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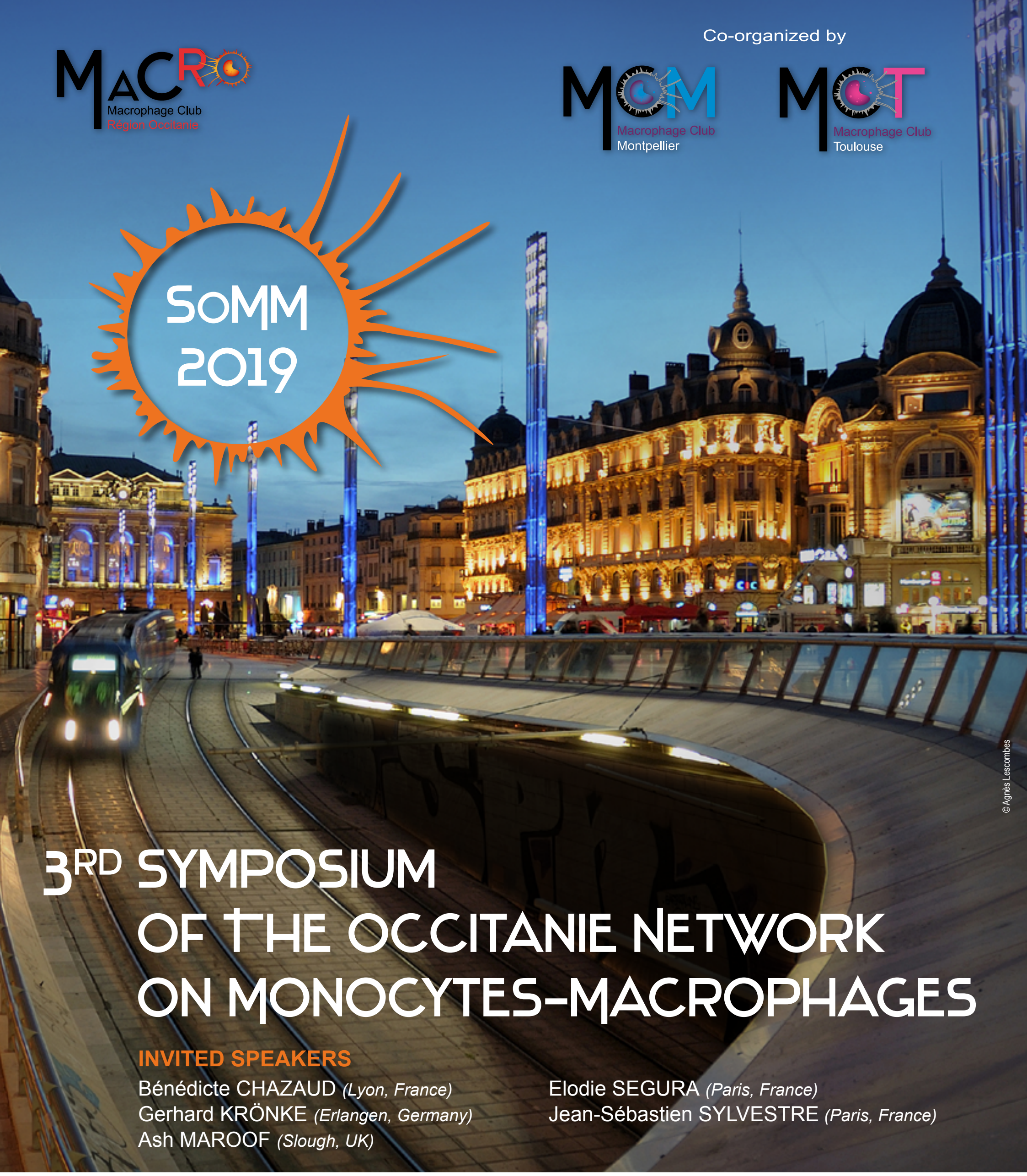

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3RD SYMPOSIUM OF THE OCCITANIE NETWORK ON MONOCYTES-MACROPHAGES

INVITED SPEAKERS

Bénédicte CHAZAUD *(Lyon, France)*
Gerhard KRÖNKE *(Erlangen, Germany)*
Ash MAROOF *(Slough, UK)*
Elodie SEGURA *(Paris, France)*
Jean-Sébastien SYLVESTRE *(Paris, France)*

FRIDAY, 29 NOVEMBER 2019 MONTPELLIER

For any question and request please contact us at: somm2019@sciencesconf.org

WEBSITE: https://somm2019.sciencesconf.org/

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GENERAL INFORATIONS

CROWNE PLAZA MONTPELLIER – CORUM

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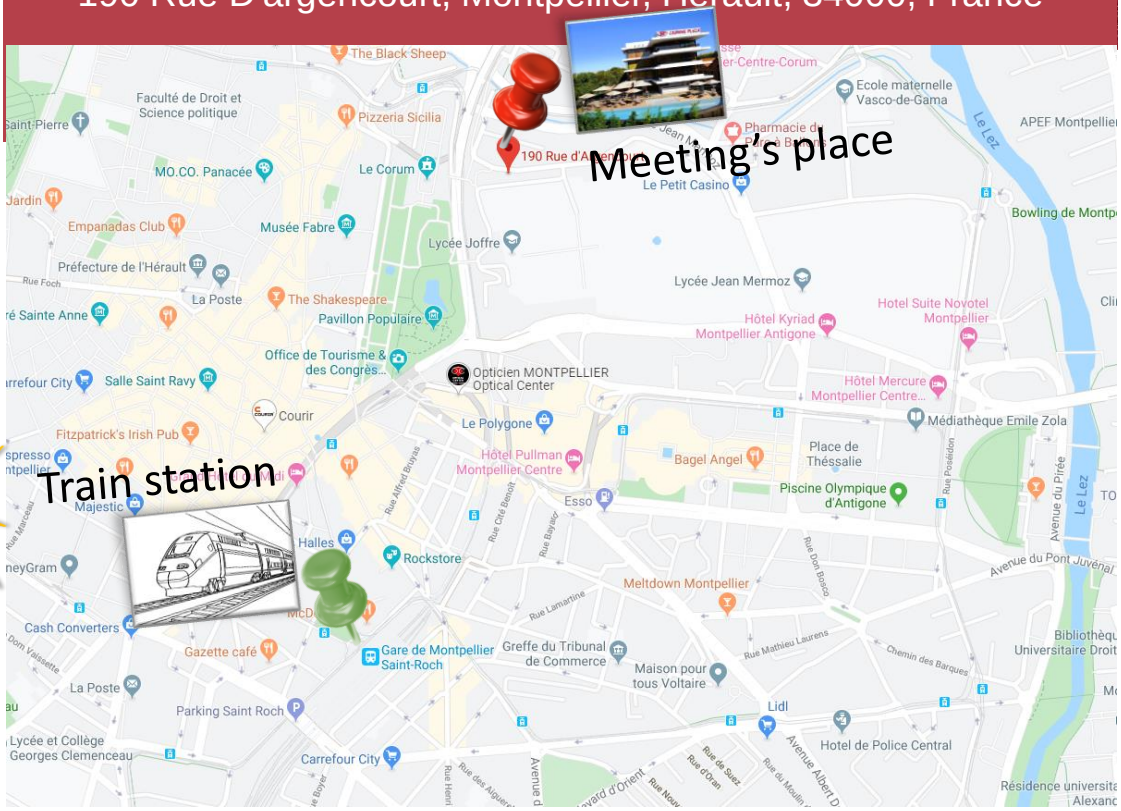

INVITED SPEAKERS

Keynotes & lectures

Bénédicte CHAZAUD
Lyon
Institut NeuroMyoGène

KEYNOTE 1

New crosstalk between signaling pathways triggering the resolution of inflammation in macrophages

Stem cell environment and skeletal muscle homeostasis

Etienne MEUNIER
Toulouse
IPBS Institut de Pharmacologie et de Biologie Structurale

LECTURE 1

How the context shapes regulated necrosis effectiveness against infections

Immune Detection and Elimination of Pathogens

Jean-Sébastien SILVESTRE
Paris
Paris Cardiovascular research Center PARCC HEGP

KEYNOTE 2

Macrophage and Cardiac Repair

Regenerative therapies for heart and vascular diseases

Asher MAROOF
Londo
ucb

LECTURE 2

Regulation of IL-17F and its role in driving chronic inflammatory diseases

Immunology/Pharmacology

Elodie SEGURA
Paris
institutCurie

KEYNOTE 3

Monocyte differentiation into macrophages versus dendritic cells in steady-state and inflammation

Immune response and cancer

Thomas HENRY
Lyon
ciri Centre International de Recherche en Infectiologie

LECTURE 3

Monocytes and inflammasomopathies

Inflammasome, infections bactériennes et autoinflammation

Gerhard KRONKE
Germany
FAU Friedrich-Alexander Universität Erlangen-Nürnberg Naturwissenschaftliche Fakultät

KEYNOTE 4

Macrophage subsets during the pathogenesis of inflammatory and autoimmune disease

Regulative mechanisms of the innate and the adaptive immune response

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PROGRAM

8h30 – 8h55	***OPENING OF THE REGISTRATION DESK***
8h55 – 9h00	***SYMPOSIUM INTRODUCTION***
	Florence Apparailly (MCM) & Francois Canonne-Hergaux (MCT)
9h00 – 10h30	**SESSION 1: MO / Mϕ and resolution of tissue inflammation**
	Chairs: Farida DJOUAD & François CANONNE-HERGAUX
9h00 – 9h35	**KEYNOTE LECTURE 1 - Bénédicte CHAZAUD (INMG, Lyon)**
	New crosstalk between signaling pathways triggering the resolution of inflammation in macrophages
9h35 – 10h00	**LECTURE 1 - Etienne MEUNIER (IPBS, Toulouse)**
	How the context shapes regulated necrosis effectiveness against infections
10h00 – 10h15	**Selected abstract 1: Raphaël GAUDIN (IRIM, Montpellier)**
	Zika virus enhances monocyte adhesion and transmigration, favoring viral dissemination to the CNS
10h15 – 10h30	**Selected abstract 2: Tamara SIPKA (LPHI, Montpellier)**
	Early wound signals mediate macrophage recruitment and polarization
10h30 – 11h00	***Coffee break / Poster Session***
(30')	***Exhibition & Sponsors***
11:00 - 12:15	**SESSION 2: MO / Mϕ and cardiovascular diseases**
	Chairs: Mary POUPOT & Gabriel COURTIES
11h00-11h35	**KEYNOTE LECTURE 2 – Jean-Sébastien Sylvestre (PARCC, Paris)**
	Macrophages and cardiac repair
11h35-12h00	**LECTURE 2 – Ash MAROOF (UCB, London, UK)**
	Regulation of IL-17F and its role in driving chronic inflammatory diseases
12H00-12H15	**Selected Abstract 3: Johanna MERLIN (C3M, Nice)**
	Myeloid cell glutaminolysis controls monocyte numbers and macrophage efferocytosis during atherosclerosis

PROGRAM

12h15-14h00	***Lunch / Networking / Poster Session***
(1H45)	***Exhibition & Sponsors***

14:00 - 15:30	**SESSION 3: MO / Mϕ and factors regulating their fate**
	Chairs: Isabelle DUROUX-RICHARD & Céline COUGOULE
14h00-14h35	**KEYNOTE LECTURE 3 – Elodie SEGURA (Inst. Curie, Paris, France)**
	Monocyte differentiation into macrophages versus dendritic cells in steady-state and inflammation
14h35-15h15	**LECTURE 3 - Thomas HENRY (CIRI-Lyon, France)**
	Monocytes and inflammasomopathies
15h15-15h30	**Selected Abstract 4: Océane DUFIES (C3M, Nice)**
	Innate immune sensing of RhoGTPases activating toxins

15h30-16h00	***Coffee break / Poster Session***
(30')	***Exhibition & Sponsors***

16h00-16h45	**SESSION 4: MO / Mϕ diversity in autoimmune inflammation**
	Chairs: Karima KISSA & Florence APPARAILLY
16h00-16h15	**Selected Abstract 5: Marion STUNAULT (C3M, Nice)**
	Adipose tissue lipolysis regulates monocyte pool dynamic
16h15-16h55	**KEYNOTE LECTURE 4 - Gerhard KRONKE (Universitatsklinikum, Erlangen, Germany)**
	Macrophage subsets during the pathogenesis of inflammatory and autoimmune disease

16h55-17h00	***Concluding remarks***

Information : https://somm2019.sciencesconf.org
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Selected abstract 1

Zika virus enhances monocyte adhesion and transmigration, favoring viral dissemination to the CNS

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Résumé

Zika virus (ZIKV) invades and persists in the central nervous system (CNS), causing severe neurological diseases. However, the virus journey, from the bloodstream to tissues through a mature endothelium, remains unclear. Here, we show that ZIKV-infected monocytes represent suitable carriers for viral dissemination to the CNS using human primary monocytes, cerebral organoids derived from embryonic stem cells, organotypic mouse cerebellar slices, a xenotypic human-zebrafish model, and human fetus brain samples. We find that ZIKV-exposed monocytes exhibit higher expression of adhesion molecules, and higher abilities to attach onto the vessel wall and transmigrate across endothelia. This phenotype is associated to enhanced monocyte-mediated ZIKV dissemination to neural cells. Together, our data show that ZIKV manipulates the monocyte adhesive properties and enhances monocyte transmigration and viral dissemination to neural cells. Monocytes transmigration may represent an important mechanism requires for viral tissue invasion and persistence that could be specifically targeted for therapeutic intervention.

Mots-Clés: migration, zebrafish, organoid, infectious diseases

*Intervenant

Selected abstract 2

Early wound signals mediate macrophage recruitment and polarization

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Résumé

Tissue wounding induces rapid recruitment of leukocytes, including neutrophils and macrophages and triggers macrophage activation and polarization predominantly towards proinflammatory (M1-like) phenotypes. While macrophage activation is critical for host defense after an injury, cues that coordinate this process *in vivo* are still poorly understood. Recently, calcium and hydrogen peroxide have been shown to be two early wound signals that recruit neutrophils to the wound. However, their role in the recruitment and the activation of macrophages is still unknown. Here we used the transparent zebrafish larva as a tractable vertebrate system to decipher the signaling cascade necessary for macrophage recruitment and activation after the caudal fin injury. By using double transgenic reporter lines to track M1-like activated macrophages combined with high resolutive microscopy and pharmacological approaches, we showed that wound-induced calcium signaling, which involves intracellular Ca^2 released form the ER stores, is enrolled in macrophage recruitment and activation towards M1-like phenotype. By contrast, ROS are only necessary for macrophage activation. Using genetic depletion of neutrophils we showed that neutrophils are not essential for this process. We are now dissecting the molecular actors involved in Ca^2/ ROS axis promoting macrophage activation. This study will reveal new insights into macrophage biology and provide innovative therapeutic targets to control macrophage function during diseases.

Mots-Clés: wound healing, zebrafish, macrophage activation, macrophage polarization

*Intervenant

Selected abstract 3

Myeloid cell glutaminolysis controls monocyte numbers and macrophage efferocytosis during atherosclerosis

Johanna Merlin*[1], Stoyan Ivanov[1], Marion I. Stunault[1], Marion Ayrault[1], Rodolphe R. Guinamard[1], and Laurent Yvan-Charvet[1]

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Résumé

Background: Cardiovascular diseases (CVD) are a major public health issue in the modern society accounting for 17 million deaths per year. It is now known that monocyte count is an independent risk factor predicting disease progression and severity, and represents an attractive therapeutic opportunity. Glutamine plasma concentration is associated with increased monocyte count and increased risk of cardiovascular diseases, but the underlying mechanisms remain unknown.
Hypothesis:We hypothesized that myeloid cell glutaminolysis modulation may contribute to atherosclerosis development.

Methodsand Results: We generated myeloid cell deletion of the rate-limiting enzyme hydrolyzing glutamine into Glutamate called Glutaminase 1 (Gls1) in ApoE deficient mice.These mice had increased number of peripheral blood monocytes associated with increased plaque area as compared to control mice. Mechanistically, these mice demonstrated accelerated monocyte recruitment to the plaque although *in situ* macrophage proliferation in plaque was not affected. Transcriptomic and functional analyses revealed a defective efferocytosis in Gls1 deficient macrophages.
Conclusion: We identified myeloid cell glutaminolysis as a critical player in CVD development. A better understanding of the metabolic pathways affected by Gls1 loss could provide new therapeutic targets to control myeloid cell functions during atherosclerosis.

Mots-Clés: Macrophage, Monocyte, Gls1, Glutaminolysis, Atherosclerosis

*Intervenant

Selected abstract 4

Innate immune sensing of RhoGTPases activating toxins

Océane Dufies*[1], Anne Doye[1], Johan Courjon[1,2], Cedric Torre[1], Grégory Michel[1], Céline Loubatier[1], Arnaud Jacquel[1], Sandrine Marchetti[1], Raymond Ruimy[2], Benedicte Py[3], Mohamed Lamkanfi[4,5], Patrick Munro[1], Orane Visvikis[1], and Laurent Boyer[1]

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Résumé

The detection of the activities of pathogen encoded virulence factors by the innate immune system has emerged as a new paradigm of pathogen recognition. Much remains to be determined with regard to the molecular components contributing to this defense mechanism and its importance during infection. Here, we show the central role of the IL-1bêta signaling axis in controlling the Escherichia coli burden in the blood in response to the sensing of the RhoGTPase-activating toxin CNF1. Consistently, the innate immune response is abrogated in Caspase 1/11-impaired mice or following the treatment of infected mice with an IL-1bêta antagonist. Our latest results further revealed that CNF1 promotes the maturation of IL-1bêta and established the role of Rac2GTPase in the inflammasome regulation. Here, we reveal the importance of effector triggered immunity during mice bacteremia and we decipher the molecular mechanism of this innate immune response.

Mots-Clés: RhoGTPase activating toxin, bacteremia, innate immunity, inflammasome

*Intervenant

Selected abstract 5

Adipose Tissue Lipolysis Regulates Monocyte Pool Dynamic

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Résumé

Monocytes are mononuclear phagocytes generated in the bone marrow compartment. In the bone marrow, monocyte pool depends on chemokine gradients controlling their retention or their egress to peripheral blood vessels: CXCR4-CXCL12 interaction leads to monocyte retention in the bone marrow whereas CCR2-CCL2 interaction favors their mobilization to the blood. Once in the blood, two subsets of blood monocytes are found: The Ly6Clow (patrolling) monocytes, healing the endothelium and phagocyting debris, and the Ly6Chigh (inflammatory) monocytes that enter peripheral tissues and can differentiate into macrophages. Interestingly, blood monocytes follow circadian oscillations, but the metabolic clues involved in this phenomenon remain ill-defined. In our study, we aim to decipher the involved mechanisms and focused on fatty acid metabolism generating deficient mice for adipose tissue lipolysis (AdipoQcre/ERT2x Atgl fl/fl). Using this genetic model, we found a significant decrease in the Ly6Clow and Ly6Chigh monocyte frequency and numbers in blood and spleen. We also observed an increased brown fat monocyte numbers in AdipoQcre/ERT2x Atgl fl/fl mice compared to littermate controls. Blood monocyte pool reduction was not paralleled with modulation of CXCL12 protein levels in the serum nor CCL2. Importantly, bone marrow hematopoiesis was not affected by the loss of adipose tissue lipolysis neither during the day nor during the night in deficient mice. Currently, we are investigating the mechanisms leading to monocyte reduction by analyzing monocyte proliferation, death and retention.

Mots-Clés: Adipose Tissue Lipolysis, Atgl, Monocyte, Circadian Rhythm

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Extracellular Angiogenin polarizes macrophages toward a proinflammatory phenotype.

Yohan Bignon*[1] and Nicolas Pallet[1]

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Résumé

Introduction:
Angiogenin (ANG) is a small secreted ribonuclease expressed in many tissues. We have shown that ANG overexpression take part of the Unfolded Protein Response triggered by Endoplasmic Reticulum Stress (ERS) in human renal epithelial cells (HREC). Indeed, the ribonucleolytic activity of ANG contributes to the global translation repression during ER stress through cleavage of tRNA, leading to cell survival. Moreover, ANG is secreted by HREC during ERS, and can serve as a urinary biomarker of renal tissue damage. We hypothesized that ANG could affect the peritubular macrophages phenotype, thereby mediating the inflammatory reaction associated with ER stress in the kidney. This effect could be mediated by binding of one of its two known receptors: EGFR and PlexineB2.
Material & Methods:
Human THP-1 macrophages were incubated during 4 to 24 hours with human ANG from 3 differents origins: 1 μg/ml of commercial (ANG R&D) or homemade recombinant ANG (ANG GFH) or culture supernatant from human renal epithelial cells overexpressing a FLAG-tagged human ANG (ANG-Flag). Macrophages polarity was analyzed by RT-qPCR targeting 28 genes associated with pro-inflammatory or anti-inflammatory phenotype. Macrophages cytokine/chemokine secretion was assessed by Dot-blot micro-array targeting 40 secreted inflammation-related proteins in the culture supernatant of macrophages incubated during 24h with or without human ANG. EGFR and PlexineB2 protein expression, maturation, surface localization and regulation in THP-1 were evaluated by Western blot, flow cytometry and RT-qPCR.
Results:
Macrophages incubated with ANG have a pro-inflammatory phenotype. Indeed, 4-24 hours of incubation with human recombinant ANG induces an increase in the transcriptional expression of genes encoding iNOS and IDO enzymes, MIP-1α, MIP-1β, IL-8, MCP-1 and IL-8 chemokines and IL-1β and IL-6. The secretome of macrophages incubated with recombinant ANG contains up to 2-fold more IL-8, MIP-1α, MIP-1β, MCP-1 and CXCL10 chemokines compared with control cells. Interestingly, conditioned culture medium of ER stressed cells (which contains ANG) promoted a similar proinflammatory transcriptional profile, suggesting that ANG can mediate, at least in part, the microenvironnemental proinflammatory effects of ER stress. The mechanisms by which ANG polarizes macrophages are under investigation. We also showed that PlexinB2, but not EGFR is expressed at the plasma membrane of macrophages under its mature form cleaved by a Furin-like convertase. Finally, expression

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of PlexinB2 is reduced by 50 % after TLR4 stimulation in macrophages, suggesting that a proinflammatory microenvironnement reduces PlexinB2 expression, and maybe ANG signaling as a potent contra regulatory mechanism..
Conclusion:
ANG polarizes macrophages towards a pro-inflammatory phenotype in vitro. ANG increases chemokines transcriptionnal expression and secretion in macrophages. PlexinB2 is expressed in macrophages and regulated by TLR4-NF-B pathways. ANG/PlexinB2 axis could contribute to the immune response in the renal tissue experiencing ERS.

Mots-Clés: Macrophages, Angiogenin, ER Stress, PlexinB2, Inflammation

MIR-132/FOXP1 AXIS REGULATES MYCOBACTERIUM TUBERCULOSIS-INFECTED-MACROPHAGE METABOLISM

Pauline Bade*[1,2], Maxime Robin[1], Stéphanie Sans[2], Patricia Laboudie[2], Khadija Kissane[2], Florence Apparailly[1], Christine Roubert[2], and Isabelle Duroux-Richard[1]

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Résumé

Background and objectives: *Mycobacterium tuberculosis* (Mtb), the etiological agent of tuberculosis (TB), kills millions every year. As macrophages (M) are Mtb's main host cells, it is crucial to understand their interactions with Mtb and how they develop strategies for the control of TB. Mtb reroutes M metabolism to its own benefit and thus influences M polarization. During the early infection, M harbor a M1-like polarization profile with a quiescent metabolism. They later shift to an anti-inflammatory M2-like polarization with an increased mitochondrial respiration within which Mtb can persist many years. Previous studies have shown that (i) M microRNAs influence M polarization and that (ii) Mtb is able to modulate microRNAs expression in M. In this study, we unveil how miR-132 and its target *foxp1* might modulate M respiration and polarization during Mtb infection. MiR-132 modulation and influence in infected-M will help us to unravel the specificity of host –pathogen interactions in Tuberculosis diseases.

Materials and Methods: We analyzed the miRNome of Mtb-infected THP-1, using a TaqMan Array Human MicroRNA A+B Cards Set. The monocyte cell line THP-1 was stimulated with 0,1 μg/ml LPS, or differentiated into M1 or M2 macrophages following IFN/LPS or IL4/IL13 stimulation, respectively. miRWalk2.0 database was used to identify putative miRNA target sequences within the 3'-UTR mRNA of *foxp1*. Using RT-qPCR, M1/M2 polarization was validated by measuring the expression of proto-typic M1 and M2 markers: the chemokine CXCL10 and the macrophage mannose receptor1 (MRC1, also known as CD206), respectively, as well as *foxp1* mRNA. We used loss-of-function method to evaluate the effect of miR-132 on CD14+ monocytes, *i.e.* its influence on macrophages classical versus alternative polarization. SeaHorse experiments were performed to measure in real time macrophage metabolism.

Results: We identified that miR-132 was upregulated in Mtb-infected THP1 monocytic

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cell line. *In silico* analyses revealed that miR-132 targeted putatively the 3'UTR mRNA of *foxp1*, a gene downregulated in Mtb infected THP-1 cell line. We performed loss-of function studies in LPS stimulated THP-1 to mimic a bacterial infection and trigger M1-like polarization, and we showed that neutralization of miR-132 led to M1-like polarization hallmark *cxcl10* repression, while *foxp1* as well as M2-like polarization hallmark *mrc1* were upregulated. SeaHorse experiments showed that neutralization of miR-132 increased mitochondrial metabolism, which was reversed by *foxp1* silencing.
Conclusion: During the early Mtb infection, miR-132 overexpression and the subsequent repression of its target *foxp1* might be involved in M1-like polarization by decreasing M mitochondrial metabolism.

Mots-Clés: tuberculosis, macrophage, polarization, Mycobacteria, microRNA

A new role for Tregs: Positive control of Tfh commitment

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Résumé

The Foxp3+ T cells, broadly known as regulatory T cells (Tregs), orchestrate and maintain tolerance to self and foreign antigens (Ag). Several studies have reported that Tregs control the immune response by restraining the magnitude of activated T cells. Here, we investigated how Tregs control the humoral immunity by controlling the priming of T follicular helper cell (Tfh), the cognate regulators of B cells. Using the DEREG mouse model, we found that transient depletion of Treg at the time of immunization repressed the Tfh lineage at the early stage of immune response. This correlated with a defect of the Tfh-controlled germinal center (GC) reaction and, more precisely, a decrease of the Ag-specific high-affinity antibodies. IL-2 signaling through the IL-2Ra (CD25) is one known mechanism to suppress the Tfh lineage by limiting Bcl6 expression. However, Achaete-scute homologue 2 (Ascl2) has been described as the transcription factor that initiates the CXCR5 expression in a Bcl6-independent manner. We comprehensively demonstrated that Treg promote Tfh commitment through a Bcl6-independent mechanism by promoting pro-Tfh -cDC2. Overall, these results suggest that Tregs control Tfh lineage at the early stage of an immune response during priming after protein vaccination. This study opens new perspectives in manipulating and enhancing the high-affinity antibody production in vivo.

Mots-Clés: Dendritic cell, TFH, Treg, Germinal Center

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Optimized 3D biopolymer scaffold for macrophages culture and implantation

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Résumé

Biomaterial cell-based therapy holds great hope in the field of regenerative medicine including soft tissues. The main advantage of cell delivery through a 3D scaffold is to provide them at the same time a biomimetic environment and a protection to implantation stress. . Those past years immunomodulatory approaches have raised growing interest thanks to their ability to target inflammation, which is known to play a central role in tissue repair. More precisely, macrophages are crucial for tissue homeostasis and show great plasticity to environmental cues. Modulation of macrophage polarization is a key feature in tissue repair process. In this context, we have developed original 3D scaffolds which can be seeded with M2-polarized macrophages, with the aim to optimize the results of cell therapy of soft tissues. The success of such strategy lies on the scaffold's design, as its biocompatibility and architecture may influence host's reaction and seeded cells' fate.
Recently, CIRIMAT has developed a family of macroporous biopolymer 3D scaffolds based on a combination of polyelectrolyte complex of opposite charge formation and production processes. According to operating conditions, the resulting scaffolds present various 3D architecture whose beneficial influence on cells fate and secretion properties has been previously demonstrated in collaboration with the I2MC [1,2]. Based on alginate and chitosan, two well-known biocompatible polymers, the scaffolds exhibit an excellent biocompatibility and have shown pro-angiogenic properties by itselves [3].

It is now recognized that the paracrine effects of cells are primarily responsible for their beneficial effects in tissue regeneration. The modulation of macrophages polarization *in vitro* induces a broad spectrum of pro- and anti-inflammatory cytokines secretion. The challenge is now to optimize the scaffolds' architecture and physico-chemical properties according to the targeted macrophage phenotype. Here our strategy is to preserve a M2-polarized

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macrophages phenotype inside our 3D biopolymeric scaffold. The first necessary steps to develop our strategy are to study macrophages' retention, viability and phenotype after seeding within the scaffold. Our preliminary *in vitro* results show that a minimum retention rate of 84% of seeded cells within the scaffold can be reached. The seeded macrophages' viability can be followed by confocal fluorescence imaging as hydrated scaffolds are translucent. RT-PCR assays can be performed to evaluate macrophages phenotype. Further characterization is required such as the study of pro- and anti-inflammatory cytokines secretion. Those assays are currently under way at the I2MC and are performed according to scaffolds' polymer ratio and drying process.

This work underlines the potentialities of our 3D biopolymer scaffolds as macrophages cell culture system and carrier for tissue regeneration. The most promising formulation will be tested *in vivo* to put in evidence the influence of the seeded scaffolds on tissue regeneration, with a particular emphasis on angiogenesis and inflammation.

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Bushkalova R, Farno M, Tenailleau C, Duployer B, Cussac D, Parini A, Sallerin B, Girod Fullana S. Alginate-chitosan PEC scaffolds: A useful tool for soft tissues cell therapy. International Journal of Pharmaceutics 571 (2019) 118692

Mots-Clés: Biopolymer, tissue engineering, macrophages

Role of Tubulin β6 on microtubule dynamics and podosome belt organization.

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Résumé

Bone is a dynamic tissue constantly renewed through the activity of osteoclasts, which resorb bone and of osteoblasts, which form the new bone. Osteoporosis is caused by excessive activity of osteoclasts. Their unique ability to resorb bone dependents on the formation of an actin-rich belt (sealing zone). It is composed of podosomes. Our goal is to characterize new actors and mechanisms regulating bone resorption. We showed that the inhibition of the exchange factor Dock5, prevents the organization of the podosome belt, osteoclast activity and bone loss in mice. Thus, preventing the organization of the podosome belt could be a novel approach to decrease bone resorption in patients with osteoporosis. The dynamics of the actin and microtubule cytoskeleton plays an essential role in the organization of osteoclast podosomes. In order to find new molecular mechanisms controlling the activity of osteoclasts, we performed global transcriptomic and proteomic approaches. Those data compared osteoclasts to monocytes and dendritic cells. These three myeloid cell types have podosomes, but only the osteoclast can organize them into a belt. Thus, we looked for genes induced in osteoclasts as compared to both other types. We found that osteoclasts express two tubulin β isotypes: β6 and β5, but only tubulin β6 gene is overexpressed in osteoclasts. Recently, we showed that the inhibition of tubulin β6 in osteoclasts affects microtubule dynamics, prevents the organization of the podosome belt and the bone resorption activity. Different papers showed that tubulin isoform composition can affects microtubule dynamics. Thus, we want to understand how tubulin β6 can affects the podosome belt organization. For this, the first aim is to study in vitro the intrinsic properties of microtubules according to their tubulin β6 and β5 content. The second goal will be to how tubulin β6 and β5 can impact on microtubule associated proteins in osteoclasts.

Mots-Clés: Osteoclast Bone resorption Cytoskeleton Tubulin β6

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Identification of new species involved in bone resorption by osteoclasts and impact of the ß-tubulin isotype repertoire

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Résumé

Osteoclasts belong to the monocyte lineage of myeloid hematopoietic cells and can be differentiated from macrophage precursors. Osteoclasts are major actors of bone resorption. Excessive activity of osteoclasts leads to bone loss and is found in pathological situations as osteoporosis, rheumatoid arthritis and bone metastases. To resorb bone, osteoclasts reorganize their cytoskeleton, develop their vesicular traffic and adhere on bone by a typical structure, the sealing zone: a belt of podosomes, which is the backbone of the resorption apparatus. This study aims to characterize new actors and mechanisms regulating bone resorption. To that end, we identified proteins and genes preferentially present in osteoclasts. These species may participate to bone resorption and be studied as therapeutic targets to decrease osteoclasts activity in osteolytic diseases.
To achieve this aim, the general principal of this work was to use two high-throughput methods, differential SILAC quantitative proteomics and RNAseq transcriptomics. We compared osteoclasts to two closely related myeloid cell types, immature dendritic cells and macrophages. Indeed, these cell types present podosomes, but only osteoclasts are able to organise them into a belt and to degrade bone. By bioinformatic and statistic analyses, we identified species specific to each cell type, especially osteoclasts, and the associated cellular pathways. To identify among these species potential actors of osteoclast biology, osteoclasts were transfected with siRNAs of interest during their differentiation.

A total of 2793 proteins and 17199 genes were identified among the three cell types in this study. Although transcriptomic allows deeper analysis, the correlation between proteomic and transcriptomic results is weak (around 0.3). So, these two methods are complementary for the identification of new regulators of bone resorption.

Hierarchical clustering analysis of transcriptomics data revealed 5 mean gene expression profiles between osteoclasts, dendritic cells and macrophages. A total of 1207 genes were defined as preferentially expressed in osteoclasts, including genes with known functions in osteoclasts. Enriched GO terms are mainly linked to mRNA translation and to energy metabolism. We also determined two major interaction gene networks among the osteoclast

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signature, which also contain genes involved in mRNA translation.

Regarding proteomics, we defined a 145-protein osteoclast signature and classified them according to their function. Expectedly, there were 27 known regulators of osteoclast activity, 6 vATPase subunits and 41 proteins participating in energy metabolism, mainly mitochondrial. We also found 12 more mitochondrial proteins and 14 proteins involved in translation. Finally, we could highlight 45 proteins which function was unknown in osteoclasts, including 16 proteins regulating cytoskeleton or vesicular traffic and 5 proteases and protease inhibitors. Enriched GO terms associated to the osteoclast signature are linked to energy metabolism, known to be elevated in osteoclasts.

Among these 45 proteins, 38 were not found enriched in osteoclasts derived from RAW 264.7 cell line in a previous study (An et al, 2014). Among them, we selected 17 proteins with a variety of functions to investigate their participation in osteoclast biology by transfection with siRNAs. For 5 proteins, depletion strikingly leads to the appearance of very large unusual structures in osteoclasts, which we defined as vacuole-like structures. A hypothesis we emit to explain the formation of these structures was a disturbance of osteoclast intracellular trafficking.

Moreover, our SILAC and RNAseq data revealed that the repertoire of Tubulin isotypes was different between osteoclasts, macrophages and dendritic cells. Among all α and β tubulin genes, myeloid cells expressed only 4 α and 4 β isotypes namely Tubba1a, 1b, 1c and 4a and Tubb2a, 4b, 5 and 6. The same 4 α and 4 β isotypes were expressed in the three cell types but interestingly Tubb6 protein was significantly increased in osteoclasts whereas the level of the other α and β Tubulin proteins was not different. After depletion of Tubb6, we noticed that belt formation was affected and resorption capacity of osteoclasts on mineralised matrix was diminished. This results support the idea that the specific tubulin isotype repertoire is of functional importance in myeloid cells, in particular that Tubb6 is essential to ensure correct patterning of podosomes and efficient bone resorption in osteoclasts.
In conclusion, our transcriptome and proteome-wide approach characterized new species preferentially abundant in osteoclasts. Among them, Tubb6 seems to be of particular importance. Furthermore, deeper analysis of the function of these candidates in osteoclast biology should uncover new approaches to treat osteolytic lesions.

SPONSORS

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