Research Group 3: Inflammation and Autoimmunity group.
  Chair: Silvia Gregori

  Research Group 4: Infectious disease group
  Chair: Anca Dorhoi

12:30-13:30 - Lunch (Location: Hotel Astoria)

13:30-15:30 - ECI Workshop Part III (joined with WG session)

Group of participants: All members of COST Action, ECIs

- Current research and perspectives of Mye-EUNITER Initiatives. Brief update session.

  Update on EUROPTION (20 min)
  Michaela Pekarova, Viktor Umansky

  Update on Mye-Hematology (20 min)
  Helen Papadaki

  Update on Mye-MMI (15 min)
  Sven Brandau

  Update on Mye-FUN (15 min)
  Pierre van der Bruggen, Annika Bruger

  Update on Mye-Tissue Map (15 min)
  Espen Baekkevold

  General Update on ESMRC Congress (15 min)
  Sven Brandau

  Specific aspects tbd at ESMRC, formation of Task Force (20 min)
  Debates, technical workshop, study groups for interlab validation, SOP, recommendations, etc.

15:30-17:00 - Coffee and Poster Walk Session

Group of participants: All members of COST Action, ECIs

- Guided poster tour with 5 minutes poster presentations.
  Poster Moderators: Jarek Baran, Helen Papadaki, Nikoleta Bizymi.

17:00-19:00 - ECI Workshop part IV joined with WC/MC members

Group of participants: All members of COST Action, ECIs

- Main Questions/Queries on MDSCs critically reviewed by the ECIs on April 16, will be presented and discussed by the ECI groups. Presentations to Questions/Queries that have been sent before the meeting (discussion will be chaperoned by non-MC, experienced WG member). Presentations by the ECI course participants for Mye-EUNITER members.
  Chairs: Panos Verginis, Nikoleta Bizymi.

  1. **Question/Query: Early stage MDSCs: Are these cells a third MDSC-subset?**
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  2. **Question/Query: Epigenetic studies for MDSCs.**
  Discuss their usage. Provide literature.
  3. **Question/Query: MDSCs as biomarkers: Is it possible in the clinical setting?**
  Discuss their usage. Where is the field now? What is feasible to investigate? Suggest experimental ways to investigate them as biomarkers (molecular signatures, functional assays).
  4. **Question/Query: Animal models for MDSCs: Pros and Cons**
  Give the main animal models. Explain the pros and cons. How to translate the results into humans. Give your own experience based on your research and how you deal with it.

5. **Question/Query: Can MDSCs work as therapeutic targets? Identify 5 (at least) agents (new or old) that are known to affect their number or function.**
   Provide papers supporting your answer. Explain the pathways affected by the evidence and how this can facilitate the therapeutic strategy. Focus on older agents now found to affect also MDSCs.

20:00 - Dinner

END OF THE ECI MEETING

Wednesday, April 18 2018

Group of participants: MC and WG Members

9:00-11:00 - Current research and perspectives of Mye-EUNITER WGs
(Topic and content tbd by WG leaders)

Research progress of WG1 (20 min presentation + discussion)
Gosse Adema

Research progress of WG2 (20 min presentation + discussion)
Jo van Ginderachter

Research progress of WG3 (20 min presentation + discussion)
Ana E Sousa

Research progress of WG4 (20 min presentation + discussion)
Karin Loré

Research progress of WG5 (20 min presentation + discussion)
Silvia Gregori

11:00-11:30 – Coffee Break

11:30-13:00 - Brainstorming and definition session

EUROPTION
Define scientific programme and WGs, leaders, workpackages, milestones, target members, ECI concept

ESMRC Final Congress
Task Force:

i. Debates
ii. Industrial partners and contacts
iii. Technical workshops
iv. Study groups
v. Involvement of industry as co-hosts
vi. SOP

13:00-14:00 - Lunch (Location: Hotel Astoria)

14:00-16:00 - Parallel sessions

Group participants: for participating members of these initiatives only

ROOM 1	ROOM 2
Workshop on Mye-MMI Sven Brandau	**Workshop on Mye-Tissue Map** Espen Baekkevold
Workshop on Mye-FUN Pierre van der Bruggen, Annika Bruger	**Workshop on Mye-Hematology** Helen Papadaki

END OF THE MEETING

Abstracts

Knossos Palace

Oral presentations

Research Group 1: Cancer

Minoan women fresco

Gemcitabine delivery via dendrimer carriers decorated with anti-VGFR1 antibody to target tumor-induced myeloid cells

M. Alper Kursunel[a], Digdem Yoyen-Ermis[a], Kivilcim Ozturk-Atar[b], Cisel Aydin[c], Didem Ozkazanc[a], Mustafa Ulvi Gurbuz[d], Aysegul Uner[c], Metin Tulu[d], Sema Calis[b], Gunes Esendagli[a]

[a]*Hacettepe University Cancer Institute, Department of Basic Oncology, Ankara, Turkey*
[b]*Hacettepe University Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, Turkey*
[c]*Hacettepe University Medical Faculty, Department of Pathology, Ankara, Turkey*
[d]*Yıldız Technical University, Faculty of Arts and Sciences, Department of Chemistry, Istanbul, Turkey*

While reshaping its microenvironment, tumors are also capable of influencing systemic processes such as myeloid cell production. Therefore, the tumor-induced myeloid cells, such as myeloid-derived suppressor cells (MDSCs) which are characterized with pro-cancer properties, became another target in order to increase success of the therapy. Drug delivery systems such as dendrimers are preferred to enhance solubility, stability and biocompatibility of the drugs, and to lower their cytotoxic side effects. Dendrimers are also useful to endow with tumor-homing agents of guidance such as antibodies aiming the molecules that are enriched in the malignant microenvironment thus increase the local concentration of the drug. VEGF is a pivotal mediator for mobilization and recruitment of bone marrow-derived leukocytes and hampers the differentiation of these myeloid cells. So, this study evaluated the capacity of a novel dendrimeric drug delivery platform decorated with VGFR1 antibody to eliminate tumor-induced myeloid cells in the reticulo-endothelial system. Preparation and characterization of dendrimeric structures and complex formation with gemcitabine first completed by our pharmaceutical collaboration. Subcutaneous tumors were established into CD-1 Nude mice. The tumor-bearing animals (approximate tumor diameter 0.5 cm) were administered intraperitoneally (2x/week) with 0.09% NaCl in dH_2O (control group), gemcitabine solution, gemcitabine loaded into PAMAM dendrimers or αFlt1-couped PAMAM dendrimers. The change in tumor growth and weight of animals were followed and the organs were dissected and macroscopically evaluated after termination of the experiments. PBMCs from cell suspensions (from the spleen, the liver, blood and the bone marrow) were obtained and analyzed in flow cytometry. Tumor samples were fixed and tissue sections then histopathologically analyzed. Anti-Flt1 antibody-conjugated polyethylene glycol (PEG)-cored poly(amidoamine) (PAMAM) dendrimers improved the efficacy of gemcitabine against pancreatic cancer. Biodistribution studies showed that these dendrimeric structures accumulated into the compartments that became rich in myeloid cells in the pancreatic tumor-bearing mice. When gemcitabine was loaded into the dendrimer complexes, the number of myeloid cells were significantly reduced while the percentage distribution of granulocytic and monocytic myeloid cells was not always significantly altered. The $CD11b^+Ly6G^-Ly6C^+$ monocytes were more severely affected from the treatments than $CD11b^+Ly6G^+Ly6C^+$ granulocytes. Immune infiltration levels in the tumor tissue was also altered. Myeloid cells in the spleen and $F4/80^+$ macrophages of the liver were protected. The compartments such as the liver and the bone marrow, which are known with high vascular endothelial growth factor (VEGF) - Flt1 pathway activity, were particularly targeted by gemcitabine when delivered through anti-Flt1 antibody-conjugated PAMAM dendrimers. The gemcitabine-loaded anti-Flt1 antibody-conjugated PEG-cored PAMAM dendrimers' success in the reduction of tumor mass were accompanied by the elimination of tumor-induced myeloid cells in various compartments. Therefore, this novel approach can not only be regarded as a promising strategy to diminish pancreatic cancer growth but also to target myeloid cells propagated under the influence the tumor mass.

Induction of myeloid-derived suppressor cells via tumor-derived extracellular vesicles in malignant melanoma

Viktor Fleming, Xiaoying Hu, Peter Altervogt, Jochen Utikal, Viktror Umansky

Skin Cancer Unit, German Cancer Research Center (DKFZ), Heidelberg and Department of Dermatology, Venereology and Allergology, University Medical Center Mannheim, Ruprecht-Karl University of Heidelberg, Mannheim, Germany

Malignant melanoma (MM) accounts for almost 80% of all skin tumors deaths. The accumulation of highly immunosuppressive myeloid-derived suppressor cells (MDSCs), which arise from immature myeloid cells (IMC) in the bone marrow, play a significant role in the immunosuppression and in the resistance to immunotherapy in MM. it was shown that melanoma cells could generate MDSC by secreting extracellular vesicles (EVs). Those are small membrane vesicles, which have been proven to be essential in intercellular communication. In addition, EVs promote the progression, invasion and metastasis of cancer. However, the mechanisms of MDSC generation and activation by EVs MM retain to be explored.

We have shown that the treatment of IMCs with EVs induced the secretion of inflammatory cytokines such as IL-1β, IL-6, IL-10, TNF-α and COX2. In addition, a strong upregulation of PD-L1 was measured. By studying myD88- and TLR2/4/7-knockout mice, we found that these alterations were mediated by the stimulation of the NFκB activation mainly by the TLR4 signaling pathway. Moreover, TLR4 signaling was shown to be mainly triggered by heat-shock proteins, which are predominantly sorted into EVs by cells undergoing stress. By inhibiting heat-shock proteins on a transcriptional level, we could completely abrogate the EVs mediated PD-L1 upregulation on IMC.

Functional assays showed that EV-treated IMC become immunosuppressive. They could inhibit the proliferation of $CD8^+$ T cells and reduce the production of interferon-γ. Interestingly, the impact of EVs treated IMC on T cell functions was mainly due to PD-L1 upregulation. To confirm the importance of our results, we are performing in vivo studies in wild type, as well as knockout mice.

Our data suggest that tumor-derived EVs could convert IMC into functionally active MDSC via upregulation of PD-L1 expression mediated by TLR4 signaling.

Spatial profiling of granulocytes and T cells in the human head and neck cancer microenvironment

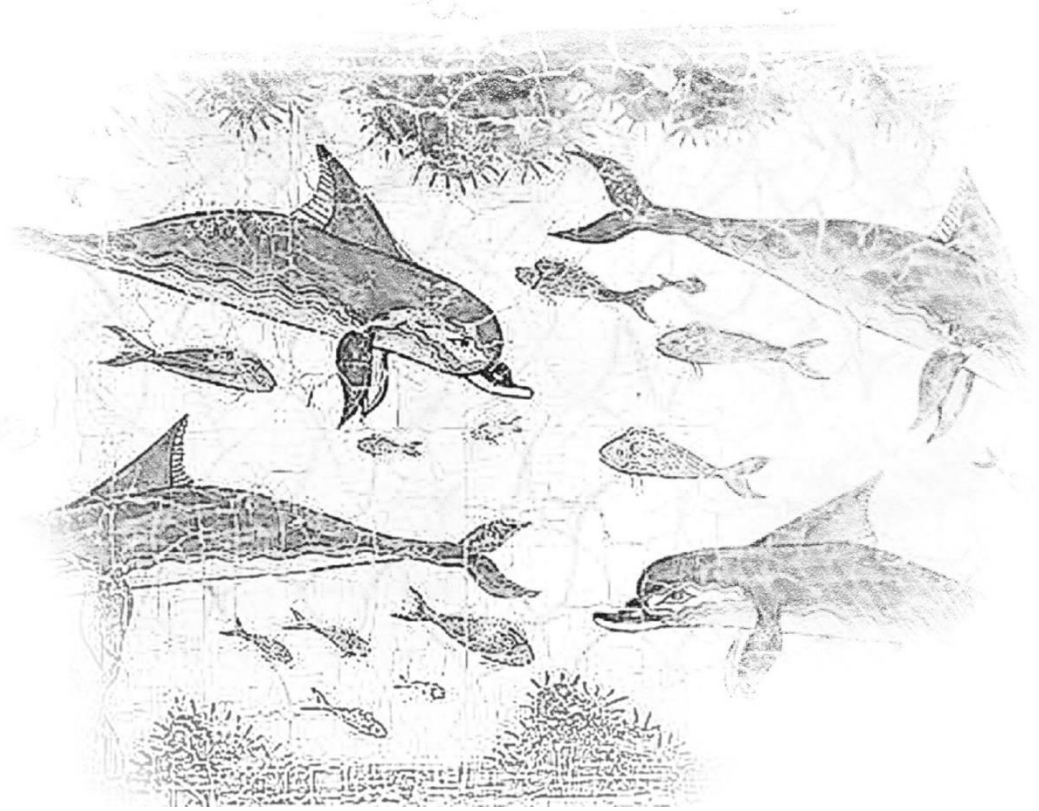
Yu Si, Anthony Squire, Stephan Lang, Sven Brandau

University Hospital Essen, Germany

New immunotherapies show promise also for HNSCC, which makes it important to better understand the local immunological tumor microenvironment in this type of cancer.
Published work associates a dense T cell infiltrate with good prognosis, while patients with tumors strongly infiltrated with tumor-associated granulocytes (TAG) have a poor outcome. It is the aim of this study to uncover the interaction of TAG and tumor-infiltrating T cells (TIL) in patients with HNSCC using digital pathology and quantitative image analysis.
The tumor tissue of patients with stage I–IV HNSCC was analyzed. Immunofluorescence was used to explore the specific phenotype and spatial distribution of TAG and TIL. Granulocyte and T cell marker CD66b and T cell markers were combined with markers indicative of cellular differentiation and activation states. The whole slide was scanned with Apotome and subjected to digital image analysis using the Definiens platform.
We developed algorithms to distinguish tumor islands from stromal regions for separate immune cell quantification. Lower tumor to stroma ratio was correlated with lymphatic metastasis and poor survival. TAG prefer to infiltrate in the stroma, but Granzyme B^+TILs in epithelial tumor islands. About 1/3 of the tumor and stromal areas are composed of mixed regions with intensive TAG/TIL interaction. Low densities of TAG and high densities of Granzyme B^+TILs predicted good survival, independent of tumor stage.
Our data suggest a functional compartmentalization of the tumor microenvironment with hot spots of Granulocyte-T cell interaction relevant for tumor progression and patient survival.

Oral presentations

Research Group 2: Haematology



Dolphins fresco

Association of Myeloid Derived Suppressor Cells (MDSCs) & Monocyte Subpopulations in Patients with Chronic Neutropenias

Nikoleta Bizymi, Maria Velegraki, Athina Damianaki, Helen Koutala, Vasileia Kaliafentaki, Irini Fragkiadaki, Aristea Batsali, Maria Ximeri, Peggy Kanellou, Irene Mavroudi, Charalampos Pontikoglou, Helen Papadaki

Haemopoiesis Research Laboratory, Medical School, University of Crete, Greece.

Introduction – Background: MDSCs are a heterogeneous population of immature immunoregulatory myeloid cells, which are elevated in various human diseases that involve chronic inflammation and tumor progression. They are divided in two subpopulations, HLA-DR$^{low/-}$CD11b^{+}CD33^{+}CD15^{+} (polymorphonuclear-PMN-MDSCs) and HLA-DR$^{low/-}$ CD11b^{+}CD33^{+}CD14^{+} (monocytic-M-MDSCs). Through activation of the enzymes arginase 1 and nitric oxide synthase 2, and production of reactive oxygen species, they lead to suppression of T-cell proliferation, inhibition of natural killer (NK) cell cytotoxicity, modulation of macrophage polarization and induction of development of regulatory T cells.
Chronic Idiopathic Neutropenia (CIN) is the prolonged, otherwise unexplained reduction in the number of PMN below the lower limit of the normal range. The main pathogenetic mechanism for CIN implicates the increased, Fas-mediated apoptosis of the CD34^{+}/CD33^{+} myeloid progenitor cells. Chronic inflammation driven by an inhibitory bone marrow (BM) microenvironment consisting of activated T-lymphocytes (oligoclonal profile) and pro-inflammatory mediators [TNF-α, (TGF-β1, Fas-ligand, IFN-γ, IL-1b, and IL-6] is also involved. Data from our lab have shown that CD14^{++}/CD16^{+} monocyte subpopulation is increased in patients, which can be associated with the enhanced antigen presentation and T cells over-activation in the disease.
Aim of the study: The study aims to explore the possible involvement of the monocyte and MDSC subpopulations in the pathogenesis of CIN, through the investigation of the number and functional characteristics of the CD16^{+} pro-inflammatory monocytes and the PMN-MDSCs and M-MDSCs, in CIN patients compared to healthy subjects.
Methodology: We studied 49 CIN patients and 23 healthy subjects. The panels for cell staining for MDSCs from PBMCs were CD33-PC7/CD15-PC5/DR-ECD/CD14-PE/CD11b-FITC, for MDSCs from BMMCs were CD33-PC7/CD15-PC5/DR-ECD/CD14-PE/CD11b-FITC & CD45-FITC/CD33-PC7/CD15-PC5/DR-ECD/CD14-PE, and for Monocytes from PB were CD14-PE/CD16-FITC. FACS analysis was done with the Kaluza software and statistical analysis was done with the Graph Pad software and the Mann-Whitney test.
Results: We found increased proportion of intermediate CD14^{++}/CD16^{+} (Donors: 7,05±0,538, Patients: 15,120±1,564, p=0,0002) and non-classical CD14^{dim}/CD16^{++} (Donors: 2,73±0,303, Patients: 4,690±1,564, p=0,411) monocytes in CIN patients and decreased proportion of M-MDSCs in the PBMC fraction of CIN patients (Donors: mean 2,085±0,521, median 1,268, Patients: mean 0,495±0,116, median 0,230, p=0.0018).
Conclusions - Discussion: CIN patients display increased number of pro-inflammatory (intermediate CD14^{++}/CD16^{+} and non-classical CD14^{dim}/CD16^{++}) monocytes in the PB that may contribute to the aberrant T-cell activation and chronic inflammation.
CIN patients display low proportions of PB PMN-MDSCs and M-MDSCs compared to the controls. These cells normally protect from uncontrolled immune responses, so the low number of these cells in CIN may contribute to the sustained chronic inflammation.
We will continue with further experiments focusing on (1) the isolation of the CD16^{+} monocytes from PB of CIN patients and controls and investigation of their transcriptional profile as regards to proinflammatory (TNFα, IL1β) cytokine production and (2) the isolation of the MDSC populations and investigation of their T-cell suppression function and production of ARG1, NOS2, COX2, TGFβ, IL6, IL10.

Monitoring monocyte subsets short term follow up post-autologous stem cell transplantation of multiple myeloma patients (work in progress)

Ida Marie Rundgren*, Elisabeth Ersvær*, Anita Ryningen* and Øystein Bruserud**

**Department of Biomedical Laboratory Sciences and Chemical Engineering, Faculty of Engineering and Natural Sciences, Western Norway University of Applied Sciences*
*** Department of Clinical Science, Faculty of Medicine and Dentistry, University of Bergen*

Multiple myeloma (MM) is a heterogeneous disease, and characterized by abnormal plasma cells secreting monoclonal immunoglobulin proteins and include signs of end organ damage [1]. MM develops from monoclonal gammopathy of undetermined significant (MGUS), and the more progressed intermediate state called smoldering multiple myeloma (SMM) [1].
The main strategy for managing MM in eligible patients is by autologous stem cell transplantation (ASCT) [2]. The innate immune system have received less attention than adaptive immunity with regard to MM and immune reconstitution post-ASCT.
Monocytes are important cells of the innate immune system, and constitute 10 % of total circulating peripheral blood leukocytes [3], they differentiate into macrophages or dendritic cells (DC) [4]. Studies reports that treatment with immune regulatory drugs enhance monocyte differentiation towards dendritic cells (DC) in MM patients [5].
Monocytes consists of three subpopulations, termed classical ($CD14^{bright}CD16^{negative}$), intermediate ($CD14^{bright}CD16^{dim}$) and non-classical ($CD14^{dim}CD16^{bright}$) monocytes [6, 7]. Monocytes have an immunoregulatory function, and animal studies suggest that epigenetic mechanisms may induce innate memory, which is important for the defense against infections [8-10]. These observations suggest a possible clinical usage of monocytes; however, the use of flow cytometry analyses of monocyte subsets still requires a careful standardization of sampling handling and procedures (Rundgren, 2018, submitted), along with more in depth knowledge of the effect the disease and treatment have on monocytes and the subpopulations.
We have investigated the short-term immune reconstitution for monocytes and the monocyte subsets in MM patients. We analyzed blood samples from MM patients succumbed to ASCT, collected at different days during treatment, by flow cytometry. From the preliminary data, the absolute number of monocytes declined from day -2 to 0, and further to day 6-8 post-ASCT, before reclining at day 10-12. The aim was to gain more insight on the effect of ASCT on monocyte subpopulations and investigate the clinical significance of the monocyte response to ASCT treatment.

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2. Rajkumar, S.V. and S. Kumar, Multiple Myeloma: Diagnosis and Treatment. Mayo Clin Proc, 2016. 91(1): p. 101
3. Swirski, F.K., et al., Identification of splenic reservoir monocytes and their deployment to inflammatory sites. Science, 2009. 325(5940): p. 612
4. Jakubzick, C.V., G.J. Randolph, and P.M. Henson, Monocyte differentiation and antigen-presenting functions. Nat Rev Immunol, 2017. 17(6): p. 349.
5. Costa, F., et al., Lenalidomide increases human dendritic cell maturation in multiple myeloma patients targeting monocyte differentiation and modulating mesenchymal stromal cell inhibitory properties. Oncotarget, 2017. 8(32): p. 53053
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Role of maternal micro-environment in promoting tolerogenic DC differentiation

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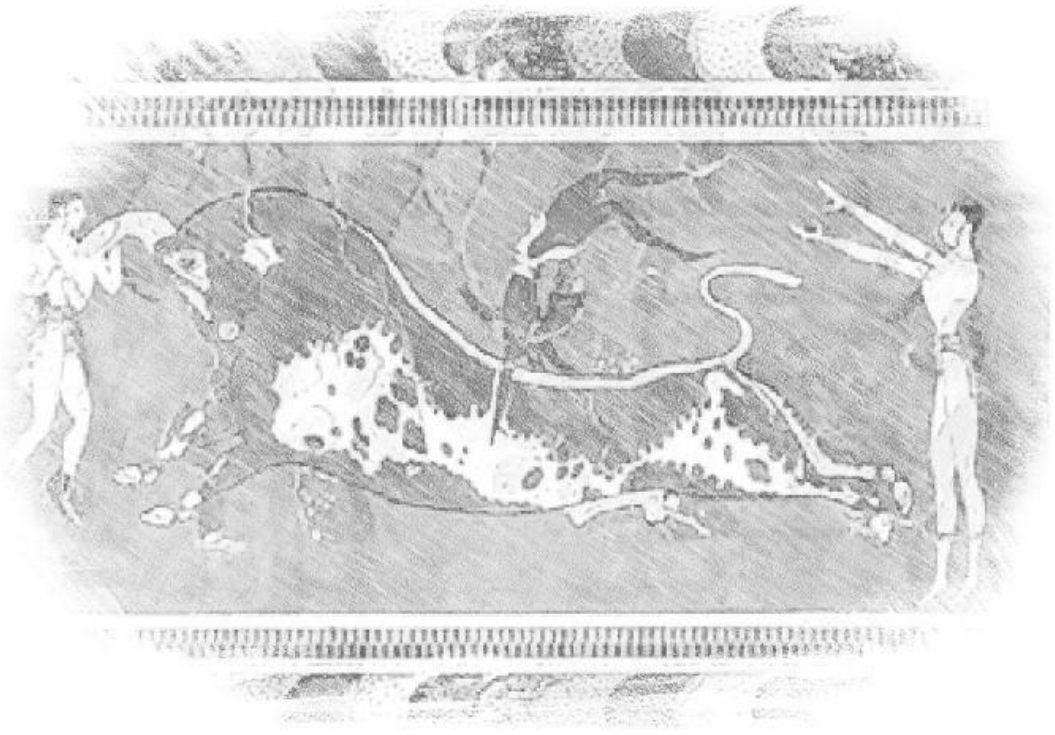
DC-10 are an inducible subset of human tolerogenic dendritic cells characterized by the ability to secrete high levels of IL-10 in the absence of IL-12, and by the expression of a specific set of markers including CD14, CD16, CD11c, and CD11b, but not CD1a, M-DC8 or CD68. DC-10 express a bunch of tolerogenic molecules such as ILT2, ILT3, ILT4, and HLA-G and promote the induction of adaptive T regulatory type 1 (Tr1) cells, a subset of inducible T regulatory cells that co-express the integrin alpha 2 subunit (CD49b) and the lymphocyte-activation gene 3 (LAG-3), and secrete IL-10, but not IL-4, and IL-17. DC-10 are present *in vivo* in both physiological and pathological conditions. Interestingly, DC-10 accumulate in decidua in the first trimester of pregnancy, and their frequency is reduced in the decidua of women with early miscarriage. Based on these observations, we postulate that DC-10 may represent the naturally-occurring DC involved in reprogramming the immune response towards tolerance during pregnancy. DC-10 accumulate in decidua during pregnancy; however, it is still not defined whether DC-10 are induced in situ from peripheral blood $CD14^+$ cells migrating into the decidua before or after embryo implantation or are recruited from peripheral blood.

The evidence that similar frequencies of peripheral blood DC-10 were observed in pregnant women and non-pregnant controls, supports the former possibility. To verify this hypothesis, we are currently testing whether the secretome of decidualizing tissues can induce the differentiation of DC-10 from $CD14^+$ monocytes. We used an immortalized human endometrial stromal cell line (T-HESCs) that, once stimulated with cAMP and medroxyprogesterone acetate, undergoes to decidualization *in vitro*. Supernatants from decidualizing cells, enriched in several mediators including prolactin (PRL), Insulin-like Growth Factor Binding Protein-1 (IGFBP-1), interleukin-6, and Tissue Factor (TF), are collected at different time points (from day 0 until day 7) and added during DC differentiation from peripheral blood $CD14^+$ monocytes cultured for 7 days with GM-CSF and IL-4. In parallel, DC-10 are differentiated as control from the same monocytes following the standard protocol of 7 days of culture in the presence of GM-CSF, IL-4, and IL-10. Differentiated DCs are characterized based on the expression of DC-10-associated markers (CD14, CD16, CD141, CD163, CD83, CD86, HLA-DR, HLA-G, and ILT4) assessed by FACS, and cytokine profile (IL-10, IL-12, IL-6) evaluated by ELISA. In addition, we are currently performing functional studies of resulting DCs by testing their modulatory activity.

Results showed that monocytes cultured with T-HESC-conditioned media collected at day 2 differentiated into DC-10. Conversely, monocytes cultured with T-HESC-conditioned media collected at day 7 displayed an intermediate phenotype between DC-10, monocytes differentiated in the presence of GM-CSF/IL-4/IL-10, and immature (i)DC differentiated with GM-CSF/IL-4. These results support our hypothesis that the local microenvironment in early phases of pregnancy allows the generation of DC-10, which sustain tissue remodeling and promote tolerance. Further experiments are needed to confirm these results and to investigate the modulatory activity of DCs obtained in the presence T-HESC-conditioned media on immune cells highly represented in the endometrium during pregnancy.

Oral presentations

Research Group 3: Inflammation and Autoimmunity



Taurokathapsia

Autophagy orchestrates the regulatory program of tumor-associated myeloid-derived suppressor cells

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Myeloid-derived suppressor cells (MDSCs) densely accumulate into tumors and potently suppress anti-tumor immune responses promoting tumor development. Targeting MDSCs in tumor immunotherapy has been hampered by lack of understanding on the molecular pathways that govern MDSC differentiation and function. Herein, we identify autophagy as a crucial pathway for MDSC-mediated suppression of anti-tumor immunity. Specifically, MDSCs in mouse tumors and melanoma patients exhibited increased levels of functional autophagy. Ablation of autophagy in myeloid compartment, significantly delayed tumor growth and endowed anti-tumor immune responses. Notably, tumor-infiltrating autophagy-deficient monocytic MDSCs (M-MDSCs) demonstrated impaired suppressive activity *in vitro* and *in vivo*. Transcriptome analysis of autophagy-deficient M-MDSCs revealed significant differences in genes related to antigen presentation and lysosomal function. Accordingly, autophagy-deficient M-MDSCs exhibited impaired lysosomal degradation and elevated levels of STAT1 that enhanced class II transactivator (CIITA) and MHC class II expression consistent with an immunogenic rather than tolerogenic phenotype. Our findings depict autophagy as a novel molecular target of MDSC-mediated suppression of anti-tumor immunity.

Evolution of CD25-positive myeloid suppressor cells in solid organ transplant recipients and relationship with rejection

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Background: Myeloid derived suppressor cells (MDSC) are immature cells with immunosuppressive capacities. Three MDSC subsets are currently defined: monocytic, early stage, and polymorphonuclear MDSC (M-MDSC, eMDSC and PMN-MDSC respectively). While MDSC increase in cancer and chronic infections and associate with poor prognosis, their role in transplantation (Tx) is unknown. Changes in MDSC in transplant patients could provide biomarkers of graft evolution, and they could be useful as immunosuppressive therapy and/or to stimulate the allograft tolerance.
Methods: Peripheral blood MDSC were identified in cohorts of kidney (n=164) and liver (n=30) recipients and healthy volunteers (HV) as $CD33^{+}CD11b^{+}HLA\text{-}DR^{lo/-}$. We characterized $CD14^{+}CD15^{-}$ (M-MDSC) and $CD14^{-}CD15^{-}$ (eMDSC) subsets. Suppression assays and measurement of surface CD25 expression (MFI) on MDSC were performed.
Results: Renal recipients MDSC were able to suppress CD4 and CD8 T cell proliferation *in vitro*. MDSC % were similar in pre-transplant, kidney transplant recipients (KTR), than in HV. However, MDSC and M-MDSC increased, mainly at 7 and 14 days post-transplant (3.48% and 3.85% vs 1.14%; 2.47% and 2.48% vs 0.24% p≤0,001 vs pre-transplant). The increase of MDSC in KTR with basiliximab (anti-CD25) as induction therapy was lower than in patients induced with thymoglobulin or without induction, and eMDSC were particularly decreased in basiliximab-treated patients (vs no induction, p≤0.05; vs thymoglobulin, p≤0.05). We confirmed that eMDSC expressed CD25, target of basiliximab. Pre-Tx, liver transplant recipients (LTR) had higher % of $CD25^{+}$ eMDSC and higher CD25 MFI than KTR and HV. In LTR and KTR without basiliximab, % and MFI of CD25 in eMDSC increased after transplant. In KTR cohort, 7% of patients suffered acute rejection (AR). Absolute numbers of MDSC at 7 days post-transplant (measured before the rejection event) were significantly lower in AR than in nonrejectors (25.84 cells/uL vs 53.18 cells/uL respectively, p≤0,05).
Conclusions: MDSC and M-MDSC increase post-Tx except in patients receiving basiliximab (anti-CD25). Transplant recipients eMDSC express CD25 which upregulates after Tx, however, if eMDSC express a complete and functional IL-2R is still unknown. MDSC were lower in AR patients and low MDSC counts significantly preceded rejection.

In vivo anti-tumour activity of LXRs correlates with changes in chemokine expression in tumour-associated macrophages

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Liver X Receptors (LXRs) are ligand-dependent transcription factors that regulate multiple physiological processes such as metabolism, proliferation and immune responses. LXRs can be activated by oxidized forms of cholesterol and by synthetic high-affinity agonists. Growing evidence indicates that LXR activity inhibits tumour progression through its regulatory role in tumour cell lipid metabolism and proliferation. We have confirmed that LXR activation inhibits proliferation of different human and murine cells lines *in vitro*, including Lewis lung carcinoma (3LLR) cells. However, discrepancies exist about the effect of LXR activation in the tumour microenvironment. We have used an *in vivo* model of tumour development in immunocompetent mice in which the animals are treated with an LXR agonist or vehicle 7 days after establishment of the primary 3LLR tumour. In this model, LXR activation is able to limit tumour growth in wildtype but not in LXR-deficient mice, suggesting that, despite direct anti-proliferative actions of LXRs on tumour cells, LXR expression in the microenvironment is essential for the anti-tumoral response *in vivo*. Dissection of the cellular components within the tumour microenvironment indicates that LXR activation does not affect myeloid cell frequencies in the tumour. However, further analysis revealed that treatment with the LXR agonist reshapes the transcriptional response of tumour-associated macrophages, counteracting the production of chemokines with important roles in the establishment of an immunesuppressive environment.

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Oral presentations

Research Group 4: Infectious Disease

Griffin Fresco

Rhesus Macaque Myeloid-Derived Suppressor Cells Demonstrate T Cell Inhibitory Functions and are Transiently Increased After Vaccination

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Myeloid-derived suppressor cells (MDSCs) are major regulators of T cell responses in several pathological conditions. Whether MDSCs increase and influence T cell responses in temporary inflammation, such as after vaccine administration, is unknown. Using the rhesus macaque model, critical for late-stage vaccine testing, we demonstrate that monocytic (M)-MDSCs and polymorphonuclear (PMN)-MDSCs can be detected using several of the markers used in humans. However, while rhesus M-MDSCs lacked expression of CD33, PMN-MDSCs were identified as $CD33^{+}$ low-density neutrophils. Importantly, both M-MDSCs and PMN-MDSCs showed suppression of T cell proliferation in vitro. The frequency of circulating MDSCs rapidly and transiently increased 24 hrs after vaccine administration. M-MDSCs infiltrated the vaccine injection site but not vaccine-draining lymph nodes. This was accompanied by upregulation of genes relevant to MDSCs such as arginase-1, IDO1, PDL1 and IL-10 at the injection site. MDSCs may therefore play a role in locally maintaining immune balance during vaccine-induced inflammation.

Keywords: Rhesus Macaques, Myeloid-Derived Suppressor Cells, Low-Density Neutrophils, CD33, Vaccination

Staphylococcal enterotoxins dose-dependently modulate the generation of myeloid-derived suppressor cells

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Staphylococcus aureus is one of the major human bacterial pathogens that can cause a broad spectrum of serious infections including skin and orthopedic infections, pneumonia and sepsis. Myeloid-derived suppressor cells (MDSC) represent an innate immune cell subset capable of suppressing T-cell responses in cancer, infectious and inflammatory diseases. Their role in the pathogenesis of *S. aureus* infections has been incompletely understood. The aim of this study was to determine the influence of different *S. aureus* strains and associated virulence factors on human MDSC generation. Using an *in vitro* MDSC generation assay we demonstrate that low concentrations of supernatants of different *S. aureus* strains led to an induction of functional polymorphonuclear MDSC (PMN-MDSC), whereas increased concentrations reduced MDSC numbers. In addition, the concentration-dependent reduction of MDSC correlated with T cell proliferation and cytotoxicity. Staphylococcal enterotoxins A and B showed the same concentration-dependent MDSC induction and inhibition, T cell proliferation and cytotoxicity as complete supernatants of *S. aureus* strains. Furthermore, a mutated enterotoxin-deficient *S. aureus* strain exhibited an approximately 100-fold weaker effect in the modulation of MDSC compared to its wildtype *S. aureus* strain. A distinct *S. aureus* strain (NCTC 8325), which was unable to reduce MDSC numbers at increased supernatant concentrations, hardly expressed any enterotoxins. Taken together, we identified staphylococcal enterotoxins as main modulators of MDSC generation. Interestingly, the inhibition of MDSC by increased concentrations of enterotoxins strikingly outweighed the previously reported MDSC induction by GM-CSF, *Pseudomonas aeruginosa*, *Aspergillus fumigatus* and IL-2. The inhibition of MDSC generation by staphylococcal enterotoxins might represent a novel therapeutic target in *S. aureus* infections and in cancer patients.

The expansion of myeloid derived suppressor cells after combination therapy with anti-PD-L1 antibody and depletion of regulatory T cells during acute retroviral infections

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Cytotoxic CD8 T cells eliminate some malignancies and acute viruses. During the chronic phase of viral infection like HIV and HBV and in patients with growing tumours, the virus-specific $CD8^+$ T cells become exhausted. The exhausted cells enhance the expression of inhibitory receptors like PD-1. Regulatory T cells (Tregs) and the subpopulation of myeloid cells (MDSCs) also regulates the functionality of effector $CD8^+$ T cells. The treatment directed on inhibitory receptors PD-1 and CTLA4 is applied in the therapy of some tumors and proposed for treatments of chronic infections. The combination of treatments directed on inhibitory receptors with treatments directed on Tregs is the one possible development of antitumor and antiviral immunotherapy. In order to define the influences of this combination therapy (CT) on the MDSCs the Friend retrovirus (FV) mice model was used. The Friend retrovirus induces an antiviral $CD8^+$ T cell response during acute FV infection; however during the chronic phase virus-specific $CD8^+$ T cells become exhausted. The treatment was performed during the acute phase of FV infection in order to enhance the elimination of virus and prevent viral chronicity. The CT was associated with an expansion of cytotoxic $CD8^+$ T cells accompanied with an expansion of granulocytic and monocytic MDSCs. Both populations of MDSCs expressed high levels of inhibitory ligands (CD270, PD-L1, CD80 and MHC II). Understanding the interaction of different immunoregulatory mechanisms and compensatory effects during combination checkpoint blocking immunotherapy will help to develop novel and safe treatments for chronic infections and malignancies.

Poster presentations

Research Group 1: Cancer

Fisherman fresco

An increased level of MDSCs in peripheral blood of patients with colorectal cancer after endoscopy treatment

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Introduction: Many cases of early stage of colorectal cancer (CRC) are treated endoscopically, however the risk of recurrence is still high. Poor prognosis and reduction of the therapy's effectiveness in cancer are often associated with myeloid derived suppressor cells (MDSCs). MDSCs are a heterogeneous population characterized by immature state and ability to suppress immune response revealed by suppression of T effector cells and induction of regulatory T cells. MDSCs may be divided into two main subpopulations of monocytic Mo-MDSCs and granulocytic Gr-MDSCs origin. It has been shown that Gr-MDSCs preferentially settle the peripheral lymphoid organs, whereas Mo-MDSCs prevail in the tumor site.

Aim: Compare the level of MDSCs in peripheral blood of patients with colorectal cancer before and after endoscopy.

Materials and Methods: Flow cytometry analysis of peripheral blood mononuclear cells isolated by density gradient centrifugation of peripheral blood from 46 adult patients with CRC and 24 adult healthy controls was performed. 17 CRC patients from the study group were subjected for endoscopy. Immunophenotyping of MDSCs was performed with the following monoclonal antibodies: anti-CD11b-BV510, anti-CD14-FITC, anti-CD15-PECY7, anti-HLA-DR-PerCp. Populations of MDSCs were characterized as HLA-DR$^-$, CD11b$^+$, CD15$^+$ or CD14$^+$, describing granulocytic and monocytic MDSCs, respectively.

Results: The level of Gr-MDSCs and Mo-MDSCs was significantly higher in the blood of patients with CRC when compare to healthy controls and the level of Mo-MDSCs positively correlated with the level of Treg cells. After endoscopy, the level of Mo-MDSCs increased comparing to that detected before the treatment. This was associated with a significantly higher risk of the tumor recurrence in this group of patients.

Conclusion: Increased level of Mo-MDSCs in peripheral blood after endoscopy may be related to their release from tumor site and may be responsible for more frequent tumor recurrence.

Keywords: myeloid-derived suppressor cells (MDSCs), colorectal cancer (CRC), flow cytometry

Clinical relevance of MDSC in head and neck squamous cell carcinoma (HNSCC)

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Myeloid-derived suppressor cells (MDSC) are a heterogeneous group of pathologically expanded myeloid cells with immunosuppressive activity. In human disease three major MDSC subpopulations can be defined as monocytic M-MDSC, granulocytic PMN-MDSC and early stage e-MDSC, which lack myeloid lineage markers of the former two subsets. Within the PMN-MDSC additional subsets comprising different stages of activation and differentiation exist. At present, the relevance of each of these subsets for immunosuppression and disease outcome is not clear.
We determined the frequency of PMN-MDSC, M-MDSC and e-MDSC in the peripheral blood of patients with head and neck cancer and found that a high frequency of PMN-MDSC most strongly correlated with poor overall survival. T cell suppressive activity was higher in PMN-MDSC compared with M-MDSC and e-MDSC. Expression of CD11b and CD16 was used to define PMN-MDSC subsets. $CD66b^+/CD11b^+/CD16^+$ defined a subpopulation of mature cells, which were superior to the other subsets in suppressing proliferation and cytokine production of T cells in multiple test systems. High levels of this $CD11b^+/CD16^+$ PMN-MDSC, but not other PMN-MDSC subsets, strongly correlated with adverse outcome.
In patients with head and neck cancer, we identified the circulating MDSC subset with the strongest immunosuppressive activity and the highest clinical relevance.

Comparative analysis of transcriptomic data obtained from breast and colorectal cancer granulocytic-like myeloid derived suppressor cells (G-MDSCs)

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Even though certain markers are associated with the myeloid-derived suppressor cells (MDSCs) which interfere with anti-tumor immunity and facilitate tumor progression, conflicting results have been reported basically due to the heterogeneity of this cell type. Not only the differences in laboratory practice but also the distinct biology of the cancers may be responsible for this variation. Here, the transcriptomic differences between peripheral blood G-MDSCs obtained from breast and colorectal cancer were evaluated following a stringent characterization with immunophenotype and functional analyses. For this purpose, peripheral blood leukocytes were isolated using by Ficoll1077 gradient centrifugation. Myeloid cells were labelled with anti-CD45, -CD11b, -CD66b, -CD14, -CD33, -CD125, -CD14, -CD16, -HLA-DR, -CD15 antibodies and $CD66b^{+}CD33^{mo}$ cells in $CD45^{+}CD11b^{+}CD125^{-}CD14^{-}HLA\text{-}DR^{-/lo}$ sub-population were purified by FACS. CD16 and CD15 expression was tested as immunophenotype confirmation. Morphological aspects were evaluated by May-Grünwald Giemsa staining. Suppressive function of the patient-derived purified G-MDSCs was tested in a CFSE-based T cell proliferation assay where healthy-donor $CD8^{+}$ T cells employed. Subsequently, following total RNA extraction and RNA-seq library preparation, next-generation sequencing (NGS) was performed to reveal G-MDSCs' transcriptomic profile. Here, we report distinctions and similarities between mRNA profiles of G-MDSC populations (which were isolated with same procedures and immunophenotype and whose suppressive function were confirmed) from two different cancer types. These data indicate the heterogeneity of MDSCs which are isolated/determined with current immunephenotyping strategies.

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Different signalling pathways govern the emergence of macrophages and neutrophils with anti-tumour functions

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Macrophages and neutrophils can represent over 50% of the immune tumour infiltrate, and are usually associated with poor prognosis. However, both of these myeloid cells are remarkably versatile and, in fact they can also act as powerful anti-tumour effectors. Regrettably the effective induction of anti-tumour functions in the innate compartment of myeloid cells remains a major scientific and clinical challenge. Thus, we decided to explore the signaling pathways promoting selectively macrophages and neutrophils to display anti-tumour effector functions within tumours.

We used a preclinical model of triple negative mouse mammary tumour. We found that (up to 100 mm^3) intratumour injection, of costimulatory agonist antibody with any of the following agonists for TLR1/2 (Pam3CSK4), TLR2/6 (Pam2CSK4), TLR3 (PolyI:C), TLR4 (LPS) and TLR9 (CpG) consistently led to complete remission in most treated animals. Strikingly, while the protective effect of PolyI:C treatment disappeared with the ablation of macrophages, tumour regression induced by LPS was impaired upon neutrophil depletion. Noteworthy, neutrophils are dispensable for the elimination of tumour triggered by the other TLR ligands Pam3CSK4, Pam2CSK4, PolyI:C and CpG. This suggests that the local signaling pathways capable of shaping macrophage and neutrophil responses to the tumour are different and non-overlapping.

We went on to characterise further the effect of PolyI:C on tumour-infiltrating macrophages. Seventy two hours after treatment anti-tumour effectors amongst $CD11b^+Ly6C^+F4/80^+$ macrophages were induced. This was revealed by a significant increase in the proportion of $TNF\text{-}\alpha^+IL\text{-}1\beta^+$ producing and MHC class II expressing macrophages, paralleled by a decrease of PD-L1high macrophages within regressing compared to progressing tumours. In addition, tumour-free survivors were resistant to tumour re-implantation indicating the generation of a long-lasting adaptive immunity. This was consistent with reduction of dysfunctional $PD\text{-}1^+CTLA\text{-}4^+Lag\text{-}3^+$ CD8 T cells, and regulatory T cells, concomitant with induction of $IFN\text{-}\gamma^+TNF\text{-}\alpha^+$ granzyme B^+ CD8 T cells.

Altogether this demonstrates that treatment targeting myeloid subsets can shape the tumour microenvironment through alteration of the tumour-infiltrating macrophages and neutrophils. These results lay the groundwork for further studies that will combine unbiased approaches (transcriptomics) with *in situ* assessment of the biology, functionality and differentiation program of anti-tumour macrophages and neutrophils. Our data will shed new light on the remarkable potential that shaping myeloid cell subsets offers to design novel avenues for immunotherapy.

Modulation of neutrophil functions by gold nanoparticles

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Gold nanoparticles (AuNPs) are promising agents for diverse biomedical applications such as drug- and gene delivery, bio imaging and cancer treatment. Understanding the interaction of AuNPs with immune cells is a key point for the development of safe and efficient therapeutic applications. Therefore, this project aims to analyze the molecular interaction of different types of AuNPs with neutrophil granulocytes, a subset of leukocytes with professional phagocytic activity.
In preliminary experiments we coated spherical and rod-shaped AuNPs with non-toxic polyethylene glycol derivates (PEG) with several functional endings (amino, hydroxy and carboxy) to investigate their uptake by neutrophil granulocytes. We observed that AuNPs accumulated in the lysosomes of neutrophil granulocytes and found no evidence for capturing by NETs. AuNPs were non-cytotoxic to neutrophils over a wide range of concentrations and exposure times.
Future experiments will uncover the molecular and cell biological effects triggered by the interaction of AuNPs with neutrophil granulocytes.

Molecular mechanisms of CCR5 regulation on MDSC in melanoma

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Melanoma microenvironment is characterized by a strong immunosuppressive network, where myeloid-derived suppressor cells (MDSC) play a major role. MDSC represent a heterogeneous population of myeloid cells that fail to differentiate into granulocytes, macrophages or dendritic cells. They were shown to inhibit anti-tumor activity of T and NK cells and stimulate regulatory T cells during tumor progression. MDSC migrate and accumulate in the tumor microenvironment due to the interactions between chemokine receptors and their ligands produced by tumor and stroma cells. We found previously a significant accumulation of MDSC expressing chemokine receptor CCR5 in skin melanoma lesions and metastatic lymph nodes as compared to the peripheral blood and the bone marrow of melanoma-bearing *ret* transgenic mice. This enrichment was associated with increased concentrations of CCR5 ligands and tumor progression. Importantly, tumor-infiltrating $CCR5^+$ MDSC displayed higher immunosuppressive activity than their $CCR5^-$ counterparts. Blocking CCR5/CCR5 ligand interactions increased survival of tumor-bearing mice associated with a reduced migration and immunosuppressive potential in tumor lesions. In melanoma patients, $CCR5^+$ MDSC were enriched at the tumor site that was correlated with enhanced production of CCR5 ligands. Furthermore, $CCR5^+$ MDSC were also enriched in the blood of melanoma patients compared to healthy donors and showed higher production of immunosuppressive molecules than their $CCR5^-$ counterparts.

Here we are deciphering the molecular mechanisms of CCR5 upregulation on MDSC leading not only to their recruitment into melanoma lesions but also to stimulation of their immunosuppressive activity. Studying the effect of cytokine, chemokine and TLR ligand-induced signaling as well as tumor-derived exosomes on CCR5 expression, we found a significant upregulation of CCR5 on immature myeloid cells mediated by TLR2 stimulation. In addition, the in vitro differentiation of MDSC from immature myeloid cells by IL-6 and GM-CSF was accompanied by CCR5 upregulation. Interestingly, $CCR5^+$ tumor-infiltrating MDSC showed increased levels of STAT3 phosphorylation compared to their $CCR5^-$ counterparts.

Altogether, our findings define a critical role for CCR5 in the recruitment and activation of MDSC. We suggest that the targeting of CCR5-positive MDSC could represent a novel strategy for melanoma treatment.

Role of tumor-derived extracellular vesicles in immunosuppression in malignant melanoma patients

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Malignant melanoma is one of the most dangerous forms of skin cancer and accounts for majority of all skin cancer deaths. The accumulation of highly immunosuppressive regulatory leucocytes, especially myeloid-derived suppressor cells (MDSC), plays a significant role in resistance to immunotherapy of malignant melanoma. Extracellular vesicles (EVs) are membrane-bound carriers with complex cargos containing proteins, lipids, and nucleic acids. They include microvesicles, exosomes and apoptotic bodies. Tumor-derived EVs can promote the progression, invasion and metastasis of cancer. In particular, they can trigger cytokines and chemokine production by immune cells. However, the role of tumor-derived EVs in immune suppressive mechanisms in malignant melanoma and its interaction with MDSCs remain to be explored. The aim of this investigation is to study molecular mechanisms of interactions of tumor-derived EVs with myeloid cells in melanoma patients leading to their conversion into MDSC and to further stimulation of their immunosuppressive functions. We found that tumor-derived EVs can induce the anti-apoptosis ability of human $CD14^+$ monocytes from healthy donor via the upregulation of BCL-2. Moreover, they could upregulate the PD-L1 expression and activate the NF-κB signaling pathway in these cells, which is mediated by toll-like receptor (TLR) 4. In addition, specific miRNA were shown to be inserted into tumor-derived EVs and taken up by immature myeloid cells. We found also that melanoma-derived EVs expressed proteins and miRNA with regulatory functions. We suggest that EV measurement will help to establish their prognostic value in melanoma patients treated with various immunotherapies.

Poster presentations

Research Group 2: Haematology

Festos disk

IL-10 causes emergency myelopoiesis

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Hematopoiesis is a highly complex and dynamic process. Cell fate decisions during this process depend on external cues, some of which are provided by the bone marrow (BM) microenvironment. Immunologic stress, for example during cancer and infection, changes the hematopoietic output to guarantee a proper supply of immune cells. IL-10, produced during most if not all immune responses, stands out as a major inhibitor of inflammation. During infection, the production of IL-10 is critical to manage the delicate balance between suppressing and activating host responses, hence between the establishment of chronicity or of pathogen clearance often accompanied by tissue damage detrimental to the host. Understanding the various implications of IL-10 to immune homeostasis is of unquestionable importance, due to potential IL-10 administration for clinical therapy of inflammatory diseases. Interestingly, several studies have shown an association between IL- 10 and the pathogenesis of hematopoietic disorders, such as B cell lymphoma, thus suggesting a possible involvement of IL-10 as a regulator of the hematopoietic process.
Using a mouse model of inducible IL-10 over-expression (pMT-10) we show that an excess of IL-10 in the organism drives profound hematological alterations, most notably increased myeloid cell production by the BM, development of anemia and extramedullary myelopoiesis with splenomegaly. The hematologic alterations observed required signaling through the IL-10 receptor (IL-10R) complex, since pMT-10 animals deficient for the IL-10Rα chain display a normal phenotype upon induction of IL-10 expression. Further genetic manipulation of the pMT-10 model combined with reconstitution experiments support a key role for T cells in the mechanism of IL-10-driven myelopoiesis.
Overall, our data shows that IL-10 over-expression changes the normal hematopoietic output, triggering myelopoiesis. These data add to the complexity of emergency hematopoiesis and to our understanding of hematopoietic deregulation by inflammation and infection. We are now further exploring our findings by identifying the IL-10 primary target population, assessing the existence of other molecular mediators involved in the phenotype and investigating the potential of the cellular subsets matured in an IL-10 conditioned environment as myeloid-derived suppressor cells.

Myeloid-derived suppressor cells frequency in myeloproliferative neoplasms

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Myeloproliferative neoplasms (MPN) are a group of hematopoietic stem cell-derived clonal disorder with defective regulation of myeloid cell proliferation. It encompasses three disorders: essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF). Patients with low risk of thrombohemorrhagic complications commonly require aspirin therapy, while for high-risk patients cytoreductive therapy with hydroxyurea (HU) is recommended as a first-line drug choice. Although circulating myeloid-derived suppressor cells (MDSC) are significantly increased, the mechanism of MDSC expression and the effects of therapy on the level of MDSC in MPN patients are not sufficiently investigated. In this study we determine the frequency of MDSC in bone marrow (BM) of 50 patients including 23 PMF, 16 PV, 11 ET and 2 healthy donors of BM, as well as in peripheral blood (PB) of patients with de novo MPN before and after therapy with aspirin and HU. The level of $CD33^{+}$/HLA-DR^{low}, monocytic $CD14^{+}/CD15^{-}$ or polymorphonuclear $CD15^{+}/CD14^{-}$ MDSC was determined by flow cytometry. We found that both PB and BM MDSC levels in MPN patients were increased compared to control samples. The highest number of PB and BM MDSC was observed in PMF ($p< 0.01$). HU therapy decreased the percentage of PB MDSC, while reduction was not observed neither in PB of patients receiving only aspirin nor in PB of patients with disease progression. Also HU inhibits in vitro MDSC induction from either PB or BM monocytes of five healthy donors with interleukin-6 or tumor growth factor beta with stem cell factor and granulocyte-macrophage colony stimulating factor. Functional test showed that depletion of $CD33^{+}$ cells in MPN samples recovery the autologous T-cell proliferation under CD3/CD28 stimulus, which suggests the immunosuppressive role of $CD33^{+}$ in MPN. Further studies are necessary to understand whether MDSC can be usefully as predictive marker for disease progression, risk and resistance to HU treatment, their role in the molecular classification of MPN and their significance as potential marker in immunotherapy.

Poster presentations

Research Group 3: Inflammation and Autoimmunity

Bull's head Rhyton

Activation of the LXR pathway interferes with the IRF4-CCL17/CCL22 axis in macrophages

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CCL17 and CCL22 are chemokines that are produced within the tumour microenvironment to promote T-regulatory cell recruitment. We have explored transcriptional mechanisms regulating the expression of these chemokines in macrophages. In primary murine bone marrow-derived macrophages, stimulation with IL-4 or GM-CSF induced the expression of both CCL17 and CCL22 in an interferon regulatory factor 4 (IRF4)-dependent manner. These effects were rather selective, as the induction of other genes associated to the acquisition of a macrophage alternative phenotype, such as Arg1, Mgl1 and Mrc1, were upregulated by IL-4 independently of IRF4. Transcriptional activation by IRF4 may occur in collaboration with a number of additional heterodimeric partners, including members of the AP1 family. By using macrophages derived from genetically modified mice, we conclude that JunD and JunB do not collaborate with IRF4 in the induction of CCL17 and CCL22. Interestingly, activation of the LXR pathway with selective agonists inhibits the expression of CCL17 and CCL22 in macrophages stimulated with either IL-4 or GM-CSF and these effects correlated with repression of IRF4 expression without affecting activation of its upstream regulator Stat-6. We have identified and used in reporter assays an enhancer region upstream of the Ccl17 promoter that is responsive to either IL-4 stimulation or IRF4 overexpression. In these studies, LXR activation repressed the activity of the Ccl17 enhancer in response to IL-4 and these effects were abolished upon overexpression of IRF4. Taken together, these results provide relevant mechanistic data about the elements involved in transcriptional regulation of CCL17 and CCL22 in macrophages, which can be targeted by activation of the LXR pathway.

This work was supported by grants from the "Ministerio de Economía y Competitividad" SAF 2010-14989, SAF 2011-23402 and SAF 2014-57856-P to A. Valledor. JM Carbó was granted an APIF fellowship 2014 from University of Barcelona.

Characterisation of populations of circulating neutrophils in patients with psoriasis

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Psoriasis is one of the most common dermatological and autoimmune diseases that affects about 1 – 2 % of the world population. Various neutrophil phenotype and function alterations have been reported in autoimmune patients, suggesting that dysfunctional neutrophils contribute to pathogenesis of psoriasis. At least two populations of circulating neutrophils have been described in humans; low density granulocytes (LDGs), and high or normal density granulocytes (PMNs). Here we demonstrate that LDGs are present in elevated levels in psoriasis patients when compared to healthy individuals. We also show that shape of LDG nucleus and the profile of surface molecule expression was consistent with a mature neutrophil phenotype of LDGs. Given that in psoriasis patients LDGs were found to display higher propensity for neutrophil extracellular traps (NET) formation compared with PMNs, we hypothesized that LDGs and PMNs differ in levels of unrestrained elastase (NE) that supports NET generation. Here we demonstrate that LDGs are much more immunoreactive for NE and less for the main NE inhibitor in neutrophils, SLPI, compared with PMNs. However, the difference in immunoreactivity did not result from different protein levels of these proteins nor manifested in higher proteolytic activity of NE in LDGs. These distinct attributes may provide an independent measure of the total contribution of LDGs and PMNs to psoriasis conditions.

Genetic influence on frequencies of blood cell subpopulations in mouse

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Inborn differences among individuals in frequencies of blood cell subpopulations might influence outcome of many acute and chronic conditions such as susceptibility to infections, atopic and cardiovascular diseases and cancer.
We have analyzed percentage of cells subpopulations in the spleens of mouse strains O20, C57BL/10 and B10.O20 using flow cytometry. Mice were kept in SPF conditions. We observed tendency to higher frequency of T cell lineage cells and lower numbers of myeloid derived cells in O20 in comparison with C57BL/10. The strain B10.O20, carrying 3.6% of genes of the O20 strain on C57BL/10 background, had dramatically lower frequency of T cell subpopulations and higher frequency of myeloid derived cells than both parents.
To determine the location of O20 gene(s) responsible for differences in blood cells frequencies in B10.O20, we analyzed cell frequencies in spleens of F2 hybrids between C57BL/10 and B10.O20. B10.O20 carries O20-derived segments on four chromosomes. They were genotyped in the F2 hybrid mice and their linkage with frequencies in blood cell subpopulations was tested by analysis of variance (ANOVA). We have sequenced genomes of C57BL/10 and O20 using next generation sequencing and performed bioinformatics analysis of the chromosomal segments exhibiting linkage with frequencies in blood cell subpopulations.
Linkage analysis revealed three novel loci. The most precise mapping (2 Mb) was achieved for locus on chromosome 1, which controls numbers of eosinophils ($CD11^+Gr1^-Siglec\text{-}F^+$) and in interaction with the locus on chromosome 17 frequency of CD11bSiglechi subpopulation. Locus on chromosome 18 regulates numbers of $CD19^+$ and $CD19^+CD22^+$ cells. Analysis of these loci for polymorphisms between O20 and C57BL/10 that change RNA stability and genes' functions led to detection of 36 potential candidate genes, 2 of them carrying a non-sense mutation in the O20 strain. These genes will be focus of future studies not only in mice but also in human.

Interplay between LXRs and CCAAT/enhacer-binding protein β (C/EBPβ) in the transcriptional regulation of CD38 during inflammation

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Macrophages are essential players of the innate immune response against pathogens. Following recognition of Pathogen-associated molecular Patterns (PAMPs), they produce inflammatory mediators and trigger phagocytosis to fight against infection. However, some bacteria have evolved to survive within phagolysosomes and use macrophages as a niche to proliferate avoiding humoral immunity. This is the case of *Salmonella*, an enteric bacterium that not only takes profit of phagocytosis by macrophages but also fosters its own uptake by non-phagocytic cells, such as epithelial cells. Liver X Receptors (LXRs) are ligand-activated transcription factors with relevant activities in the regulation of metabolism and the immune response. Recently, our group identified a new role for LXRs reducing macrophage infection by invasive *Salmonella Typhimurium* through transcriptional activation of the multifunctional enzyme CD38 (Matalonga et al., Cell Rep 2017). CD38 is a type II transmembrane glycoprotein that catalyzes the conversion of NAD^+ into nicotinamide, ADPR and cADPR. Remarkably, pharmacological treatment with an LXR agonist ameliorated clinical signs associated to *Salmonella* infection *in vivo* and these effects were dependent on CD38 expression in bone marrow-derived cells. Interestingly, inflammatory mediators and bacterial components act synergistically with LXR agonists to induce CD38 expression and NADase activity in macrophages. Luciferase reporter studies indicated that an LXR response element located 2kb upstream of the CD38 transcription initiation site is important for the synergistic induction of CD38 by LPS and an LXR agonist. In order to explore mechanisms for cooperation between LPS and the LXR pathway, we have investigated the role of the transcription factor CCAAT/enhancer-binding protein β (C/EBPβ) in the transcriptional control of CD38. C/EBPβ is highly expressed in macrophages and exerts complex regulatory functions on target genes, as transcriptional activator and repressor isoforms co-exist and they can heterodimerize with other members of the C/EBP family. Mice with myeloid C/EBPβ deficiency were generated by crossing LysMCre and C/EBPβ$^{fl/fl}$ mice. Interestingly, CD38 expression underwent de-repression in C/EBPβ-deficient macrophages in comparison to wildtype cells, whereas induction by inflammatory cytokines, bacterial lipopolysaccharide (LPS) or LXR agonists was drastically impaired. Altogether, these results suggest significant crosstalk between LXRs and C/EBPβ to tightly regulate CD38 expression in the inflammatory context.

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Monocytes from patients with coeliac disease display suppressed inflammatory potential

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Coeliac disease (CD) is an autoimmune enteropathy precipitated by gluten in genetically predisposed individuals. CD affects approximately 1% of the population, can develop at any age, with many cases undiagnosed and untreated for years. Infiltration of monocytes into duodenal mucosa is a rapid and prominent part of the immune response associated with gluten exposure in individuals with CD. However, monocyte role in this response and their contribution to the pathology of CD is not well defined. By transcriptional analysis of purified $CD14^+$ monocytes from 14 untreated CD patients and 14 controls we showed that circulating monocytes from individuals with CD displayed gene expression profiles compatible with attenuated pro-inflammatory potential, exemplified by lower expression of Jun and Fos family transcription factors. These changes in gene expression were stable and detectable in $CD14^+$ monocytes from the same patients six months after diagnosis and introduction of gluten-free diet. Additionally, analysis of purified $CD14^+$ monocytes from 16 treated CD patients (median 109 months of gluten-free diet, range 26 - 239) and 12 controls showed similar downregulation of pro-inflammatory pathways. Preliminary data showed lower spontaneous and stimulated production of pro-inflammatory cytokines in $CD14^+$ monocytes from CD patients that have been on gluten-free diet for years. Together, our results point towards a suppression of the inflammatory potential of $CD14^+$ monocytes from CD patients that persists over time even when the disease is not active.

Role of mTOR in inducing trained immunity and ROS generation in oxLDL primed monocytes

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Cardiovascular diseases (CVDs) remain one of the leading causes of death in the world. There are increasing evidences that the cells of innate immune system particularly monocytes/macrophages are pivotal players both during the initial insult and the chronic phase of CVDs. Recently it has been shown that oxidized low-density lipoprotein (oxLDL) induces long term pro-inflammatory responses in monocytes due to epigenetic and metabolic reprogramming which is part of an emerging new mechanism called trained immunity. Priming of human monocytes with oxLDL lead to significantly increased TNF-α and IL6 production following PAM3 stimulation. However, this trained immunity was abolished in the presence of the specific mTOR inhibitor, Torin1. While trained monocytes displayed significantly increased reactive oxygen species (ROS) production, the level of ROS was decreased in the Torin1 treated group. Finally, treatment with an HDAC-inhibitor, which has been shown to increase ROS production in monocytes, was sufficient to induce a long-term pro-inflammatory response similar to oxLDL priming.
Altogether, our data suggest that mTOR activity and ROS production control oxLDL-induced trained immunity in human monocytes. Pharmacologic modulation of these pathways might provide a promising approach to modulate inflammation during atherosclerosis development**.**

Standardizing functional MDSC co-culture assays with T-cells

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Myeloid-derived suppressor cells are immature cells of myeloid origin that contribute to the immunosuppressive microenvironment within tumors. As there are no known MDSC specific markers yet, functional assays are required to assess their immunosuppressive potential. In humans, T cell proliferation assays are most commonly used to show MDSC suppressive activity. However, the protocols for this assay vary widely among the different research groups, making the comparison of data difficult. We propose to develop a standardized and sharable T-cell proliferation assay protocol. To achieve this, we collaborate with four Mye-EUNITER laboratories. We identified four critical parameters: the purity of the sorted T cells, the sorting method used to isolate T effector cells, the method of T cell stimulation and the use of appropriate controls.

To optimise and compare the assay between the four participating laboratories we used frozen allogenic CD3 positive cells isolated from hemachromatosis donor blood as effector T cells. The cells were exchanged between groups. We observed that T-cell purity was a critical parameter, as cells with high purity (>99% $CD3^+$) were unable to proliferate properly when they were stimulated with anti-CD3 and anti-CD28 antibodies. However, adding small amounts of myeloid cells (dendritic cells, MDSCs or monocytes) restored proliferation capacities. These data indicate that T-cells require the presence of myeloid cells to proliferate. To compare directly between laboratories, we propose to supplement 5 to 10% irradiated THP-1 myeloid cells. However, this approach was unsuccessful in the other participating labs. We are thus currently exploring using allogenic mature dendritic cells instead.

In addition, the T-cell sorting method impacts T-cell proliferation. We compared three different sorting methods (CD3 positive selection, NK depletion on CD2 positive fraction and CD4/CD8 positive selection) and we observed that using anti-CD3 magnetic beads decreased the proliferation of the sorted T-cells, probably by masking the CD3 epitopes or causing low-level CD3 triggering during sorting. Thirdly, we observed that the choice of the stimulus was also critical. Indeed, we noted that anti-CD3/anti-CD28 beads were phagocyted by MDSCs and macrophages, which could then reduce the amount of beads available to the T-cells, decreasing the proliferation rate and inducing a bias in the experiment. We instead recommend to use plate-bound or soluble anti-CD3 and anti-CD28 antibodies for T cell stimulation.

Finally, we observed that only functional positive and negative controls (i.e. unstimulated and stimulated T cells without MDSC) are commonly used. We instead propose to use biological controls in the form of T cell activating mature dendritic cells and immunosuppressive DC-10s in addition.

We continue to work towards a standardized and exchangeable T cell proliferation assay to assess MDSC immunosuppressive functions more comparably. We identified several key parameters that influence the success of the assay. We continue to optimise the protocol in collaboration with the other four laboratories.

Roles of IRF-1 in the control of LXR-mediated gene expression

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Lipid metabolism has emerged as an important modulator of immune cell fate and function. Liver X receptors (LXR) are druggable members of the nuclear receptor family of transcription factors that regulate lipid and glucose homeostasis and exert multiples roles at the interface between metabolism and the immune system. Pharmacological LXR activation with a high affinity synthetic agonist induces the expression of genes involved in cholesterol efflux (e.g. ABCA1 and ABCG1), and downregulates the expression of Idol, an E3 ubiquin-ligase that mediates degradation of the low density lipoprotein receptor. Recent work from our group has revealed that LXR activation also induces the expression of the enzyme CD38 in macrophages. CD38 is a type II transmembrane glycoprotein that catalyzes the conversion of NAD^+ into second messengers (e.g. cADPR and NAADP) for intracellular $Ca2^+$ mobilization. However, the main role of CD38 enzymatic activity in mammals seems to be the control of NAD^+ levels in tissues. We have previously reported reciprocal crosstalk between IFN-γ signaling and the LXR pathway in macrophages. IFN-γ involves activation of the JAK-STAT1 pathway to promote the expression of primary IFN response genes, among them the transcriptional regulator IFN regulatory factor-1 (IRF-1). Our aim was to characterize the role of IRF-1 in the crosstalk between IFN-γ and LXR activation. We compared the effects of IFN-γ stimulation on the expression of LXR target genes involved in cholesterol and fatty acid homeostasis and in NAD metabolism in macrophages from wildtype and IRF-1-deficient mice. IFN-γ signaling resulted in reduced induction of the cholesterol transporter ABCA1 and the apoptosis inhibitor AIM by LXR, but these effects were independent of IRF-1. In contrast, IFN-γ signaling and LXR cooperated in transcriptional induction of CD38 and, to our surprise, such cooperation was drastically enhanced in IRF-1-deficient macrophages. These results suggest that IRF-1 serves to fine-tune the expression of CD38 during the macrophage response to IFN-γ.

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Poster presentations

Research Group 4: Infectious Disease

Minoan seal

Human monocytic suppressive cells promote replication of Mycobacterium tuberculosis and alter stability of in vitro generated granulomas

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Tuberculosis (TB), a bacterial disease primarily affecting the lung, causes high rates of morbidity and mortality worldwide. Despite extensive research, TB pathogenesis remains incompletely understood. Our research focuses on myeloid cells, including myeloid derived suppressor cells (MDSCs), and employs various models to investigate roles of these cells in TB. To understand the function of human MDSCs in TB we have generated monocytic MDSCs and analyzed their interactions with *Mycobacterium tuberculosis* (Mtb), the causative agent of TB. MDSCs retain suppressive properties after Mtb infection. More recently, we have utilized an *in vitro* granuloma model, which mimics to some extent human TB pathology to analyze dynamics of human MDSCs within granulomas. We observed that the MDSCs alter the structure and affect bacterial containment properties of these granuloma like structures in a process depending on their capacity to release IL-10. Further, we found that the differential upregulation of distinct signaling pathways in MDSCs, when compared to monocyte-derived macrophages, underlies their heightened propensity to produce this regulatory cytokine. Moreover, we observed an MDSC-PDL-1 dependent CD8 suppression in granuloma like structures, albeit with negligible effects on Mtb replication. A comprehensive characterization of roles of human MDSCs in TB will help design novel, host-directed therapies against this deadly infection.

The effect of dialysable leucocyte extract on myeloid cells population in experimental larval cestodiasis

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Larval cestodiases are chronic parasitic infections, which are still very common in developing countries. These life-threatening diseases are caused by larval stages of cestodes (metacestodes) or tapeworms. The growth of metacestodes leads to the hepatic tissue destruction and finally to liver failure of their host. Infections with larval stages of tapeworm are usually asymptomatic for a long period of time and metacestodes expansion in hosts' tissues is associated with suppression of immune response of their host.
A potential immunomodulatory effect of low molecular weight leukocyte extracts from human donors (IMMODIN, ImunaPharm, Slovakia) was investigated on mice with experimental *Mesocestoides vogae* infection.
Preliminary results show that standard anthelmintic therapy in combination with leucocyte extract contributes to the reduction of the $CD11b^{high}Gr\text{-}1^{+}$ myeloid cells in mice suffering from larval cestodiasis. In addition, the population of $CD11b^{high}Gr\text{-}1^{high}$ myeloid cells expresses higher levels of F4/80 and MHC class II molecules. Moreover, decreased concentration of the immunosuppressive cytokine TGFβ in the peritoneal cavity of treated mice is correlated to the change of myeloid cells number. The results of this study will be confirmed by molecular methods.
Based on this, we assume that the adjuvant therapy by leucocyte extract may contribute to the elimination of heterogeneous population of myeloid cells and moreover to support their differentiation into maturate cells.

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The effect of TGF-beta receptor 2 signaling on LysM^{+} myeloid cells in Mycobacterium tuberculosis infection in mice

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Mycobacterium tuberculosis (Mtb) caused 10.4 million recorded cases of tuberculosis (TB) and 1.7 million recorded deaths in 2016. Understanding of the optimal immune responses required for bacterial killing and the mechanisms regulating lung pathology and bacterial spreading is incomplete. The cytokine TGF-beta (TGF-β) is known to be upregulated in active pulmonary TB and negatively affects the ability of macrophages to kill bacteria *in vitro*. However, TGF-β is also thought to induce a suppressor macrophage phenotype that could be important in downregulating excessive immune responses that play a role in lung pathology. Therefore we generated LysM^{cre}TGFβR2$^{lox/lox}$ mice, which lack TGFβR2 signaling on monocytes, macrophages and neutrophils, to elucidate the role of TGF-β signaling on these cells during TB. Male and female LysM$^{+/+}$, LysM$^{+/-}$ and LysM$^{-/-}$ TGFβR2$^{lox/lox}$ mice were infected with 200 cfus Mtb H37RV by aerosol. Interestingly, bacterial loads were significantly higher in lungs of mice with defective TGF-βR2 signaling on LysM^{+} cells. Pathology was also more severe in the lungs of LysM$^{+/+}$TGFβR2$^{lox/lox}$ mice, with increased cell infiltration. At day 28 post infection, LysM$^{+/+}$TGFβR2$^{lox/lox}$ mice had increased Ly6C^{high} myeloid cells and neutrophils in the lungs, and increased numbers of infected neutrophils, suggesting an effect of TGF-β signaling on neutrophil function. Alveolar macrophages and neutrophils from infected LysM$^{+/+}$, LysM$^{+/-}$ and LysM$^{-/-}$ TGFβR2$^{lox/lox}$ mice and controls were isolated by cell sorting, and we are currently performing transcriptomics analysis to investigate the effect of TGF-β signaling on gene expression in these cells.

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Notes

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