MACRO
Macrophage Club
Région Occitanie

Co-organized by

MCM
Macrophage Club
Montpellier

MCT
Macrophage Club
Toulouse

SOMM
2018

2ND SYMPOSIUM OF THE OCCITANIE NETWORK ON MONOCYTES-MACROPHAGES

INVITED SPEAKERS

Charlotte Scott *(Ghent, Belgium)*
Hal Drakesmith *(Oxford, UK)*
Elisa Gomez-Perdiguero *(Paris, France)*
Dominique Baeten *(Amsterdam, Netherlands)*
Sandrine Henri *(Marseille, France)*
Audrey Varin *(Toulouse, France)*
Florence Perrin *(Montpellier, France)*
Hélène Authier *(Toulouse, France)*
Arnaud Jacquel *(Nice, France)*

FRIDAY, 30 NOVEMBER 2018 TOULOUSE

Auditorium Institut Universitaire du Cancer Toulouse (IUCT) Oncopole

For any question and request please contact us at: somm2018@sciencesconf.org

EDITORIAL

Welcome to Toulouse

The Macrophage Club of Toulouse (MCT) and Macrophage Club of Montpellier (MCM) are pleased to organise the Second Symposium of the Occitanie network of Monocytes-Macrophages (SoMM2018). This meeting is the expression of a joined initiative between the scientific communities of Montpellier and Toulouse aiming at gathering experts of the novel district named Occitanie around the biology of monocytes/macrophages. We launched a new logo MACRO for MAcrophage Club of Region Occitanie to illustrate the objective of our scientific community to sustain exchanges in our region on monocyte and macrophage biology.

In an intense day meeting of 100 junior and senior researchers from the academic and private fields, cutting edge work on monocyte/macrophage biology will be presented. This second edition of SoMM in Toulouse will address through 5 Keynotes, 4 lectures, 6 selected oral short communications and 8 posters, numerous appealing topics in monocyte and macrophage biology including differentiation, ageing and tissue identity, inflammation, infection, iron & immunity, microglia, as well as melanophage in the skin.

We hope that SoMM2018 will be an interesting meeting for you and that you will enjoy your stay in Toulouse.

On behalf of the scientific Committee,

Scientific Committee
Florence APPARAILLY (Montpellier)
& François CANONNE-HERGAUX (Toulouse)

MCM Macrophage Club Montpellier

MACRO Macrophage Club Région Occitanie

MCT Macrophage Club Toulouse

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VENUE

IUCT ONCOPOLE

1, avenue Joliot-Curie - IUCT-O
31 059 Toulouse Cedex 9

How to get to the IUCT Oncopole?

By bus

- Bus route 3 (Saint Cyprien - République/Oncopole) Oncopole bus stop
- Bus route 11 (Basso Cambo / Empalot) IUC bus stop
- Bus route 52 (Empalot / Roquettes église or Pinsaguel Mairie) - IUC bus stop
- Bus route 50 (Basso Cambo / Roques acacias) Larrieu bus stop
- Bus route 117 (Muret railway station - Potier Oncopole) Silos bus stop (direct connection)

Before leaving, check routes and schedules on the site www.tisseo.fr

Some connecting lines (south-west Toulouse area):
- Bus route 49 (Basso Cambo – Portet railway station)
- Bus route 53 (Basso Cambo – Tibaous / Portet)

By coach

'Arc-en-ciel' intercity network bus route Nos. 18, 19, 58, 59, 61, 64 and 80 stop at the IUCT Oncopole site at the Silos bus stop.

By train

TER rail route 16 (Auch-Colomiers-Toulouse) stops at the Galliéni Cancéropole Train Station, 3 km from the IUCT Oncopole. The station is connected to the bus network (routes 11 and 52).

By car

Take the ring road and take exit no. 25 or 38. There is drop-off area outside the main entrance of the building. Possibility to park for free at the IUT car park (700m from the congress venue).

INVITED SPEAKERS

Charlotte SCOTT

Department of Biomedical molecular biology, University of Gent, Gent, Belgium
Postdoctoral Researcher
(Martin Guilliams - ONSET group)

Research description:

ONTOGENY AND FUNCTIONAL SPECIALIZATION OF MYELOID CELL SUBSETS (ONSET)

Our research focuses on the development and functional specialization of macrophages (MΦs) and dendritic cells (DCs). To unravel the role of MΦs and DCs in the regulation of immune responses in vivo we have constructed novel DTR- or CRE- expressing knock-in mice that allow to deplete a particular MΦ or DC subset specifically in vivo or to knock-down genes of interests specifically within these cells. These novel knock-in mouse models include mice specific for liver resident Kupffer Cells and lung-resident Alveolar Macrophages. We are particularly interested in: (i) identifying the transcription factors that drive DC and MΦ development, (ii) unraveling how tissue-resident macrophages participate to the maintenance of tissue homeostasis and (iii) understanding how inflammation influences the development and function of DC and MΦ subsets.

GHENT UNIVERSITY

KEYNOTE LECTURE 1:
Zeb2 and maintenance of macrophage tissue-specific identities

Arnaud JACQUEL

U1065, C3M, Nice, France
Researcher CRCN INSERM

Research description:

DISSECTING THE MECHANISMS INVOLVED IN MACROPHAGE DIFFERENTIATION OF MONOCYTES AND THEIR ALTERATIONS IN CMML

The differentiation of peripheral blood monocytes into resident tissue macrophages can be recapitulated ex vivo by CSF-1. We recently established that proper macrophagic differentiation of monocytes required both caspase and autophagy activation and pinpointed a novel and highly specific mechanism of caspase activation during this process. This may explain the limited number of cleaved substrates at sites different from those cleaved during apoptosis observed during monocyte differentiation. In this context, the main objectives of the project are to decipher this original mechanism of caspase activation, understand the specificity of cleavage and the role of the cleaved fragments and define the interplay between caspases and autophagy during CSF-1 mediated monocyte differentiation. We expect to highlight a new mode of caspase activation in a non-apoptotic context, i.e. the physiological differentiation of monocytes and more widely during hematopoietic cell differentiation. Finally, understanding these mechanisms of differentiation will allow us to better understand the pathophysiology of the chronic myelomonocytic leukemia (CMML) characterized by defect in monocyte differentiation.

LECTURE 1:
Molecular characterization of the mechanisms involved in macrophage differentiation of monocytes

INVITED SPEAKERS

Dominique BAETEN

Amsterdam, Netherland, UCB (originally Union Chimique Belge)
MD PhD, Professor

Research description:

Keeping a part-time position as Professor of Rheumatology at the University of Amsterdam, Dominique Baeten joined UCB in August 2016 where he first lead the New Patient Value Mission in the Immunology Patient Value Unit and is currently Vice-President and Head of the Immuno-Bone Therapeutic Area. His major interest is in early clinical development (bringing solutions to the right patient population) and translational medicine (linking unmet needs back to the cellular and molecular pathways).

His translational research focuses on the immunopathology of chronic inflammatory arthritis, in particular rheumatoid arthritis and arthritis). He aim to identify the immune alterations triggering, driving and perpetuating these diseases in order to a) understand the pathophysiology, b) develop biomarkers for prediction, diagnosis and prognosis (including treatment response), and c) develop and validate innovative targeted therapies.

KEYNOTE LECTURE 2:
Macrophage-derived pro-inflammatory cytokines in chronic arthritis: how one single mediator can lead to completely different phenotypes

Audrey VARIN

Stromalab, Toulouse, France
Researcher

Research description:

Audrey Varin did her thesis in the laboratory of Prof. Georges Herbein at the University of Franche-Comté where she worked on the control of the replication of HIV in macrophages by the accessory proteins of HIV. Then, she left 2 years post-doc at Oxford University in the team of Pr Siamon Gordon where she worked on alternatively activated macrophages and their responses to different pathogens such as bacteria Neisseria meningitidis or H5N1 influenza virus, part of the work done at the University of Hong Kong. This work was continued for 1 year in the laboratory of Prof. Giorgi Trinchieri at the National Cancer Institute in Frederick, Maryland, USA. Since 2011, she is a researcher in the STROMALab unit in Toulouse where she work on the interaction between the innate immune system and mesenchymal stromal cells and its role in tissue regeneration.

LECTURE 2:
Interaction with pro-inflammatory macrophages modulated immunosuppressive function of human mesenchymal stromal cells

Elisa GOMEZ PERDIGUERO

Institut Pasteur, Paris, France
Laboratory Director

Research description:

MACROPHAGES AND ENDOTHELIAL CELLS

Our team is interested in the role of 'resident' macrophages during development, homeostasis and tissue repair. Macrophages are professional phagocytic cells that scavenge dead/apoptotic cells, debris, macromolecules and pathogens, and also produce cytokines, both concurring to tissue homeostasis and repair. They belong to the innate immunity arm of the hematopoietic system.

Within the hematopoietic system that produces all blood cells, tissue 'resident' macrophages are a lineage of myeloid cells that arise from yolk sac-derived progenitors and that self-maintain in their tissue of residency, independently of adult hematopoietic stem cells (HSC).

Resident macrophages from the same lineage, such as liver Kupffer cells, brain microglia, epidermal Langerhans cells, lung alveolar macrophages…, display tissue-specific phenotypes, perform tissue-specific functions and have distinct gene expression profiles. Thus, resident macrophages are a unique system where the respective contributions of ontogeny and environment can be investigated. We will combine methods from the fields of immunology, developmental biology and angiogenesis to understand in vivo the development and lineage-specific function(s) of resident macrophages, thereby opening new venues of research into the interaction between macrophages and endothelial cells during development and in response to tissue damage.

Institut Pasteur

KEYNOTE LECTURE 3:
Development of resident macrophages and their maintenance during ageing

Florence PERRIN

INSERM U1198, Université de Montpellier, France
Laboratory Director, Professor

Research description:

We investigate molecular mechanisms that underlie neurodegeneration processes in the context of spinal cord pathologies. We are particularly interested in spinal cord injury (SCI), a pathology with no current therapy.

One means of analyzing the molecular substrate of degeneration is to identify in each cell type of the spinal cord, changes induced in gene expression levels. In this line, we are developing a genomic integrative analysis to decipher the specific involvement of different cell populations as well as cell-cell interactions in mechanisms and pathogenesis of SCI and other spinal cord pathologies. Using this approach will allow the identification of cell specific candidate genes that may be involved in the mechanisms and pathogenesis of spinal cord diseases.

We have a specific interest in glial cells (such as astrocytes and microglia), the most abundant cells in the mammalian central nervous system. Yet our knowledge about their function in health and disease has been limited but during the last years, more and more evidence show that they are playing a crucial role in several spinal cord and brain pathologies.

MMDN

LECTURE 3:
RNA-Seq analysis of microglia reveals time-dependent activation of specific genetic programs following spinal cord injury

INVITED SPEAKERS

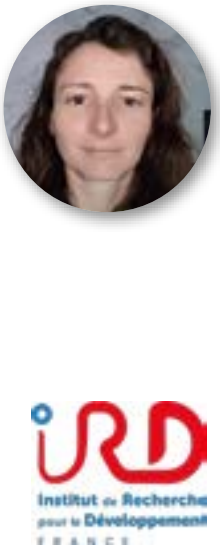
Hal DRAKESMITH

University of Oxford, UK
Associate Professor of immunology

Research description:
IRON AND INFECTION

My lab at the Weatherall Institute of Molecular of Medicine studies the role of iron in infectious diseases and the immune response. Iron is critical for the biochemistry of cells, and is needed equally by host and pathogen; indeed the 'battle for iron' is a key determinant of the outcome of infection. We study the molecular basis of this battle, focusing on hepcidin, the iron regulatory hormone (1, 2). Hepcidin controls iron homeostasis analogously to how insulin controls glucose, but unlike insulin, hepcidin is also an acute phase response gene and is upregulated by inflammation. This innate immune activity of hepcidin reflects the importance of iron regulation for host-pathogen interactions.

KEYNOTE LECTURE 4:
Iron, hepcidin and macrophages: implications for innate and adaptive immunity



Hélène AUTHIER

IRD Pharma-Dev, Toulouse, France
Associate Professor

Research description:
INFLAMMATORY BOWEL DISEASES AND MACROPHAGES POLARIZATION

We are studying the role of macrophages in inflammatory bowel diseases and particularly their polarization and implication of C-type lectin receptors in the course of intestinal chronic inflammation. We identify signaling pathways and molecular mechanisms that control the M1/M2 polarization of macrophages.

LECTURE 4:
Dectin-1 on macrophages promotes inflammatory responses contributing to the severity of inflammatory bowel diseases

Sandrine HENRI

CIML, Marseille, France
Researcher CRCN INSERM
(Bernard Mallissen - group)

Research description:

Sandrine HENRI is a resident scientist who joined the CIML Institute in 2002 after a post-doctoral training in Ken Shortman's laboratory (WEHI, Melbourne, Australia). As a project leader, she supervised postdoctoral fellow, PhD student and technical assistant and made major contributions to decipher dendritic cell and macrophage subsets within peripheral tissues (ie skin and gut) and to the understanding of their origins and functions.

With the use of innovative knock-in mouse models, she is interested to further understand the role of each specific DC and macrophage subsets in the control of adaptive T cell responses and to apply this knowledge to improve vaccination and desensitization.

KEYNOTE LECTURE 5:
Dermal Macrophages: Failure of the phagocytosis process

PROGRAM

FRIDAY, 30 NOVEMBER

08:30 - 08:55 am	**OPENING OF THE REGISTRATION DESK - WELCOME COFFEE**
08:55 - 09:00 am	**SYMPOSIUM INTRODUCTION**
09:00 - 10:30 am	**SESSION 1** Chairs: Céline COUGOULE, Mary POUPOT
09:00 - 09:45	**KEYNOTE LECTURE 1** Charlotte SCOTT (Ghent, Belgium) **Zeb2 and maintenance of macrophage tissue-specific identities**
09:45 - 10:15	**LECTURE 1** Arnaud JACQUEL (C3M, Nice, France) **Molecular characterization of the mechanisms involved in macrophage differentiation of monocytes**
10:15 - 10:30 am	**SELECTED ABSTRACT** Nguyen Chi Mai (DIMNP, Montpellier, France) **Pro-regenerative Protectin D1 controls immune cell function during epimorphic regeneration**
10:30 - 10:45	***Coffee Break***
10:45 - 12:30 pm	**SESSION 2** Chairs: Florence APPARAILLY, Yoann ROMBOUTS
10:45 - 11:30	**KEYNOTE LECTURE 2** Dominique BAETEN (UCB pharma, Amsterdam, Netherlands) **Macrophage-derived pro-inflammatory cytokines in chronic arthritis: how one single mediator can lead to completely different phenotypes**
11:30 - 12:00	**LECTURE 2** Audrey VARIN (Stromalab, Toulouse, France) **Interaction with pro-inflammatory macrophages modulated immunosuppressive function of human mesenchymal stromal cells**
12:00 - 12:15 pm	**SELECTED ABSTRACT** Rémi Planès (IPBS,Toulouse, France) **Localization of Pseudomonas dictates differential NLRC4 inflammasome-conferred host immunity**
12:15 - 12:30 pm	**SELECTED ABSTRACT** Sindhu Kotagudda (Biotis, Bordeaux, France) **Low molecular weight hydrogels as injectable scaffolds for tuning the foreign body reaction**
12:30 - 01:45	***Lunch / Networking***
01:45 - 03:30 pm	**SESSION 3** Chairs: Yannick DEGBOE, Christel VEROLLET
01:45 - 02:30	**KEYNOTE LECTURE 3** Elisa GOMEZ-PERDIGUERO (Institut Pasteur, Paris, France) **Development of resident macrophages and their maintenance during ageing**
02:30 - 03:00	**LECTURE 3** Florence PERRIN (IBN, Montpellier, France) **RNA-Seq analysis of microglia reveals time-dependent activation of specific genetic programs following spinal cord injury**

FRIDAY, 30 NOVEMBER

03:00 - 03:15 pm	**SELECTED ABSTRACT** Solène Accarias (IPBS, Toulouse, France) **Generation of an immortalized murine macrophage cell line: characterization and functional study**
03:15 - 03:30 pm	**SELECTED ABSTRACT** Virginie Gavioli (Stromalab, Toulouse, France) **Macrophages derived from endogenous adipose tissue hematopoiesis drive regeneration in adult mice**
03:30 - 03:45	***Coffee Break***
03:45 - 05:15 pm	**SESSION 4** Chairs: François CANONNE-HERGAUX, Céline DERAISON
03:45 - 04:30	**KEYNOTE LECTURE 4** Hal DRAKESMITH (Weatherall Institute of Molecular of Medicine, Oxford, UK) **Iron, hepcidin and macrophages: implications for innate and adaptive immunity**
04:30 - 05:00	**LECTURE 4** Hélène AUTHIER (IRD, Toulouse, France) **Dectin-1 on macrophages promotes inflammatory responses contributing to the severity of inflammatory bowel diseases**
05:00 - 05:15 pm	**SELECTED ABSTRACT** Vanesa Ayala Nunez Nilda (IRIM, Montpellier, France) **Transendothelial migration of HIV-infected monocytes: a role for monocytic tight junction-associated proteins?**
05:15 - 05:30 pm	**SESSION 5** Chairs: Florence APPARAILLY, François CANONNE-HERGAUX
05:15 - 06:00	**KEYNOTE LECTURE 5** Sandrine HENRI (CIML, Marseille France) **Dermal Macrophages : Failure of the phagocytosis process**
06:00 - 06:15 pm	**PRICES AND CONCLUSIONS**

ABSTRACTS

KEYNOTE LECTURE I

ZEB2 AND MAINTENANCE OF MACROPHAGE TISSUE-SPECIFIC IDENTITIES

Charlotte SCOTT [1]
[1] *Ghent, Belgium*

The ZEB family of transcription factors are best known for their roles in EMT, however more recently their roles in the immune system have started to be elucidated. Using novel CRE models in combination with Zeb1fl/fland Zeb2fl/fl, we have identified crucial roles for these factors in mononuclear phagocyte biology. By crossing Zeb2fl/fl mice to CD11cCREmice, we identified a role for ZEB2 in the development and maintenance of pDCs and cDC2s, while its absence is critical for the development of cDC1s. Using novel Kupffer cell specific CRE mice (Clec4FCRE), CD11cCREand CD64CREmice crossed to Zeb2fl/fl we have also found that Zeb2 is required across the macrophage lineage to maintain the tissue-specific identities of these cells. The loss of Zeb2 from different macrophages significantly alters the tissue-specific transcriptomes of the cells, rendering them less equipped for their niche and leading to their subsequent death by necroptosis. Finally, we are currently investigating the role of Zeb1 in mononuclear phagocyte biology, using the XCR1CREmice we found Zeb1 to be a major TF regulating cDC1s and Macrophages in the spleen. Mice lacking Zeb1 specifically in cDC1s completely lack splenic mϕs while also showing a significant reduction in cDC1s. Additionally, the phenotype of the remaining splenic cDCs is augmented in these mice with the remaining cDC1s lacking CD8α expression and expressing CD103 compared with WT splenic cDC1s, while the cDC2s displayed a decreased expression of ESAM compared with WT littermates. Analysis of BM chimeras revealed that the effects of Zeb1 loss are dominant, also affecting WT cells in this competitive setting. Using RNA sequencing, we are currently investigating the mechanisms at play.

LECTURE I

MOLECULAR CHARACTERIZATION OF THE MECHANISMS INVOLVED IN MACROPHAGE DIFFERENTIATION OF MONOCYTES

Arnaud JACQUEL [1]
[1] *Nice, France*

The differentiation of peripheral blood monocytes into resident tissue macrophages can be recapitulated ex vivo by CSF-1. We recently established that proper macrophagic differentiation of monocytes required both caspase and autophagy activation and pinpointed a novel and highly specific mechanism of caspase activation during this process. This may explain the limited number of cleaved substrates at sites different from those cleaved during apoptosis observed during monocyte differentiation. In this context, the main objectives of our project are to decipher this original mechanism of caspase activation, understand the specificity of cleavage and the role of the cleaved fragments and define the interplay between caspases and autophagy during CSF-1 mediated monocyte differentiation. We expect to highlight a new mode of caspase activation in a non-apoptotic context, i.e the physiological differentiation of monocytes and more widely during hematopoietic cell differentiation.

KEYNOTE LECTURE 2

MACROPHAGE-DERIVED PRO-INFLAMMATORY CYTOKINES IN CHRONIC ARTHRITIS: HOW ONE SINGLE MEDIATOR CAN LEAD TO COMPLETELY DIFFERENT PHENOTYPES

Dominique BAETEN [1]

[1] *Amsterdam, Netherlands, UCB pharma*

TNF plays a key role in immune-mediated inflammatory diseases including rheumatoid arthritis (RA) and spondyloarthritis (SpA). It remains incompletely understood how TNF can lead to different disease phenotypes such as destructive peripheral polysynovitis in RA versus axial and peripheral osteoproliferative inflammation in SpA. We observed a marked increase of transmembrane (tm) versus soluble (s) TNF in SpA versus RA together with a decrease in the enzymatic activity of ADAM17. In contrast with the destructive polysynovitis observed in classical TNF overexpression models, mice overexpressing tmTNF developed axial and peripheral joint disease with synovitis, enthesitis, and osteitis. Histological and radiological assessment evidenced marked endochondral new bone formation leading to joint ankylosis over time. SpA-like inflammation, but not osteoproliferation, was dependent on TNF-Receptor I and mediated by stromal tmTNF overexpression. Collectively, these data indicate that TNF can drive distinct inflammatory pathologies. We propose that tmTNF is responsible for the key pathological features of SpA.

LECTURE 2

INTERACTION WITH PRO-INFLAMMATORY MACROPHAGES MODULATED IMMUNOSUPPRESSIVE FUNCTION OF HUMAN MESENCHYMAL STROMAL CELLS

Audrey VARIN [1]

[1] *Toulouse, France*

Mesenchymal stromal cells (MSCs) are adult multipotent stromal cells present in all tissues but mostly isolated from bone marrow (BM-MSCs) and adipose tissue (ASCs). Increasing clinical and basic investigations have demonstrated the successful use of MSCs to treat inflammatory-mediated disorders and autoimmune diseases. Indeed, the benefits of MSCs are associated with their broad immunoregulatory properties, which modulate both adaptive and innate immunity. Mesenchymal stromal cells sense microenvironment through soluble factors but direct effect of innate immune cells and more especially macrophages (MΦ) are still unsolved. In our work, we described an unconventional but functional interaction between pro-inflammatory classically activated macrophages (M1MΦ) and MSCs. BM-MSCs and ASCs both interacted with M1-MΦ however this specific interaction have a complete different effect on the immunosuppressive capacities of the MSCs. To confirm the effect of macrophage in MSC function, we analyzed effect of MΦ-primed ASCs on acute inflammation using caecal ligature and puncture model of sepsis. We demonstrated that MΦ-primed ASCs improved the survival of mice compared at ASCs. This improvement is associated with a modification of immune cells response in the peritoneum as well as in the different organs and is associated with a decrease of general inflammation in treated mice.

Therefore, we demonstrated that macrophages interact physically with MSCs and this cross-talk modulates the immunosuppressive functions of MSCs. This finding opens new perspectives for MSC-based cell therapy but also highlight the possible role of the MSC-MΦ interaction in tissue homeostasis.

ABSTRACTS

KEYNOTE LECTURE 3

DEVELOPMENT OF RESIDENT MACROPHAGES AND THEIR MAINTENANCE DURING AGEING

Elisa GOMEZ-PERDIGUERO [1]
[1] *Paris, France*

Macrophages are phagocytic cells that orchestrate homeostatic and innate immune functions, via the scavenging of cells and pathogens, and the production of cytokines. Because macrophage diversity is not well understood, their functions in vivo have not been well characterized. Within this diversity, there are now two independent lineages based on ontogeny. Tissue 'resident' macrophages self-maintain independently of hematopoietic stem cells (HSC) in contrast to monocyte-derived macrophages. The originality of resident macrophages lies in their proliferation within their tissue during development and in response to injury. To critically assess the contribution of resident macrophages to tissue repair/regeneration, our group aims to determine their developmental pathway and the underlying molecular mechanisms that control their renewal and functions in vivo.

LECTURE 3

RNA-SEQ ANALYSIS OF MICROGLIA REVEALS TIME-DEPENDENT ACTIVATION OF SPECIFIC GENETIC PROGRAMS FOLLOWING SPINAL CORD INJURY

Florence PERRIN [1]
[1] *Montpellier, France*

Spinal cord injury (SCI) induces a pronounced neuroinflammation driven by resident microglia and infiltrating peripheral monocyte-derived macrophages. Microglia/infiltrating monocytes play both positive and negative roles on axonal regeneration, raising the question whether their response depends on lesion severity and/or time post-lesion.The first part of the presentation will focus on microglia-specific molecular alterations induced by a lateral hemisection of the spinal cord. Using cell-specific RNA-sequencing at several time-points after two lesion severities, we demonstrated that activation of microglia is time-dependent but is independent of lesion severity. Early transcriptomic response of microglia after SCI involves proliferation and neuroprotection, which is then switched to neuroinflammation at later stages. Moreover, we identified that SCI induced the expression of astrocytic markers in 6% of the microglia. We also identified the potential involvement of DNA damage and in particular tumour suppressor gene breast cancer susceptibility gene 1 (Brca1).The second part of the presentation, will focus on the effect of chronic pharmacological depletion of microglia proliferation on behavioral and histological outcomes following SCI. Using GW2580, an inhibitor of the colony-stimulating factor 1 receptor (CSF1R), we first demonstrate that the depletion of microglia proliferation improved several parameters of motor recovery in spinal cord injured mice. Then, using in and ex vivo magnetic resonance imaging (MRI) we established that GW2580 treatment had no effect on lesion size. However, histological analyses revealed that GW2580-treated animals had reduced gliosis and microcavity formation following SCI.Thus, limiting early microglial proliferation may offer therapeutic approach to improve functional recovery following SCI.

KEYNOTE LECTURE 4

IRON, HEPCIDIN AND MACROPHAGES: IMPLICATIONS FOR INNATE AND ADAPTIVE IMMUNITY

Hal DRAKESMITH [1]
[1] *UK, MRC HIU*

Iron is required for the growth of almost all infectious organisms but is also needed for host immune function. The iron regulatory hormone hepcidin controls both total body iron levels and the distribution of iron; hepcidin inhibits release of iron from macrophages that express the iron efflux protein, ferroportin. Hepcidin expression is regulated by the balance of several signals, chief among them being iron status, inflammation, and erythropoietic drive. Interestingly, iron appears to be the only nutrient that is controlled by a hormone that responds both to nutrient levels and to infection, underscoring the importance of iron in host-pathogen interactions. Here I will discuss the role of hepcidin and iron in infectious diseases and innate and adaptive immunity. There is marked heterogeneity in how hepcidin is regulated during different types of infection, and the effect of hepcidin-mediated iron distribution on the outcome of different infections (for example, intra- versus extra-cellular bacteria, malaria) is also highly variable. The innate immune response to most infections involves an acute and profound hepcidin-mediated decrease in serum iron levels. Recent genetic evidence links lack of iron acquisition by lymphocytes from serum to severe immunodeficiency. Therefore, a currently underappreciated important aspect of iron and hepcidin in the context of infection is that serum iron levels strongly influence the adaptive immune response.

LECTURE 4

DECTIN-1 ON MACROPHAGES PROMOTES INFLAMMATORY RESPONSES CONTRIBUTING TO THE SEVERITY OF INFLAMMATORY BOWEL DISEASES

Hélène AUTHIER [1], Mélissa PRAT [1], Etienne MEUNIER [1], Mohamad ALAEDDINE [1], Mouna RAHABI [1], Godefroy JACQUEMIN [1], Bénédicte BERTRAND [1], Lise LEFÈVRE [1], Khaddouj BENMOUSSA [1], Philippe BATIGNE [1], Agnès AUBOUY [1], Johan AUWERX [2], Sylvain KIRZIN [3], Delphine BONNET [4,5], Marie DANJOUX [6], Bernard PIPY [1], Laurent ALRIC [1,4]*, Agnès COSTE [1]*

[1] UMR 152 Pharma Dev, Université de Toulouse, IRD, UPS, France
[2] Metabolic signaling, Institute of Bioengineering, Ecole Polytechnique Fédérale de Lausane, Lausane 1015, Switzerland
[3] Department of Surgery and Digestive Diseases, CHU Purpan, Université de Toulouse, France
[4] Department of Internal Medicine and Digestive Diseases, CHU Purpan, Toulouse, France
[5] IRSD, Université de Toulouse, INSERM, INRA, ENVT, UPS, Toulouse, France
[6] Department of Pathology, CHU Purpan, Université de Toulouse, France
** These authors contributed equally to this work*

Colonic macrophages are considered the main effectors of pathology in inflammatory bowel diseases (IBD). Although the control of gut inflammation through the activation of C-type lectin receptors on macrophages is an emerging concept in IBD pathogenesis, their contribution is still controversial. We show that loss-of-function of Dectin-1 in macrophages prevents colitis in mice, while the loss of MR exacerbates it via the Dectin-1 overexpression, supporting Dectin-1 as an important mucosal inflammatory regulator during IBD. Dectin-1 on macrophages increases the Ly6ChighCCR2high blood monocyte subset through CCL2 production and its recruitment to the inflamed colon as inflammatory macrophage precursors. Dectin-1 promotes inflammasome-dependent IL-1β secretion through the modulation of LTB4 production. These pathways also operated during IBD in humans. This study provides important insights into the role of Dectin-1 on macrophage in the development of mucosal inflammation and offers breakthroughs in terms of therapeutic strategies to control the imbalanced inflammatory response in IBD.
Keywords: Mannose receptor / C-type lectin receptors / colitis / mucosal immunity / innate immune response.

KEYNOTE LECTURE 5

DERMAL MACROPHAGES : FAILURE OF THE PHAGOCYTOSIS PROCESS

Sandrine HENRI [1]

[1] Marseille, France

The skin is a major barrier protecting the body frommicrobial invasion and dendritic cells (DCs) and macrophages are key players of the immune system in theskin. Lately, we contributed to decipher the mononuclear phagocyte complexity. Indeed, we identified severalmonocyte, macrophage and DC subsets in the skin and defined reliable markers to disentangle them. Dermalmacrophages survey the skin by scavenging and degrading microorganisms, foreign bodies as well as selfmacro-molecules. To assess further their contribution to tissue homeostasis, we developed a novel mousemodelto deplete macrophages allowing us to assess their dynamics in vivo. Using our new mouse model, weidentified melanin-laden cells in skin as well as in melanocytic melanoma, with specific macrophage features.Those cells have been referred to as melanophages in humans. Moreover, we revisited the tattoo persistencedogma and showed how cycles of pigme.

SELECTED ABSTRACT 1

PRO-REGENERATIVE PROTECTIN D1 CONTROLS IMMUNE CELL FUNCTION DURING EPIMORPHIC REGENERATION

Mai NGUYEN CHI [1], @, Patricia LUZ-CRAWFORD [2], Laurence BALAS [3], Rafael CONTRERAS [2], Georges LUTFALLA [1], Thierry DURAND [3], Christian JORGENSEN [4], Farida DJOUAD [4]

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Specialized pro-resolving mediators (SPM) are a family of lipids controlling the resolution of inflammation and playing a role in many processes including organ protection and tissue repair. While synthetic SPM are potent bioactive molecules in vivo, their role in epimorphic regeneration of organs in vertebrates has never been proved. Using the zebrafish larvae as a robust regenerative vertebrate system, we showed that the SPM Protectin D1 (PD1) improves the regeneration of the zebrafish caudal fin after amputation, presumably by increasing cell proliferation at the stump. We showed that PD1 acts in a narrow time window during regeneration. Using fluorescent transgenic lines, we demonstrated that PD1 accelerates the resolution of inflammation without affecting the initial kinetic of neutrophils infiltration but instead, promoting their reverse migration potential. In addition, PD1 induces macrophage polarization switch toward non-inflammatory states in both zebrafish and mammalian system. Thus, our findings strongly support the development of pro-resolving mediators to improve tissue regeneration in mammals with inflammatory and degenerative diseases.

SELECTED ABSTRACT 2

LOCALIZATION OF PSEUDOMONAS DICTATES DIFFERENTIAL NLRC4 INFLAMMASOME-CONFERRED HOST IMMUNITY

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The NAIP-NLRC4 inflammasome is activated by cytosolic bacterial flagellin and type-3 secretion system (T3SS) components. While inflammasome activation promotes host defense against numerous bacteria, the opportunist bacterium Pseudomonas aeruginosa (P. aeruginosa) exploits NLRC4-driven IL-1 cytokine production to escape immune clearance. Here, we investigated both the host and bacterial determinants underlying this deleterious response. We show that T3SS-mediated P. aeruginosa extracellular localization inhibits its uptake by inducing NLRC4-dependent pyroptosis of macrophages. Surprisingly, T3SS-deficient P. aeruginosa still promote residual NAIP5-NLRC4 activity, a process that requires bacterial phagocytosis and cytosolic flagellin leakage. Remarkably, intracellular P. aeruginosa are trapped into pyroptotic corpses, which sensitized them to additional stress. Yet, T3SS-expressing P. aeruginosa escape imprisonment by injecting flagellin through the macrophage plasma membrane. Finally, acute infection of mice reveal that depletion of alveaolar macrophages and genetic inactivation of NLRC4 protect mice against T3SS-expressing P. aeruginosa whereas it rendered them susceptible to T3SS-deficient strain.Overall, our results reveal that P. aeruginosa hijacks NLRC4-driven pyroptosis, a process mainly efficient against intracellular bacteria.

SELECTED ABSTRACT 3

LOW MOLECULAR WEIGHT HYDROGELS AS INJECTABLE SCAFFOLDS FOR TUNING THE FOREIGN BODY REACTION

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Introduction: One of the major challenges for the use of implantable biomaterials is to develop strategies that moderate the innate host inflammatory reaction. Among biomaterials that are considered for clinical applications, hydrogels have gained a significant interest due to their tunable mechanical properties. However, only a few are used in clinics because hydrogels, like other biomaterials, induce FBRs after implantation. The severity of the hydrogel-induced FBR is different for each biomaterial. To identify modifications that suppress the FBR, we developed four novel low molecular weight supramolecular hydrogels that were tested and compared in a mouse model of subcutaneous implantation. As the goal of the biomaterial has often centered on promoting the cellular infiltration, degradation, and vascularization, we demonstrate here the injectable hydrogels with innately promotes all of these regenerative responses when subcutaneously implanted into the mice.

Methods: Subcutaneous implantation of hydrogels was performed in mice to assess different aspects of the FBR. The type of inflammatory cells in the surrounding tissue, as well as within the hydrogels, was determined by histology. Specific markers for angiogenesis, macrophage polarisation, and fibroblast were labeled to assess the FBR. Mass spectrometry was used to characterize degradation products of the hydrogel biomaterial.

Results and Conclusion: Our results indicate that all novel low molecular weight hydrogels showed low inflammatory response by modulating the macrophage polarisation, enhancing vascularisation in the surrounding tissue, and absence of fibrous capsule formation. A potential angiogenic property of the degradation product is currently under investigation. The results indicate that hydrogels are potential biomaterial candidate for various applications such as tissue engineering, drug delivery, and medical devices.

Keywords: Foreign body reaction, macrophages, inflammation, fibrosis, hydrogels.

SELECTED ABSTRACT 4

GENERATION OF AN IMMORTALIZED MURINE MACROPHAGE CELL LINE: CHARACTERIZATION AND FUNCTIONAL STUDY

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Functional study of macrophages generally requires the use of mouse bone-marrow derived macrophages (BMDM). This model has several drawbacks, including a limited life span, a limited number of cells and a difficulty to generate stable mutants. To overcome these drawbacks and the use of animals, we decided to produce an immortalized hematopoietic progenitor cell line. This cell line provides unlimited amount of macrophages that are readily genetically modifiable and constitute a valuable tool for both in vitro and in vivo studies.
The homeogene Hoxb8 has been shown to arrest myeloid differentiation and enforce self-renewal (Wang and al., Nat Methods, 2006). By inducing expression of estrogen-regulated Hoxb8 gene in murine bone marrow progenitors (Hoxb8-myeloid progenitors), it is therefore possible to obtain unlimited expansion of myeloid precursors without inducing their transformation. The use of the CRISPR/Cas9 technology on this immortalized cell line then allows generating stable mutants. We aim at identifying effectors of macrophage migration in 3D environments (Gui and al, Cancer Immunol Res, 2018). Thus, our strategy has been to use Hoxb8 cells coupled to CRISPR/Cas9 technology as a convenient approach to target potential regulators of macrophage migration. Here we present data on the characterization Hoxb8-myeloid progenitors and validate their use as tools to study macrophage functionalities in vitro and in vivo.
First, we have analyzed the ability of Hoxb8-myeloid progenitors to differentiate into functional macrophage compared to BMDM. We have examined macrophage differentiation markers by flow cytometry, the cell phenotype by microscopy (morphology and presence of podosomes as specialized cell structures of macrophages) and several macrophage activities (NADPH oxidase, phagocytosis, 3D migration, M1/M2 polarization). Our results show that, compared to BMDM, differentiated Hoxb8-myeloid progenitors have all the features of mature macrophages.
Second, CRISPR/Cas9 system extinction was used to target the WASP gene, a known effector of BMDM migration (Park and al, J Biol Chem, 2014). Western blot analysis confirmed the efficient extinction of the protein, which is associated with a functional defect in podosome formation and migration capacity of cells.
In conclusion, the Hoxb8 cell line represents a valuable model to identify new effectors of macrophage functionalities with a large scale of applications.

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SELECTED ABSTRACT 5

MACROPHAGES DERIVED FROM ENDOGENOUS ADIPOSE TISSUE HEMATOPOIESIS DRIVE REGENERATION IN ADULT MICE

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Unlike prenatal tissues that can regenerate (perfect repair without any remnant scar at the wound site), adult tissue repair typically leads to formation of a fibrotic scar which affects normal tissue architecture and ultimately can disable proper functioning of tissues. Emerging evidences suggest that the quality of repair (regenerative vs scar healing) relies on both inflammation and its resolution. At the cellular level, the key steps for the inflammation resolution program are conditioned by macrophage function. However, it is now evident that macrophages form a heterogeneous population, with distinct origins and specific tissue distribution, transcriptional profiles and functions. We have previously shown that in adipose tissue (AT), resident macrophages arise from both circulating monocytes and an endogenous hematopoietic process based on the presence of peculiar AT-hematopoietic stem cells. The aim of this study was to determine the precise roles of macrophages according to their origin, in the early phases of the regeneration process in adult mammals. By using a newly-developed model of AT resection and regeneration in adult mice, and hematopoietic reconstitution assays, our results show that rapid and efficient resolution of inflammation is required for regeneration. This is specifically achieved by macrophages derived from endogenous AT hematopoiesis, through their ability to perform efficient efferocytosis. The replacement of AT-derived macrophages by their medullar counterparts inhibits inflammation resolution and induces scar healing. Altogether, these results highlight the causative role of macrophages in the early phases of the regeneration process in adult mammals, and uncover the specific involvement of macrophages derived from endogenous AT-hematopoiesis in this process.

SELECTED ABSTRACT 6

TRANSENDOTHELIAL MIGRATION OF HIV-INFECTED MONOCYTES: A ROLE FOR MONOCYTIC TIGHT JUNCTION-ASSOCIATED PROTEINS?

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Infection by the Human Immunodeficiency Virus (HIV) is a major health problem worldwide. Despite its vast clinical impact, it is still not clear how the virus establishes viral reservoirs that contribute to a persistent infection that lasts the life of the host. Within the HIV tissue reservoirs, the Central Nervous System (CNS) stands out for its inaccessibility, which allows the virus to be better protected from the immune system and antivirals. It is proposed that, to invade this tissue, HIV hitches a ride on monocytes that will cross the blood-brain barrier (BBB) and later differentiate into macrophages. This “Trojan Horse” mechanism takes place via transendothelial migration, process where the HIV-infected monocyte locally perturbs the highly impermeable BBB endothelial layer to cross it. The molecular partners involved, the role of tight junction-associated proteins (TJAPs) and the impact of HIV in this process is not fully understood.

This project aims to characterize the molecular mechanisms involved in monocyte transmigration through the BBB, and the influence of HIV-1 during this process. Interestingly, human monocytes express a subset of TJAPs, even though they are unable to form actual tight junctions. We hypothesized that monocytic TJAPs (mTJAPs) may play a role in para- and/or trans-cellular diapedesis and that HIV affects their expression to favor the Trojan horse strategy.

Using a siRNA screen, we observed that the expression of a subset of mTJAPs are important for successful monocyte transmigration through a layer of blood-brain barrier-derived endothelial cells. In particular, we identified mTJAP1 as a candidate protein involved in monocyte transmigration. We confirmed the expression of mTJAP1 in primary human monocytes and we observed that its expression levels were modulated upon HIV infection. Additionally, we showed that primary human monocytes carry HIV virions and no viral replication takes place. And, even in the absence of viral replication, we observed a 3-4 fold enhanced transmigratory capacity of HIV-carrying monocytes compared to monocytes that were not in contact with the virus.

We are now using a combination of advanced 3D live-cell imaging and CRISPR/Cas9 gene editing to investigate the detailed dynamics of transmigration of HIV-infected monocytes. In particular, we will quantitatively characterize the various steps of the transmigration process (attachment, rolling, diapedesis) of infected cells to better understand how HIV impacts the transmigration process.

This work provides new molecular insights into some biological aspects of HIV persistence and reservoir establishment, supplying the seminal knowledge for a rational design of a novel class of antivirals.

POSTERS 1

AN INFLAMMATION LEADS TO AN UNEXPECTED INSULIN RESISTANCE IN NOONAN SYNDROME

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The "gain in function" mutations in the gene encoding Ptpn11 for SHP2 are responsible for the Noonan Syndrome (NS), a rare disease that occurs through several developmental defects, including growth, heart disease and myeloproliferative disorders. SHP2 is a tyrosine phosphatase that regulates major signaling pathways (MAPK, PI3K), playing pleiotropic roles in development, homeostasis and metabolism. Although the developmental consequences induced by SHP2 mutations are well known, their impact on metabolism has never been studied. The study was performed on a mouse model of NS (Ptpn11D61G / +). We found an unexpected metabolic phenotype, associated with reduced adiposity and insulin resistance intolerance, both in the mouse model and in patients. Although this is no sign of lipid ectopic deposits, NS mice develop an inflammation of their metabolic tissues. A bone marrow transplant or macrophage depletion with clodronate is used to normalize glucose intolerance. We identified an original metabolic phenotype that resulted in insulin resistance caused by inflammation of the adiposity aspect. This opens new perspectives in the pathophysiology of SN and beyond the context of rare diseases, in understanding the mechanisms involved in metabolic diseases.

POSTERS 2

POTENTIAL OF AUTOFLUORESCENT INTRACELLULAR NADH AND FAD TO BE USED AS BIOMARKERS OF THE VULNERABILITY OF THE ATHEROMATOUS PLAQUE

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Atherosclerosis is an inflammatory disease characterized by lipids' deposition into the arterial wall and its subsequent thickening significantly compromises the lumen and/or eventually leads to plaque rupture and thrombosis conducing to cardiovascular diseases. Lipids-activated endothelium induces the persistent and massive infiltration of blood monocytes in the intima of the arterial wall where they differentiate into macrophages that phagocyte oxidized Low-Density Lipoprotein (oxLDL) inducing their transformation toward foam cells. Foam cells are unable to manage cholesterol efflux and produce cytokines and proteases inducing sustained inflammation and plaque vulnerability. Apoptosis then necrosis of these cells create the pro-thrombotic necrotic lipid core, key component of the vulnerable plaques. Macrophages are therefore characteristic of the plaques but they represent a quite heterogeneous and plastic population gathering different subpopulations presenting, through slight variations of global gene expression, a dual functionality ("heal" or "fight"). The extremities of this continuum are represented by the well-characterized in vitro models, classically activated pro-inflammatory M1 and alternatively activated immunoregulatory M2 macrophages (Mantovani, et al. 2004). Markers of these functional subtypes of macrophages can be found in atherosclerotic lesions (Toutouzas, et al. 2015) and their relative proportion and their specific localization in the plaque could be predictive factors of plaque rupture. If fluorochrome-coupled antibodies directed against M1 or M2 markers do exist, none of such antibodies are validated for in vivo colocalization imaging in humans. Besides, recent immunometabolism data suggested that the functional duality of these cells could be related to their differential utilization of metabolism pathways to produce energy, which may result in different concentrations of intracellular autofluorescent co-enzymes NADH and FAD (Pal & Konkimalla 2016). Therefore we expected that a local disequilibrium of the M1/M2 ratio will result in a modification of NADH and FAD autofluorescence (AF) and of the related Optical Redox Ratio (ORR= I(FAD)/I(NADH)).

POSTERS 3

DEVELOPMENT OF AN TAM TARGETING ANTIBODY FOR ANTICANCER THERAPIES

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Purpose: Tumor microenvironment (TEM) is highly involved in the tumor development and chemoresistance. Among cellular components of the TEM, tumor associated macrophages (TAM) are modified macrophages educated by the tumor to favour its development. Numerous studies aim to target TAM which are present in most cancers. Being able to eliminate or deactivate these cells is a challenge today in anticancer therapies. We recently produced a new antibody specifically directed against TAM. We chose the chronic lymphoblastic leukaemia (CLL) as model which is a malignant hemopathy with only 50% of complete remission and a deleterious effect of the treatment on the immune system of the patients (Ysebaert 2010). The resistance of the residual disease is due to the intrinsic properties of cancer cells but also to their close contact in lymph nodes with nurse like cells (NLC). We defined these cells as CLL's TAM, infiltrating the lymph nodes and associated with the aggressiveness of the disease in a contact dependant manner (Ysebaert 2011, Boissard 2016a and 2016b). Target these cells would be a new therapeutic insight in cancer.

Experimental Design and results: NLC are easily produced in vitro by the culture of PBMC from CLL patients by the differentiation of monocytes in contact with CLL cells. After a mouse immunization with these NLC, we selected one antibody specifically targeting NLC (patent Inserm). This antibody called 6-25 does not bind to leukemic cells or healthy B and T lymphocytes and monocytes. Moreover, immunohistochemistry and immunofluorescence analyses of different human tumors proved that our antibody can target different TAM.

Conclusion: The anti-CD115 antibody is today the only anti-TAM antibody used in clinical trials. The advantage of our anti-TAM antibody is the specificity of targeting. Indeed, the CD115 receptor being expressed on TAM but also on monocytes, M2 macrophages and on microglia, which could have negative effects on inflammatory processes, bone turnover and on immune responses in central nervous system. Here, we developed an antibody specifically directed against TAM with a promising future in anticancer therapies.

POSTERS 4

DYSREGULATION OF THE TYPE-I INTERFERON SIGNALING PATHWAY BY MYCOBACTERIUM TUBERCULOSIS LEADS TO HIV EXACERBATION IN HUMAN MACROPHAGES

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Clinical and epidemiological evidence identify tuberculosis (TB) as an increasing factor of HIV-1 infected patients morbidity and mortality. Mycobacterium tuberculosis (Mtb), the etiological agent for TB, and HIV-1 are known to synergize and trigger each other's progression in co-infected patients. However, the mechanisms by which HIV-1 infection is enhanced in the context of coinfection with Mtb remain poorly understood. Both Mtb and HIV-1 are able to impair the host immune system, and particularly lung macrophages, representing the convergent cellular target for these pathogens. In order to establish the role of macrophages in HIV-1 exacerbation in a coinfection context, we established a relevant in vitro model to mimic TB-associated microenvironment, consisting of Mtb-infected macrophages supernatant, called cmMTB. Under these conditions, we showed that HIV-1 infection and spread is increased. To understand the mechanisms involved in this phenomenon, we performed a global transcriptomic analysis of cmMTB-treated macrophages. Strikingly, instead of an expected set of viral permissive gene expression profile, we found that these cells exhibit a type I interferon (IFN-I) signature, notably characterized by a high induction of IFN-stimulated genes (ISG), certain of them known to be antiviral, like Mx1, Mx2 and ISG15. As IFN-I is known to be deleterious for the host in chronic infections, by changing cell's response to IFN-I exposure, we hypothesized that cmMTB treatment dysregulate IFN-I signaling pathway, rendering cmMTB-treated macrophages susceptible to HIV-1 infection. We have several evidence that, in our model, conditioning with cmMTB, enriched with IFN-I, is deleterious for HIV-1 control, because of a chronic exposure to IFN-I. Indeed, STAT1 silencing during cmMTB conditioning reduced HIV-1 exacerbation in macrophages. Moreover, this chronic exposure to IFN-I during cmMTB conditioning rendered the cell response sub-optimal to exogenous IFN-I stimuli ; we found a diminished STAT1 activation and a decreased ISG induction in cmMTB treated cells compared to controls, along with a diminished expression of antiviral genes during the course of HIV-1 infection. In addition, we identified CD169 (Siglec-1), a pro-viral gene involved in HIV-1 capture and transfer, as cell surface molecule overexpressed upon CmMTB treatment. Importantly, silencing of CD169 lead to a partial but significant inhibition of HIV-1 exacerbation and to a strong reduction in viral cell-to-cell transfer between macrophages, suggesting a role for this receptor in the co-infection context. Altogether, our results suggest that TB-associated microenvironment renders cells less responsive to IFN-I during HIV-1 infection, lowering the expression of antiviral factors over time to enact a protective role against viral infection in macrophages, but sufficiently maintaining the expression of pro-viral factors such as CD169.

POSTERS 5

MEFV/MIR-326 AXIS INVOLVEMENT IN HUMAN MACROPHAGE POLARIZATION

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Background and objectives: Familial Mediterranean fever (FMF) is an inherited autoinflammatory disease, characterized by acute self-resolving attacks of fever and serositis, which mainly prevails in populations around the Mediterranean sea. It is caused by mutations in the MEFV gene, which encodes the pyrin protein. The alteration of MEFV mRNA expression in monocytes is related to both genotype and phenotype of the disease, suggesting that the pathophysiology of FMF can be regulated on a quantitative defect of MEFV mRNA. Since microRNAs (miRNAs) are implicated in a number of diseases including FMF, the present study aimed at identifying miRNA regulators of MEFV expression involved in monocyte inflammatory response.

Materials and Methods: miRWalk2.0 database was used to identify putative miRNA target sequences within the 3'-UTR mRNA of MEFV. Human primary CD14+ monocytes were sorted from peripheral blood of healthy donors using magnetic microbeads and differentiated into M1 or M2 macrophages following IFNγ/LPS or IL4/IL13 stimulation, respectively. Using RT-qPCR, M1/M2 polarization was validated by measuring the expression of prototypic M1 and M2 markers: the chemokine CXCL10 and the macrophage mannose receptor 1 (MRC1 also known as CD206), respectively, as well as the MEFV mRNA. We used loss-of-function method to evaluate the effect of candidate miRNA on CD14+ monocytes, i.e. its role on macrophages classical versus alternative polarization. IL-10 expression was quantified using ELISA.

Results: In silico analyses revealed that miR-326 targets putatively the 3'UTR mRNA of MEFV. miRNAs and mRNAs quantification in polarized macrophages showed that miR-326 is mainly expressed by the M2-type macrophages, and MEFV by the M1-type macrophages.Loss-of-function studies showed that neutralization of miR-326 in M2 macrophages induced the expression of MEFV and CXCL10 while reducing MRC1 expression level. Furthermore, enforced expression of miR-326 in M1 macrophages significantly repressed MEFV expression and induced the production of IL-10.

Conclusion: A miR-326/MEFV axis seems to be implicated in macrophage polarization and might explain the observed monocyte versatility in FMF.

POSTERS 6

CSF1R INHIBITION REDUCES MICROGLIA PROLIFERATION, PROMOTES TISSUE PRESERVATION AND IMPROVES MOTOR RECOVERY AFTER SPINAL CORD INJURY

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Spinal cord injury (SCI) induces neuroinflammation driven by activation and proliferation of resident microglia as well as infiltrating peripheral monocyte-derived macrophages. Depending on the time post-lesion, positive and detrimental influences of microglia/macrophages on axonal regeneration had been reported after SCI, raising the issue whether their modulation may represent an attractive therapeutic strategy. Colony stimulating factor 1 (CSF1) regulates microglia/macrophages proliferation, differentiation and survival thus, pharmacological treatments using CSF1 receptor (CSF1R) inhibitors had been used to ablate microglia. We characterized in this study the effect of microglia proliferation inhibition after SCI using GW2580, a CSF1R inhibitor, from behavioral to tissular and cellular levels.

POSTERS 7

WHAT DOES DORASAY?

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The San Francisco Declaration on Research Assessment (DORA), initiated at the 2012 Annual Meeting of the American Society for Cell Biology by a group of editors and publishers of scholarly journals, recognizes the need to improve the ways in which the outputs of scientific research are evaluated.

POSTERS 8

SINGAPORE STATEMENT ON RESEARCH INTEGRITY

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The value and benefits of research are vitally dependent on the integrity of research. While there can be and are national and disciplinary differences in the way research is organized and conducted, there are also principles and professional responsibilities that are fundamental to the integrity of research wherever it is undertaken.

POSTERS 9

ARCHITECTURE AND MECHANICS OF THE MACROPHAGE PODOSOME

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Macrophages infiltration in body tissues is critical for immune protection, but it also helps progression of inflammatory diseases and cancers. We have shown that to migrate within the cancer stroma, macrophages use the mesenchymal migration mode that involves adhesion cell structures called podosomes. Thanks to the development of a method called Protrusion Force Microscopy, we have observed that podosomes apply protrusive forces onto the extracellular substrate. We could estimate the protrusive force generated by podosomes and show that it requires combined acto-myosin contraction and actin polymerization. Podosomes are composed of a core of F-actin surrounded by adhesion complexes. The scope of our study is to decipher the podosome architecture to determine how it generates protrusive foces.

We have recently demonstrated that the adhesion complex proteins talin, vinculin and paxillin, located at the podosome ring, sustain protrusion force generated at the podosome core and shown that talin is stretched attesting for mechanical tension at the ring. We are now questioning the organization and regulation of actin filaments in podosome cores using a combination of force measurements, optical nanoscopy and cryo-electron tomography. We aim to reveal the specific localization and function of actin crosslinkers, alpha actinin 1, filamin A, fascin and L-plastin, and how filaments are collectively organized.

Progressing in the knowledge of podosomes may help to identify pharmacological targets to limit tissue infiltration of macrophages in several diseases.

POSTERS 10

OMEGA-3 PUFAS AND ISOPROSTANOIDS REGULATE INFLAMMATION IN MICROGLIAL CELLS

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Obesity is an energy balance disorder that increases the susceptibility of developing metabolic diseases and that is characterized by a state of chronic low-grade inflammation. This type of inflammation is not limited to peripheral tissues (liver, adipose tissue, muscle) as it extends to the CNS, leading to the development of neuroinflammation. Microglia, the resident immune cells of the brain, represent a novel way to target neuroinflammation in order to potentially mitigate obesity and its health consequences.

Thus, we investigated the impact of PUFAs in microglial cells on markers of inflammation and oxidation. Based on the close link between inflammation and oxidative stress, we focused specifically on non-enzymatic lipid oxidative products.

Our studies could be divided into two parts:

- First, in primary microglia cultures under inflammatory conditions induced by LPS, we determined by LC-MS/MS the qualitative and quantitative profiles of isoprostanoids in cells and media, on the basis that these metabolites constitute excellent biomarkers of oxidative stress and exhibit also a wide range of bioactivities.

- Then, under similar conditions, we evaluated the putative protective effects of PUFAs and some of their oxidized metabolites on LPS-induced inflammatory responses. To this end, we quantified by qPCR and ELISA the expression and secretion of pro-inflammatory cytokines (IL-1b, IL-6, TNFa, MCP-1).

To our knowledge, we demonstrate for the first time that LPS increased the production of oxidized metabolites of EPA and DHA in primary microglial cells. Our results also show that EPA, DHA and oxidized metabolites decrease the expression and secretion of pro-inflammatory cytokines.

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