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Amphithéâtre Denis Escande - IRS-UN

SoMM2024

7th Symposium of the network on MONOCYTES & MACROPHAGES

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Symposium of the Network on Monocytes & Macrophages

9:00 AM– Participant Reception
9:30 AM– Introduction

9:45 AM – **Session "Myeloid Cells and Metabolism"**

Pascale Jeannin, Angers
« *Impact of lactic acid and acetoacetate on human macrophages* »

- Maaike SUURING (CR2TI, Nantes) : Comparative analysis of transcriptional and metabolic profiles in ATDC and DC10 used in myeloid regulatory cell-based immunotherapies
- Najia JEROUNDI (Innate Immunity and Cancer, Angers): Glycogenesis and glyconeogenesis from glutamine, lactate and glycerol support human macrophage functions
- Alison DUMONT (CR2TI, Nantes) : Human Tolerogenic Dendritic Cells used in clinic regulate CD8+ T cells by their secretome

11:00 AM – Break and Poster Session

11:30 AM – **Session "Roles of Myeloid Cells in Disease"**

Gaëtan Juban, Lyon
« *Efferocytosis-mediated resolution of inflammation during normal and pathological skeletal muscle regeneration* »

- Olivier BORTOLOTTI (IRBM, Montpellier) : Age-Associated Macrophage Deficits Drive Fibrotic Remodeling in Synovial Joints
- Nicolas GAIGEARD (RMeS, Nantes):Single-cell transcriptomic analyses of OA synovial macrophages reveal pathotype-specific profiles.
- Monika MYKHAYLISHYN (CR2TI, Nantes) :Macrophage dysfunction in Crohn's Disease

12:45 PM – Lunch and Poster Session

2:15 PM – **Session "Myeloid Cells & Infections"**

Molly Ingersoll, Paris
« *Macrophages in the Bladder Mucosa* »

- Thomas OGOR (CR2TI, Nantes) : Absence of the innate immune checkpoint CLEC-1 prevents the establishment of the immunosuppressive state of myeloid cells during sepsis in mice
- Camille DAVID (CEPR, Tours): Influenza virus induces distinct alterations in human and mouse alveolar macrophages: a comparative analysis
- Alexandre MAYE (CR2TI, Nantes) : Study of glycolytic mononuclear phagocytes in the tumor microenvironment

3:30 PM – Break and Poster Session

4:00 PM – **Session "Myeloid cells in Inflammation and Resolution"**

Sylvain Perruche, Besançon
« *Dysregulation and Reengagement of Resolution* »

- Agathe DEPAIRE (Immunoconcept, Bordeaux):IL-1β and microvascular endothelial cells synergize to favor altered macrophage efferocytosis: implication in SSc
- Tiphaine DELAUNAY (CRCI2NA, Nantes):Influence of pleural mesothelioma tumor cells on the macrophage subpopulations present in the tumor microenvironment.
- Dima GHANNOUM (Institut de Cancérologie de l'Ouest, Angers):Shedding light on tumor-monocyte interactions with 3D cell culture models of triple negative breast cancers

5:15 PM– Conclusion

Oral presentations

Comparative analysis of transcriptional and metabolic profiles in ATDC and DC10 used in myeloid regulatory cell-based immunotherapies.

Maaike Suuring * [1], Amodio Giada [2], Mathieu Rouel [1], Mikaël Croyal [3], Axel Raux [4], Thomas Delhaye [5], Anne-Lise Royer [4,6], David Rondeau [5], Francesca Santoni De Sio [2], Silvia Gregori [2], Aurélie Moreau [1]

[1] Team 1 : Mononuclear phagocytes, Immunopathology, Immunovirology – Centre de Recherche en Transplantation et Immunologie - Center for Research in Transplantation and Translational Immunology – France

[2] San Raffaele Telethon Institute for Gene Therapy (SR-TIGET) – Italy

[3] Université de Nantes,CHU nantes, CNRS, Inserm, l'institut du thorax, Nantes, France – Centre de Recherche Inserm – France

[4] Laboratoire d'étude des Résidus et Contaminants dans les Aliments – Oniris, INRAE, LABERCA, UMR 1329 – France

[5] Institut d'Électronique et des Technologies du numéRique – Université de Rennes, Institut National des Sciences Appliquées - Rennes, CentraleSupélec, Centre National de la Recherche Scientifique, Nantes Université - pôle Sciences et technologie – France

[6] MetaboHUB-Grand-Ouest – INRAE – France

Tolerogenic dendritic cells serve essential immunological roles, as prevention of inflammation and maintenance of self-tolerance by modulating the immune response. This modulation makes them a promising therapeutic target across a wide range of clinical therapeutic applications, including, chronic inflammatory disease, autoimmunity and organ transplantation. Over the last years, various human tolDCs have been generated ex vivo, each displaying specific phenotypical and functional characteristics. Our study aimed to compare two well-known tolDCs, autologous tolerogenic dendritic cells (ATDC) (1) and IL-10 induced dendritic cells (DC10) (2), on their phenotype, metabolic secretion, transcriptomic profile and their ability to inhibit T cell responses. Our results indicate that both ATDC and DC10 are efficient in inducing tolerance, but operate different mechanisms. Interestingly, even if both tolDCs regulate T cell proliferation, they exhibit highly distinct transcriptomic and metabolomic profiles. The data suggest that DC10 regulates tolerance in an antigen-specific manner and/or through cytokines and protein mediators, whereas ATDC mediate tolerance via metabolic adaptations or small secreted immunomodulators. On metabolic level, both tolDCs excrete high levels of lactate, which has been shown to regulate the inhibition of CD4+ T cell response (1). Understanding these specific regulatory mechanisms is crucial for their application in cell therapy to target immunological diseases and potentially enhance therapeutic outcomes.

1. Marin E, Bouchet-Delbos L, Renoult O, Louvet C, Nerriere-Daguin V, Managh AJ, et al. Human Tolerogenic Dendritic Cells Regulate Immune Responses through Lactate Synthesis. Cell Metab. 2019 Dec 3;30(6):1075-1090.e8.

2. Gregori S, Tomasoni D, Pacciani V, Scirpoli M, Battaglia M, Magnani CF, et al. Differentiation of type 1 T regulatory cells (Tr1) by tolerogenic DC-10 requires the IL-10-dependent

*Speaker

ILT4/HLA-G pathway. Blood. 2010 Aug 12;116(6):935–44.

Keywords: Tolerogenic dendritic cells, clinical application, metabolism, transcriptomic

Human Tolerogenic Dendritic Cells used in clinic regulate CD8+ T cells by their secretome

Maaike Suuring [1,2,3], Alison Dumont * [1,2,3], Mathieu Rouel [1,2,3], Alexandre Maye [1,2,3], Gaelle Tilly [2,3], Chloé Cloteau [4], Denisia Lavercan [5], Claire Pecqueur [6], Xavier Prieur [4], Mikaël Croyal [7], Nicolas Degauque [2,3,8], Aurélie Moreau [1,2,3]

[1] Team 1 : Mononuclear phagocytes, Immunopathology, Immunovirology – Centre de Recherche en Transplantation et Immunologie - Center for Research in Transplantation and Translational Immunology – France

[2] Nantes Université, INSERM, Center for Research in Transplantation and Translational Immunology, UMR 1064, ITUN, Nantes, France – Nantes Université, INSERM, Center for Research in Transplantation and Translational Immunology, UMR 1064, ITUN, Nantes, France – France

[3] LabEx IGO, Nantes Université, Nantes, France – LabEx IGO, Nantes Université, Nantes, France – France

[4] L'unité de recherche de l'institut du Thorax
$\mathrm{UMR}_S 1087/UMR_c 6291, INSERM, Nantes Université, CHU Nantes, 44000 Nantes, France - -L'unité de recherche de l'institut du Thorax UMR_S 1087 - - France$

[5] CRNH-O Mass Spectrometry Core Facility, Nantes, France. – CRNH-O Mass Spectrometry Core Facility, Nantes, France. – France

[6] Nantes Université, INSERM CNRS, Nantes - Angers Cancer and Immunology Research Center, Nantes France – Nantes Université, INSERM CNRS, Nantes - Angers Cancer and Immunology Research Center, Nantes France – France

[7] Université de Nantes,CHU nantes, CNRS, Inserm, l'institut du thorax, Nantes, France – Centre de Recherche Inserm – France

[8] Team 4 : Deciphering organ immune regulation in inflammation and transplantation (DORI-t) – Centre de Recherche en Transplantation et Immunologie - Center for Research in Transplantation and Translational Immunology – France

Our study addresses the critical issue of allograft rejection in kidney transplantation, where current immunosuppressive drugs pose significant risks. We propose a novel approach of cell therapy using *autologous tolerogenic dendritic cells* (ATDC) to promote graft acceptance. Based on our encouraging results in rodents, our team developed a GMP-compliant ATDC manufacturing process and this cell therapy was evaluated in a first-in-man phase I/II clinical trial. In vitro characterization of ATDC highlighted that these cells exhibit an enhanced glycolytic activity and the abundant produced lactate was responsible for the suppression of CD4+ T cell proliferation and activation. In our trial, a reduced CD8+ T cell activation and an increased FoxP3 expression were observed in the circulating immune cells of kidney transplant recipients treated with ATDC. This study aims to decipher the regulation of CD8+ T cells by ATDC. Our *in vitro* experiments demonstrated that ATDC-derived metabolites suppress naïve and effector/memory CD8+ T cells proliferation through contact-independent mechanisms. However, unlike CD4+ T cells, lactate was not involved in this suppression. Metabolic and transcriptomic analyses highlighted that ATDC environment promotes oxidative phosphorylation in naive CD8+ T cells, associated with their reduced activation. Furthermore, the critical role of indoleamine-2,3-dioxygenase produced by ATDC to suppress CD8+ T cells was evidenced using a specific inhibitor, suggesting

*Speaker

the regulation of the tryptophan pathway. Moreover, ATDC reduce the migratory capacity of these cells through a contact-dependent mechanism. In conclusion, our findings support the efficacy of ATDC as a therapeutic strategy in kidney transplantation, particularly in suppressing CD8+ T cell-mediated allograft rejection.

Keywords: transplantation, tolerogenic, metabolism

Age-Associated Macrophage Deficits Drive Fibrotic Remodeling in Synovial Joints

Olivier Bortolotti * [1], Gabriel Courties [2], Florence Apparailly [2]

[1] Cellules Souches, Plasticité Cellulaire, Médecine Régénératrice et Immunothérapies (IRMB) – Centre de Recherche Inserm – France
[2] Cellules Souches, Plasticité Cellulaire, Médecine Régénératrice et Immunothérapies (IRMB) (IRMB U1183) – Centre Hospitalier Régional Universitaire [Montpellier], Institut National de la Santé et de la Recherche Médicale, Université de Montpellier – Institute for Regenerative Medicine and Biotherapy - 80 rue Augustin Fliche 34295 Montpellier - Cedex 5, France

Aging is associated with the decline of joint function and an increased risk of degenerative joint disorders. The synovial tissue microenvironment is composed of various cell types that work together to maintain joint integrity and function. However, the cellular and molecular mechanisms driving age-related changes in synovial tissue remain poorly characterized.
In this study, we used single-cell RNA sequencing (scRNA-seq) to map the cellular landscape of aging murine synovial tissue. We identified age-associated transcriptional changes across individual synovial cell types. In particular, synovial macrophages exhibited functional deficits in homeostasis, including impaired phagocytic activity. Further analysis revealed a significant reduction in TIM4+ VSIG4- macrophages within the interstitial sublining niche, accompanied by increased tissue fibrosis. Notably, depletion of macrophages in healthy joints accelerated fibrosis, underscoring their critical role in preventing age-related tissue remodeling.
These findings provide new insights into the physiological networks within the aging synovial compartment, offering potential therapeutic avenues for mitigating joint degeneration in older individuals.

Keywords: Macrophage, synovial tissue, aging

*Speaker

Single-cell transcriptomic analyses of OA synovial macrophages reveal pathotype-specific profiles.

Nicolas Gaigeard * [1], Anaïs Cardon [1], Astrid Ouattara [1], Claire Vinatier [1], Frédéric Blanchard [1], Denis Waast [1,2], Benoit Le Goff [1,3], Jérôme Guicheux [1], Marie-Astrid Boutet [1,4]

[1] Regenerative Medicine and Skeleton – Ecole Nationale Vétérinaire, Agroalimentaire et de l'alimentation Nantes-Atlantique, Institut National de la Santé et de la Recherche Médicale, Nantes Université - UFR Odontologie, RMeS INSERM U1229 – France
[2] CHU Nantes – Department of Orthopaedics, CHU Nantes, Nantes, France – France
[3] CHU Nantes – Department of Rheumatology, CHU Nantes, Nantes, France – France
[4] William Harvey Research Institute, Barts and the London Medical School – United Kingdom

Osteoarthritis (OA) is the most common joint disease, affecting 13.5% of the population aged over 60 y.o. worldwide, and causing pain and loss of joint function. Currently, no curative treatment exists and the mechanisms involved in OA pathogenesis are poorly understood. The involvement of synovitis in pain and disease progression is recognised, and we previously demonstrated that OA synovium can be categorized into three distinct histopathological pathotypes: pauci-immune (PI), diffuse myeloid (DM) and lympho myeloid (LM), based on the presence and distribution of macrophages, B, T and plasma cells. Interestingly, macrophages represent the main immune population in the three pathotypes. This study aims to characterize the synovial macrophage diversity in the three pathotypes in relationship with OA clinical presentation.
Synovial tissues were retrieved from 9 OA patients who underwent total knee replacement. Samples were histologically characterized and presented a PI (n=3), DM (n=3) or a LM (n=3) pathotype. Two non-OA synovial tissues retrieved from patients who underwent meniscectomy were used as controls. Following enzymatic digestion, CD45+ cells were sorted and sequenced using single-cell RNA sequencing. The analyses were carried out under R, using Seurat (V5.1.0). Multiple immunofluorescence staining was performed on paraffin-embedded tissues.
The study identified 13 macrophage populations, confirming previous studies in the context of rheumatoid arthritis. Our results showed that certain macrophage populations, such as SPP1pos, LYVE1pos, and TREM2pos, were specifically more prevalent the PI, DM, and LM pathotype respectively. Multiple immunofluorescences were optimized to visualize each population and the analyses are ongoing to confirm our findings on a larger number of samples. Spatial transcriptomic analyses using the Xenium analyser are ongoing to understand the intercellular communications between macrophages and other immune and stromal cells in the synovial microenvironment. Ultimately, this research aims ameliorate the understanding of OA pathophysiology and contribute to the development of personalized treatments.

Keywords: Osteoarthritis, Macrophages, Single, cellRNAsequencing

*Speaker

Macrophage dysfunction in Crohn's Disease

Monika Mykhaylyshyn * [1], Thomas Laurent [1], Gaëlle Beriou [1], Aurélie Joussaume [1], Nicolas Chapelle [1], Lucas Brusselle [1], Cynthia Fourgeux [1], Cécile Braudeau [2], Justine Chevreuil [2], Laurence Delbos [1], Camille Lecuroux [1], Catherine Le Berre [3], Caroline Trang [3], Théo Soude [3], Juliette Podevin [3], Jessica Le Berichel [4], Pablo Canales-Herrerias [4], Samarth Hedge [4], Cécile Girard [5], Jean-François Mosnier [5], Miriam Merad [4], Saurabh Mehandru [4], Arnaud Bourreille [3], Jeremie Poschmann [1], Ephraim Kenigsberg [4], Jérôme Martin [1,2]

[1] Center for Research in Transplantation and Translational Immunology, Nantes Université, CHU Nantes, INSERM, UMR 1064, Nantes, France. – Center for Research in Transplantation and Translational Immunology, Nantes Université, CHU Nantes, INSERM, UMR 1064, Nantes, France. – France

[2] CHU Nantes, Laboratoire d'Immunologie, CIMNA, Nantes, France – CHU Nantes, Laboratoire d'Immunologie, CIMNA, Nantes, France – France

[3] Institut Des Maladies de L'Appareil Digestif (IMAD), Nantes Université, CHU Nantes, Hépato-Gastro-Entérologie, Inserm CIC 1413, 44000, Nantes, France – Institut Des Maladies de L'Appareil Digestif (IMAD), Nantes Université, CHU Nantes, Hépato-Gastro-Entérologie, Inserm CIC 1413, 44000, Nantes, France – France

[4] Icahn School of Medicine at Mount Sinai [New York] – United States

[5] Service d'Anatomie et Cytologie Pathologiques (ACP), CHU Nantes, France – Service d'Anatomie et Cytologie Pathologiques (ACP), CHU Nantes, France – France

Crohn's Disease (CD), one of the two main forms of inflammatory bowel disease (IBD) is a disabling condition with a growing incidence worldwide. CD is associated with severe complications, frequently leading to surgical resection. Anti-TNF therapy has transformed CD outcome but efficacy is limited to a subset of patients. We previously described a cellular response enriched in inflamed ileums of non-responders to TNF blockers (Martin et al., 2019), which suggested major roles for dysregulated actions of mononuclear phagocytes (MNP). In this study, we cell-sorted MNP from resections of CD patients and characterized them by single-cell RNA sequencing (scRNA-seq). Using Metacells (Baran et al. 2019; Ben-Kiki et al. 2022) and a gene module-based analysis, we captured 17 molecular programs of coregulated genes present in the monocytes/macrophages compartment, the combinatorial expression of which allowed to resolve 10 molecular states of monocytes and macrophages. While monocyte-like cells were enriched in inflamed ileums, our approach enabled the identification of an inflammatory subset characterized by coexpression of CD274 and IL3RA that was uniquely enriched in inflamed tissues from patients with severe forms of the disease, including anti-TNF resistance and surgical recurrence. Using flow cytometry, we validated the enrichment of PD-L1+ CD123+ monocyte-like cells in inflamed ileums. Transcription factor binding motif enrichment analyses of the 17 molecular programs suggested the generation of PD-L1+ CD123+ monocyte-like cells depended on a complex inflammatory milieu, which was validated in vitro. Importantly, PD-L1+CD123+ monocyte-like cells exhibited a high JAK/STAT activity. Accordingly, the JAK inhibitor upadacitinib, which was approved by the FDA to treat patients resistant to anti-TNF therapy, interfered with the generation and activity of PD-L1+CD123+ monocyte-like cells. In summary, our study identifies a molecular state of inflammatory monocytes uniquely enriched severe in CD, as compared

*Speaker

Absence of the innate immune checkpoint CLEC-1 prevents the establishment of the immunosuppressive state of myeloid cells during sepsis in mice

Thomas Ogor * [1], Esther Porée [1], Marion Davieau [1], Javier Saenz [1], Régis Josien [1], Victor Gourain [1], Jeremie Poschmann [1], Antoine Roquilly [1], Cédric Jacqueline [1], Elise Chiffoleau [1]

[1] Centre de Recherche en Transplantation et Immunologie - Center for Research in Transplantation and Translational Immunology – Nantes université - UFR des Sciences et des Techniques – France

Pneumonia is a leading cause of hospitalization and death worldwide. Sepsis exhibits phases of hyperinflammation, alternating with immune suppression, characterized by a remodelling of the myeloid immune compartment, increasing the incidence of severe forms of secondary infections. Thus, defining new immunotherapies is crucial for the prevention and treatment of sepsis-induced immunosuppression. We previously described the C-type Lectin Receptor CLEC-1 as a receptor of necrotic cells, expressed by myeloid cells such as conventional Dendritic Cells type I (cDC1) and limiting acute immune responses during sterile inflammation. As such, we evaluated the role of this novel immune checkpoint in sepsis-induced immunosuppression.
To do so, we analysed the immune response in *Clec1a* KO mice during the acute pro-inflammatory and later resolution phases of *E. coli* pneumonia. Unexpectedly, *Clec1a* KO mice lose less weight than WT mice during the acute phase of sepsis suggesting less inflammation in the absence of CLEC-1. We found that resident myeloid cells such as cDC1 and alveolar macrophages (AM) downregulated Interferon Stimulated Genes (ISGs) and other activation markers during the early acute phase. In contrast, during the late acute phase, infiltrating myeloid cells such as neutrophils and monocyte-derived AMs displayed a higher activation state. This suggests that the absence of CLEC-1 limits the hyperinflammatory state of lung-resident myeloid cells, preventing the establishment of the immunosuppressive state later on. Indeed, we observed during a secondary infection, a higher level of class II major histocompatibility complexes (MHCII) on infiltrating macrophages of *Clec1a* KO mice compared to WT mice.
These preliminary data show that the absence of CLEC-1 balances both hyperinflammatory and immunosuppressive phases in resident and infiltrating myeloid cells to prevent sepsis-induced immunosuppression, supporting CLEC-1 as a potential therapeutic target during sepsis.

Keywords: Sepsis, Myeloid Cells, Immunosuppression

*Speaker

Study of glycolytic mononuclear phagocytes in the tumor microenvironment

Alexandre Maye * [1], Mathieu Rouel [1], Cécile Girard [2], Elvire Pons-Tostivint [3], Murielle Corvaisier Chiron [4], Myriam Ammi [5], Grégoire Justeau [5], Yves Delneste [6], Aurélie Moreau [1]

[1] Team 1 : Mononuclear phagocytes, Immunopathology, Immunovirology – Centre de Recherche en Transplantation et Immunologie - Center for Research in Transplantation and Translational Immunology – France
[2] Service Anatomocytopathologie (CHU Nantes) – Service Anatomocytopathologie (CHU Nantes) – France
[3] Centre Hospitalier Universitaire de Nantes – Centre Hospitalier Universitaire Nantes, Nantes University, Nantes, France – France
[4] UIC Oncologie Thoracique – CHU Nantes – France
[5] Centre Hospitalier Universitaire dÁngers – CHU Angers – France
[6] Centre de Recherche en Cancérologie et Immunologie Intégrée Nantes-Angers – Université d'Angers, Centre de Recherche Inserm, CNRS : UMR6075, Université d'Angers – France

Tumor microenvironment (TME) is a complex, dynamic and suppressive environment, rich in lactate, mainly produced by tumor cells. This metabolite promotes tumor escape notably through functional changes in immune cells. It has been shown to promote the polarization of mononuclear phagocytes (MNP) into pro-tumor MNP, with these cells contributing to tumor growth in mice. Furthermore, the absence of expression of the enzyme that enables lactate synthesis, specifically in monocytes/macrophages, leads to a reduction in tumor growth. The team previously generated and studied a population of *in vitro*-derived MNP with strong suppressive activity and glycolytic metabolism. In an original way, these cells inhibit the proliferation of CD4+ T cells through their high lactate secretion. Based on these properties and the literature, our hypothesis is that some population of MNPs secrete lactate which play an important role in the suppression of tumoral T cells. Preliminary *in silico* studies using public single cell RNAseq data from human lung tumors allowed to identify two macrophages clusters with glycolytic metabolism and low pro-inflammatory activity. The aim of my project is to confirm the presence of such populations *in vivo*, from human lung tumor samples, using spectral flow cytometry. First samples were used to optimize tissue dissociation's protocol and to develop a flow cytometry panel including markers allowing MNP characterization and the analysis of cell metabolism. This panel then enabled the identification of different MNP clusters, some of them being enriched in the tumor and that seem to display a higher expression of GLUT1, a glucose transporter. The majority of these clusters correlates with the literature, confirming the panel's functionality and the right choice of markers. More donors need to be included to confirm these results and to perform further analyses by spectral flow cytometry, notably by investigating the functionality of the clusters.

Keywords: Mononuclear phagocytes, Lactate, Immunosupression, Tumor Microenvironment

*Speaker

IL-1β and microvascular endothelial cells synergize to favor altered macrophage efferocytosis: implication in SSc

Agathe Depaire * [1,2], Damien Brisou [1], Damien Leleu [3,4], Matthieu Cochard [1], Charlotte Betbedat [1], Félix Blaison [1], Marie-Elise Truchetet [1,5], Sylvain Perruche [6,7], Cecile Contin-Bordes [1,8]

[1] Immunology from Concept and Experiments to Translation = Immunologie Conceptuelle, Expérimentale et Translationnelle – CNRS UMR5164, Université de Bordeaux – France
[2] MED'INN'Pharma, Besançon, France – MED'INN'Pharma, Besançon, France – France
[3] Université Bourgogne UMR1231 – UMR U1231 Université Bourgogne Franche-Comté, 21078 Dijon cedex – France
[4] Laboratoire de Biochimie Spécialisée – CHU Dijon Bourgogne – France
[5] CHU de Bordeaux Pellegrin [Bordeaux] – Rheumatology Department, Bordeaux University Hospital, France – France
[6] INSERM UMR right 1098 (Equipe TAI-IT) – EFS, Université Bourgogne Franche-Comté - UFC, Institut National de la Santé et de la Recherche Médicale - INSERM – France
[7] MED'INN'Pharma – MED'INN'Pharma – France
[8] Immunology and Immunogenetic Departement, Bordeaux University Hospital – Hopital Pellegrin - CHU Bordeaux – France

INTRODUCTION:

Systemic Sclerosis (SSc) is a chronic incurable fibrotic autoimmune disease. Chronic inflammation can convert a limited repair response into chronic fibrosis. Effective resolution of inflammation depends on macrophage (M) clearance of apoptotic cells (efferocytosis). The aim of the present work was to decipher whether microvascular endothelial cells (MECs) and IL-1β might contribute to altered M efferocytosis in SSc, and to address its consequences on fibroblast activation and endothelial to mesenchymal cell transition (EndoMT).

MATERIALS AND METHODS:

Skin MECs were purified from four healthy donors and four SSc patients. Monocyte-derived M were generated as described in (Laurent et al,AR,2022). Efferocytosis-associated receptors expression was analyzed by flow cytometry. Efferocytosis was evaluated with pH-Rhodo-stained apoptotic Jurkat cells for 24 hours using Incucyte. Healthy fibroblasts or MECs were cultured with MEC-M supernatants for 48 and 72 hours, respectively. Fibroblast and EndoMT were assessed by RT-qPCR.

RESULTS:

IL-1β-MECs-M exhibited a significant decrease of phagocytosis, that was fully restored by recombinant IL-1RA. However, addition of IL-1β during MDM0 differentiation did not altered phagocytosis. AXL, SRB1, ITGB5, CD36, and BAI1 expression was decreased in IL-1β-MECs condition, but irrespective of the origin of the MECs. IL-1RA fully restored AXL, did not affect SRB1, and only partially restored ITGB5, CD36, and BAI1 expression, suggesting that other

*Speaker

secreted factors present in MECs secretome synergize with IL-1β to modulate their expression.

IL-1β-MECs-M favored fibroblasts pro-remodeling and inflammatory phenotype, but only efferocytic supernatant induced significant additional up regulation of MMP1/TIMP1 ratio, along with aSMA and fibronectin down regulation; with a more pronounced effect in SSc. IL-1β-activated MECs-M supernatant induced a significant increase of HD MECs aSMA expression only in post-efferocytosis SSc settings.

CONCLUSION:
Altogether, these data suggest that IL-1β, which is increased in SSc skin, might alter perivascular M efferocytic ability leading to disturbed surrounding tissue homeostasis.

Keywords: Fibrosis / Inflammation / Efferocytosis / EndoMT / Myofibroblast / IL, 1β

Influence of pleural mesothelioma tumor cells on the macrophage subpopulations present in the tumor microenvironment.

Tiphaine Delaunay * [1], Guillaume Tosato [2], Sophie Deshayes [1], Judith Fresquet [1], Maya Arnould [2], Clément Meiller [2], Didier Jean [2], Christophe Blanquart [1]

[1] Inserm UMR 1307, CNRS UMR 6075, Université d'Angers, CRCI2NA, F-44000 Nantes, France – Institut National de la Santé et de la Recherche Médicale - INSERM – France
[2] Centre de Recherche des Cordeliers, INSERM, Université Paris Cité, Sorbonne Université, Functional Genomics of Solid Tumors, Paris, France – Institut National de la Santé et de la Recherche Médicale - INSERM – France

Pleural mesothelioma (PM) is an aggressive asbestos-related cancer with poor median survival and limited treatment options. Immunotherapy, especially the anti-CTLA-4/anti-PD-1 combination, benefits only 20% of patients. Finding new strategies is crucial, especially by targeting tumor-associated macrophages (TAMs) that promote tumor growth and resistance to treatments. This project aims to characterize macrophage subpopulations in PM using frozen patient tumors and multicellular tumor spheroids (MCTS), which reproduce the 3D organization of tumors in situ and, among others, the differentiation of macrophages into M2-like immunosuppressive phenotypes.
We focus our study on the molecular and functional characterization of macrophage sub-populations, analyzing their heterogeneity through single nucleus RNA sequencing (snRNA-seq) and flow cytometry. The snRNAseq analysis performed by Dr. Didier Jean's team on tumors from patients allowed us to identify 10 clusters representing the different cell types found in the tumors. Furthermore, within the cluster corresponding to myeloid cells, we identified eight distinct subpopulations. With the MCTS model, flow cytometry and RNASeq analyses show no monocyte donor-dependent effects on macrophage profiles obtained. Our preliminary results, obtained on 10 models of MCTS by flow cytometry, indicate a diversity of macrophage subpopulations depending on the PM cell lines, confirming the relevance of the approach. To conduct more in-depth functional studies, we plan to sort the predominant macrophage subpopulations in MCTS through cell sorting. We successfully conducted a preliminary experiment confirming the feasibility of sorting macrophage subpopulations from dissociated spheroids, demonstrating their viability and functionality 24 hours post-sorting.
Following the identification of the most immunosuppressive subpopulations, this project will aim to enhance PM treatments by modulating the tumor immune microenvironment through the specific targeting of these pro tumoral macrophages.

Keywords: Pleural mesothelioma, tumor, associated macrophages, multicellular tumor spheroids.

*Speaker

Shedding light on tumor-monocyte interactions with 3D cell culture models of triple negative breast cancers

Dima Ghannoum * [1], Léa Perreau [2], Clément Sanchez [3], Eva Huet [1], Marie-Françoise Heymann [4], Hamza Lasla [5], Pasqual JÉzÉquel [5], Sophie LeliÈvre [1]

[1] SCaT laboratory (Sensibility of Cancers to treatment), Institut de Cancérologie de l'Ouest (ICO), Angers. – Institut de Cancérologie de l'Ouest – France
[2] SCaT laboratory (Sensibility of Cancers to treatment), Institut de Cancérologie de l'Ouest (ICO), Angers. – Institut de Cancérologie de l'Ouest – France
[3] Gliocure - Institut de Biologie en Santé, Angers. – Gliocure – France
[4] Anatomopathological Unit, ICO, Saint Herblain. – Institut de Cancérologie de l'Ouest – France
[5] Omiques et Data Science Unit, ICO, Saint Herblain. – Institut de Cancérologie de l'Ouest – France

Triple-negative breast cancer (TNBC) is known for its aggressiveness, poor prognosis, heterogeneity, and treatment difficulty. It has been classified into four subtypes using transcriptomics, and it was demonstrated that the non-immune-activated subtypes are enriched in M2-macrophages. Because of TNBC heterogeneity, and the well-established influence of the tumor microenvironment (TME) on cancer phenotype, an in-depth analysis of TNBC architecture including TME cell content, and nuclear morphometry, is required to create personalized breast cancer models. In oncology, "nuclear morphometry" refers to quantitative and qualitative morphomic approaches used to assess alterations in the appearance of individual cell nuclei that provides clinically significant information concerning tumor features. Our project's initial focus is on TAMs that make up more than 50% of the tumor mass in the majority of solid tumors in humans. We hypothesize that gaining insight into nuclear morphometry of cancer cells and macrophages, and the interaction between these cells will contribute to the creation of a personalized platform for TNBC. Morphometry of cancer cell nuclei in tumor histological sections from a cohort of 48 TNBC patients, categorized into subtypes, reveals that at least one nuclear morphometric parameter can be used to distinguish between three of these subtypes. To investigate tumor-immune interactions highly (MDA-MB-231) and poorly (T4-2) aggressive TNBC cells were cultured in 3D collagen 1 matrix, with distinct invasive behaviors illustrated by a greater number of (and longer) invasive arms in MDA-MB-231 tumors. For coculture with tumors, monocytes were added either directly to the culture medium or to the upper compartment of a Boyden chamber with 5-micron pores. Monocytes were able to migrate and differentiate into anti-inflammatory M2-macrophages, as indicated by CD163 expression, in both types of coculture with MDA-MB-231 cells, but only in direct coculture with T4-2, which suggests a link between aggressiveness of TNBC cells and their impact on monocytes.

Keywords: TNBC, Microenvironment, 3D cell culture, Immune cells, Macrophages, Personalized medicine.

*Speaker

to anti-TNF responders, and provides a molecular rationale to help guide immunotherapy in CD.

Keywords: Inflammatory Bowel Disease, macrophage, Crohn's disease, ScRNASeq, gut

Posters

Mass cytometry immunophenotyping revealed unexpected macrophage subtypes during mouse alveolar echinococcosis

Brice Autier * [1], Christelle Manuel [2], Sarah Dion [2]

[1] Institut de recherche en santé, environnement et travail – Institut National de la Santé et de la Recherche Médicale - INSERM, École des Hautes Études en Santé Publique [EHESP], CHU Rennes, Université Rennes – France

[2] Institut de recherche en santé, environnement et travail – Institut National de la Santé et de la Recherche Médicale - INSERM, École des Hautes Études en Santé Publique [EHESP], Université Rennes – France

Alveolar echinococcosis (AE) is a parasitic disease due to the larval stage of *Echinococcus multilocularis* which develops in the human liver as a slowly growing heterogeneous mass, similarly to cancer. AE is characterized by a marked interindividual variability in host response. Factors that are responsible for resistance/susceptibility are yet partially understood. We hypothesized that periparasitic macrophages are involved in the susceptibility to the disease and we aimed at describing macrophage diversity in AE using mass cytometry immunophenotyping. C57/Bl6J mice were sequentially infected by peritoneal inoculation, in order to obtain, on the day of sacrifice, mice infected for 15 days to 3 months, together with uninfected controls. Periparasitic immune cells were collected by peritoneal lavages, stained using a 37-markers panel for mass cytometry at the HYPERION platform (UMR1227 LBAI, Brest). We observed that both resident (F4/80HiMHC-II-CCR2-) and recruited macrophages (F4/80LoMHC-II+CCR2+) polarized into atypical macrophage subpopulations called Mac1 to Mac5, as soon as 15 days p.i. Almost all macrophages ($\geq$88%) were Mac1-5 at 15 days, 1 month and 2 months p.i. At late stage of the disease (3 months p.i.), proportion of Mac1-5 decreased to 33% and were replaced by Ly6C+CD206- (23%) and Ly6C-CD206+ (33%) macrophages. Mac1-5 had profile partially similar to Tumor-Associated Macrophages (TAM), as they were invariably CD68+MERTK+TREM2+ and, depending on subgroup, expressed SIRPα for Mac3 (57% of M at 15 days p.i., $p< 0.001$), CD31 for Mac5 (38% of M at 1 month p.i., $p< 0.01$), and CD31 and DC-SIGN for Mac2 (45% of M at 2 months p.i., $p< 0.001$). Proportions of cells other than macrophages were stable, except for neutrophils which increased from 1% to 16% of total cells between 2 and 3 months p.i. ($p< 0.001$). Altogether, we showed that macrophages early polarized into TAM-like cells which are possibly involved in the chronicization of the disease.

Keywords: Echinococcus multilocularis, alveolar echinococcosis, macrophages, infectious diseases, parasite

*Speaker

Targeting synovial macrophages to develop personalized therapeutic strategies for osteoarthritis

Anaïs Cardon * [1], Lola Le Vaillant [1], Nicolas Gaigeard [1], Julien De Lima [1], Claire Vinatier [1], Frédéric Blanchard [1], Denis Waast [2], Benoit Le Goff [3], Virginie Escriou [4], Jérôme Guicheux [1], Marie-Astrid Boutet [1,5]

[1] UMR1229 Regenerative Medicine and Skeleton – Nantes University, Oniris, CHU Nantes, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, F-44000 Nantes, France – France
[2] CHU Nantes – Department of Orthopaedics, CHU Nantes, Nantes, France – France
[3] CHU Nantes – Department of Rheumatology, CHU Nantes, Nantes, France – France
[4] Unité de Technologies Chimiques et Biologiques pour la Santé – UTCBS, CNRS, INSERM, Université Paris Descartes, Sorbonne-Paris-Cité, Chimie ParisTech, PSL Research University, Paris, France – France
[5] Centre for Experimental Medicine Rheumatology, William Harvey Research Institute and Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom – United Kingdom

Osteoarthritis (OA) is the most common rheumatic disease affecting over 500 million of people worldwide. Incurable and debilitating joint disease, OA leads to cartilage degradation, bone remodelling, and synovitis, with only symptomatic treatments available. Synovitis has been shown to play an important role in OA initiation and progression. Our group recently identified that OA synovium could be stratified into three different pathotypes, based on a precise histopathological analysis of the synovial cellular infiltration. Importantly, macrophages represent the most abundant synovial immune population observed in the three pathotypes (Boutet et al., OAC, 2024).
Our preliminary data indicate distinct macrophage populations, differently represented in the OA synovium, with a particular enrichment of specific macrophage subpopulations in each pathotype. We hypothesized that the targeting of those pathotype-specific macrophage populations could provide novel personalized therapeutic approaches for OA patients, contributing to giving the "right drug to the right patient".

Therefore, we designed a lipoplexe-based targeting strategy. To individually target pathotype-specific macrophage populations, we selected relevant genes in each population and developed specific lipoplexes containing a mix of siRNA and mRNA. To validate the effective targeting of macrophages, we first confirmed the internalisation of fluorescent control lipoplexes *in vitro* in macrophages and their biodistribution *ex vivo* in synovial tissue explants, obtained from OA patients who underwent total knee replacement surgery. Next, we plan to validate *in vivo* their biodistribution and internalisation in synovial macrophages in the knee of OA murine models. Finally, using pathotype-specific lipoplexes, we aim to assess their effective modulation of gene expression in the targeted macrophage populations and ultimately evaluate their effect on OA alleviation in OA murine models.
Overall, this strategy could bring the proof of concept that the pathotype-specific modulation of synovial macrophage populations would contribute to prevent OA development and offer new therapeutic perspectives for patients.

*Speaker

Keywords: Osteoarthritis, macrophages, synovitis, inflammation, lipoplexe

The inflammatory effects of a repeated exposure of human macrophages to PM2.5 from house dust-SRM 2585 are partly reversible and alter the LPS/CD14-dependent endocytosis pathway

Valérie Lecureur * [1], Marie Lelong [1], Laura Morin [1], Ivannah Pottier [2], Véronique Andre [2]

[1] Institut de recherche en santé, environnement et travail – Université de Rennes I – France
[2] ABTE UR 4651 – Université de Caen – France

Objectives: To evaluate whether repeated exposures to house dust PM2.5, used at non-toxic concentration, activate human macrophages after one- and seven-days post-treatment, and whether such PM2.5 exposure may alter their response to LPS.
Methods: Human monocyte-derived macrophages (MDMs) were repeatedly (4 consecutive days) exposed to 1 and 10 μg/cm2 house dust particulate matter SRM 2585®. MDM phenotype and functions were evaluated by flow cytometry and ELISA one- and seven-days after the last PM2.5 exposure with or without LPS priming during the last 24 h of exposure.

Results: PM2.5 used at non cytotoxic concentrations induced a pro-inflammatory phenotype characterized by an increased expression of M1 macrophagic markers (CD40, CD80, CD86) and pro-inflammatory cytokines (IL-6, IL-8, TNFα) and a decreased expression of M2 macrophagic markers (CD163, CD204) and of the anti-inflammatory cytokine IL-10, however depending of post-exposure time and the concentration. PM2.5-induced polarization profile of MDM was reversible when they were exposed to 1 μg/cm2 but not to 10 μg/cm2. PM2.5-exposed MDMs primed by LPS showed a pro-inflammatory phenotype characterized by altered secretion of IL-8 and IL-10, a decreased expression of CD14 and scavenger receptors (SR) SR-A1 and SR-B2, and a decrease of phagocytosis and of interferon responsive gene (CXCL9/10, ISG15, MIX1) expression.
Conclusions: Repeated exposures, even to low concentration of house dust PM2.5, primed MDMs to a pro-inflammatory phenotype that is only partly reversible 7 days post-exposure. The altered response of PM2.5-pre-exposed MDMs to LPS might be due to, at least in part, impaired phagocytosis crucial for the TLR4/CD14 pathway.

Keywords: CD14, LPS, macrophage polarization, phagocytosis, PM2.5, repeated exposure, scavenger receptors

*Speaker

Study of monocytes in PBMCs of patients with multiple sclerosis : comparison of the severity of the pathology for the identification of biomarkers

Amandine Loret * [1], Laureline Berthelot [2], Léo Boussamet [2], Sita Shah [1], Alexandra Garcia [2], Dugast Emilie [3], Cynthia Fourgeux [4], Jeremie Poschmann [5], Nicolas Vince [6], Arnaud Nicot [2], David Laplaud [2]

[1] Team 5 : Neuroinflammation, mechanisms, therapeutic options (NEMO) – CHU Nantes, Nantes Université, INSERM, Centre de Recherche Translationnelle en Transplantation et Immunologie (CR2TI), UMR 1064, ITUN, Nantes – France

[2] Team 5 : Neuroinflammation, mechanisms, therapeutic options (NEMO) – Centre de Recherche en Transplantation et Immunologie - Center for Research in Transplantation and Translational Immunology, Nantes Université – France

[3] Team 5 : Neuroinflammation, mechanisms, therapeutic options (NEMO) – Centre de Recherche en Transplantation et Immunologie - Center for Research in Transplantation and Translational Immunology – France

[4] Team 6 : Impact of acute inflammation on host pathogen interactions and lung homeostasis (Team 6 - U1064 Inserm - CR2TI) – Nantes Université – CHU Nantes - Hôtel Dieu - 30 Bd Jean Monnet 44093 Nantes Cedex 01., France

[5] Centre de Recherche en Transplantation et Immunologie - Center for Research in Transplantation and Translational Immunology – Nantes université - UFR des Sciences et des Techniques – France

[6] Centre de Recherche en Transplantation et Immunologie – Institut National de la Santé et de la Recherche Médicale : U1064, Université de Nantes - UFR de Médecine et des Techniques Médicales – France

Multiple sclerosis (MS) is a complex inflammatory disease of central nervous system characterized by clinical diversity and unpredictable progression. Myeloid cells are involved in the disease, infiltrating brain and participating to demyelination through cytokine production and phagocytosis. Using single-cell transcriptomic analysis of PBMC, we explored blood monocytes of untreated MS patients with aggressive versus non-aggressive MS and compared to healthy volunteers, to explore alterations associated with disease severity. Analysis revealed 5 clusters of monocytes (1 intermediary, 1 non-classical, 3 classical monocytes). The results highlighted an increase in glucocorticoid signaling pathways in different forms of MS, type I interferons in non-aggressive MS, and lipid transport regulation in aggressive MS in untreated patients. This study provides new insights into the underlying immunological mechanisms, as well as early diagnostics and personalized treatments by identifying specific markers for aggressive and non-aggressive MS forms.

Keywords: multiple sclerosis, monocytes, PBMC, severity, biomarkers

*Speaker

Effects of crystalline silica exposure in the HOCl-induced systemic sclerosis mouse model

Laura Morin * [1], François Zimmermann [2], Marie Lelong [1], Juliette Ferrant [3], Patrice Hemon [4], Salomé Patry [1], Francine Uwambayinema [5], Yousof Yakoub [5], Erwan Dumontet [3], François Huaux [5], Alain Lescoat [2], Valérie Lecureur [1]

[1] Institut de recherche en santé, environnement et travail – Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en sante, environnement et travail) - UMR$_S$1085, $F-35042Rennes--France$
[2] Institut de recherche en santé, environnement et travail – Univ Rennes, CHU Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail) - UMR$_S$1085, $F-35000Rennes, France--France$
[3] Centre Hospitalier Universitaire de Rennes, UMR1236 – Centre Hospitalier Universitaire de Rennes, Pôle Biologie, Rennes, France, Unité Mixte de Recherche (UMR)1236, Université Rennes, INSERM, Etablissement Français du Sang Bretagne, Equipe Labellisée Ligue Contre le Cancer, Rennes, France – France
[4] Lymphocytes B, Autoimmunité et Immunothérapies – Université de Brest, Institut National de la Santé et de la Recherche Médicale, LabEX IGO Immunothérapie Grand Ouest, Institut Brestois Santé Agro Matière – France
[5] Louvain centre for Toxicology and Applied Pharmacology (LTAP), Institute de Recherche Expérimentale et Clinique (IREC), Université catholique de Louvain (UCLouvain), Brussels, Belgium – Belgium

Systemic sclerosis (SSc) is an autoimmune disease characterized by skin and lung fibrosis. Crystalline silica (SiO2), a mineral found in rocks to which construction workers are particularly exposed, is the main environmental risk factor of SSc, increasing the risk of more severe SSc in human. However, the pathophysiological mechanisms involved in silica-related-SSc remain unclear. The aim of this study was to determine the contribution of inhaled SiO2 to the severity of SSc and to identify the lung immune cells that may involve in this aggravation using the HOCl-induced SSc mouse model.
Intradermic injections of HOCl were performed five days a week during 6 weeks in female C57BL/6 mice to induce SSc. Mice were exposed twice to 1.5 mg of SiO2 by oropharyngeal instillations. Mice were divided in four groups (n=10/group): NaCl, SiO2, HOCl, HOCl+SiO2. Blood cell populations were identified by flow cytometry and pulmonary cells were studied by mass cytometry (CyTOF). Skin and lung fibrosis were analyzed by histology and inflammation by RT-qPCR.

NLRP3 inflammasome genes were synergistically overexpressed in the lungs of HOCl+SiO2 mice as compared to other groups. The percentages of lung immune cell populations, identified by CyTOF in HOCl group varied from those observed in SiO2-exposed mice. Dendritic cells and monocyte-derived alveolar macrophages were increased in SiO2-exposed mice, whereas in HOCl-mice, classical monocytes were increased, as compared to control mice. However, these cell population percentages did not appear to be more represented in co-exposed mice. Circulating classical monocytes were increased in HOCl+SiO2 mice as compared to control. NLRP3

*Speaker

inflammasome genes were overexpressed synergistically in the skin of HOCl+SiO2 mice, as compared to other groups, showing systemic effects of SiO2.
Altogether, we provide new insights on immune cell populations and related pathways, including NLRP3 inflammasome, in pathogenic mechanisms contributing to SiO2-related SSc.

Keywords: Autoimmune disease, Crystalline silica, Mass cytometry, Systemic sclerosis

Phenotypic and spatial analysis of myeloid cells in a mouse model of DLBCL

Benjamin Papail * [1], Maëlig Oresve [1], Karine Crozat [1]

[1] UMR1236 MOBIDIC – Unité Mixte de Recherche (UMR)1236, Université Rennes, INSERM, Etablissement Français du Sang Bretagne, Equipe Labellisée Ligue Contre le Cancer, Rennes, France – France

Diffuse Large B Cell Lymphoma (DLBCL) is a common, highly aggressive Non-Hodgkin's lymphoma, with a poor prognosis: 30 to 40% of DLBCL patients relapse or are refractory to standard treatments. These failures may be attributed in part to the composition and nature of the tumor microenvironment (TME). In DLBCL patients, the presence and spatial localization of immune cells from myeloid lineage in TME appear to be prognostic factors (Ferrant J et al, BioRxiv preprint, 2022; Wright et al, Blood Adv, 2023). Our aim is to determine the phenotypic and functional heterogeneity of myeloid cells infiltrating aggressive B lymphoma in a syngeneic mouse model. In parallel, we are working to map the spatial localization of myeloid populations within the lymphoma, and to analyze their interactions with T cells and stromal cells of the TME. Our preliminary analyses show that macrophagic populations, dendritic cells and non-classical monocytes are deregulated in presence of lymphoma. These results are consistent with analyses of PBMCs and biopsies from DLBCL patients. Our project will enable us to define the cellular and molecular mechanisms regulating myeloid populations in DLBCL TME, and to identify candidate therapeutic targets that could enhance current clinical treatments.

Keywords: Lymphoma, DLBCL, Myeloid cells, Tumor microenvironment

*Speaker

List of participants

- Allard Mathilde
- Apparailly Florence
- Apparailly Florence
- Autier Brice
- Autier Brice
- Babursah Ela
- Barakat Georges
- Berthelot Laureline
- Berthet Pascale
- Blanchard Frédéric
- Boiziau Claudine
- Bordes Cécile
- Bouineau Vincent
- Bourgeais Nina
- Boutet Marie-Astrid
- Boyer Cécile
- Cardon Anaïs
- Cartron Matéo
- Caussette Camille
- Courties Gabriel
- Couty Mickael
- David Camille
- De Lima Julien
- Delaunay Tiphaine
- Depaire Agathe

- Fournier Alphonse Noémie
- Gaigeard Nicolas
- Gaudin Alexis
- Ghannoum Dima
- Guicheux Jerome
- Han-Yee-Yu Jérôme
- Hascoët Emilie
- Humbert Paul
- Jamoneau Juliette
- Jeroundi Najia
- Katby Maya
- Khan Archie
- Laurent Thomas
- Le Mercier Mathilde
- Ligeron Camille
- Loret Amandine
- Maye Alexandre
- Mykhaylyshyn Monika
- Ogor Thomas
- Ouattara Astrid
- Paoletti Audrey
- Parlak Mikâil
- Perdrieau Hortense
- Riquin Kevin
- Sallé Marine
- Suuring Maaike
- Tessier Solène