PROGRAM & ABSTRACT BOOK

"DC-phering mononuclear phagocyte biology in health and disease"

Annual Meeting of the French Dendritic Cell Society

December 16-17, 2021 at the Institut Necker Enfants Malades in Paris, FRANCE

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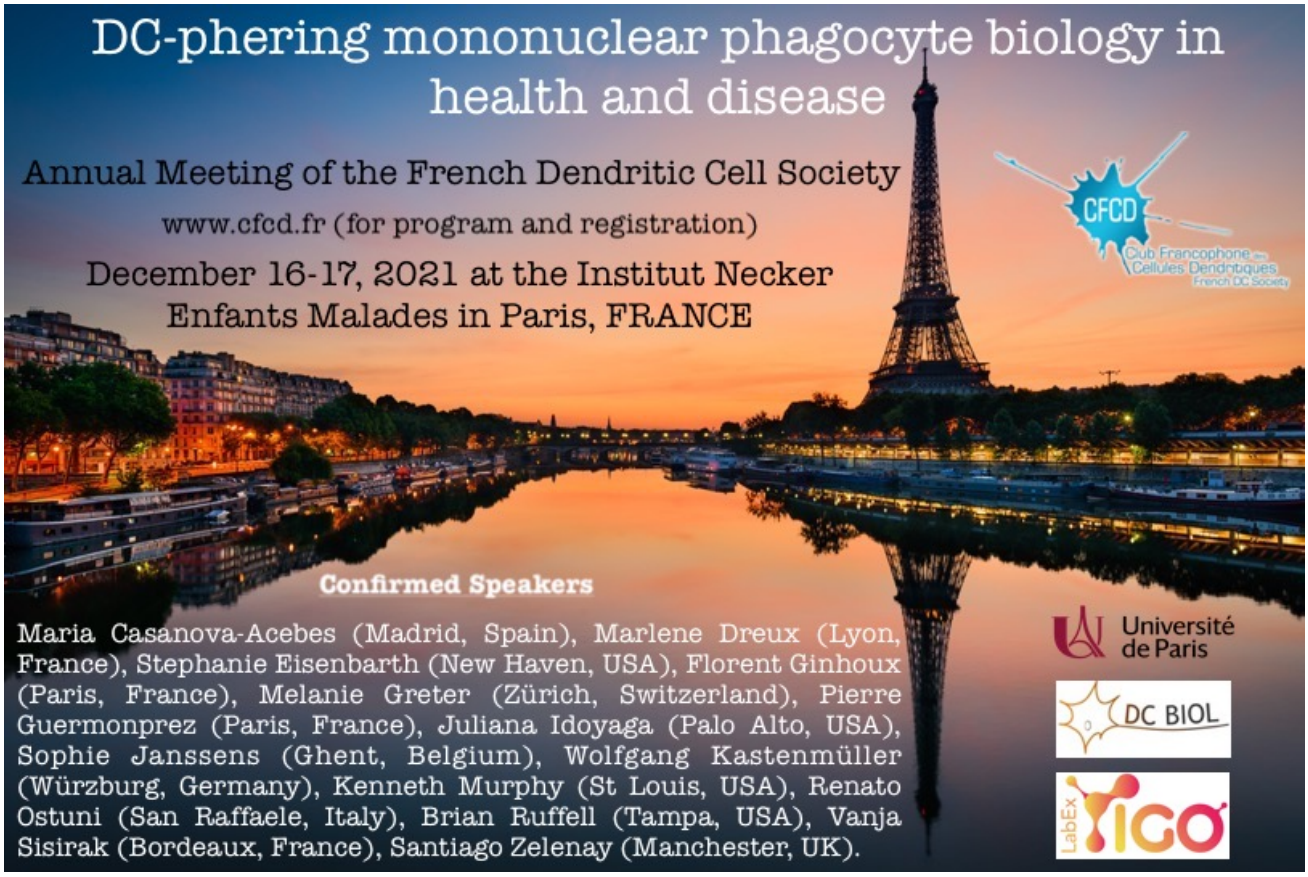

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1. PROGRAM

Thursday December 16th, 2021

8:30 Opening of the registration desk

9:15 Welcome address

SESSION I – DC subsets
Chairpersons: Bénédicte Manoury and Marlène Dreux

9:30-9:55 Kenneth Murphy *(USA)*
Genetic basis for divergence of DC progenitors

10:00-10:25 Sophie Janssens *(Belgium)*
Dendritic cell maturation revisited

10:30-11:00 Coffee Break

11:00-11:25 Pierre Guermonprez *(France)*
DC subsets and T cell memory

11:30-12:15 Selected oral presentations

11:30-11:45 **Stefanie Scheu *(Germany)***
The transcription factor BATF as novel regulator of type I interferon production in plasmacytoid dendritic cells

11:45-12:00 **Andreas Schlitzer *(Germany)***
GPR183 dictates subtissular localisation of pulmonary CD301b+ conventional dendritic cells type 2 and instructs their survival via the TSLP – TSLP receptor axis

12:00-12:15 **Javiera Villar *(France)***
ETV3 and ETV6 enable monocyte differentiation into dendritic cells during inflammation by repressing interferon-stimulated genes

12:15-12:45 Wolfgang Kastenmüller *(Germany)*
cDC1 maintain and guide the differentiation of T_{PEX} cells in distinct cellular niches

12:45-15h30 LUNCH and Poster viewing

SESSION II – Tumor-associated macrophages and DCs

Chairpersons: Julie Helft and Paula Michea

15:30-15:55 Maria Casanova-Acebes (Spain)
Getting to know the origin of tumor-associated macrophages : challenges and opportunities for therapeutic intervention

16:00-16:25: Renato Ostuni (Italy)
Transcriptional control of tumor-associated macrophages

16:30-17:00 Selected oral presentations

16:30-16:45 **Yohan Gerber-Feder (France)**
Breast cancer remotely remodel the hematopoietic niche favoring myelopoiesis and tumor growth

16:45-17:00 **Elisa Gobbini (France)**
Spatial organization and prognostic impact of dendritic cell subsets in a large cohort of triple negative breast cancer patients

17:00-17:30 Florent Ginhoux (France)
"The ever-expanding DC universe"?

17:30 Cocktail

20:00 Speaker Dinner

Friday December 17th, 2021

9:00 Opening of the registration desk

SESSION III – DCs and macrophages in tissues
Chairpersons: Héléna Païdassi and Jérôme Martin

9:30-9:55 Melanie Greter *(Switzerland)*
Macrophage development and function

10:00-10:25 Juliana Idoyaga *(USA)*
Human dendritic cell heterogeneity unraveled by high-dimensional analyses

10:30 Coffee Break

11:00-11:45 Selected oral presentations

11:00-11:15 **Maria-Graciela Delgado *(France)***

Formation of the myeloid compartment in vivo: Revealing an unexpected role for the Arp2/3 complex

11:15-11:30 **Zhana Haimon *(Israël)***
Microglia interactions with T cells in relapsing remitting EAE

11:30-11:45 **Antonin Weckel *(USA)***
Early life interactions between commensal bacteria and dermal CD301b+ cDC2s facilitate long-term immune tolerance to the skin microbiome

11:45-12:15 Stephanie Eisenbarth (USA)
Cellular mechanics of DC migration

12:15-14:30 LUNCH and Poster viewing

14:30-14:45 CFCD General Assembly

SESSION IV – DCs in diseases
Chairpersons: Sophie Laffont and Vanja Sisirak

14:45-15:10 Brian Ruffel *(USA)*
Therapeutic targeting of intratumoral dendritic cells

15:15-15:40 Santiago Zelenay *(United Kingdom)*
Manipulating inflammation to unleash cancer immune control

15:45-16:15 Selected oral presentations

15:45-16:00 **Elena Winheim *(Germany)***
Yellow fever vaccination induces distinct and overlapping gene expression programs in human blood DC and monocyte subsets

16:00-16:15 **Lucie Maisonneuve *(France)***
Regulation of IRE1α, an ER stress sensor, in dendritic cells

16:15-16h40 Vanja Sisirak (France)
Plasmacytoid dendritic cells promote DNA autoreactivity

16:45-17:10 Marlène Dreux *(France)*
Severe COVID-19 patients have impaired plasmacytoid dendritic cell-mediated control of SARS-CoV-2-infected cells

17h15 Award ceremony and concluding remarks

17:30 End of the meeting

2. ABSTRACTS

2.1. Selected SHORT TALKS (ST)

Thursday December 16th, 2021

Session I – DC subsets

ST-1

The transcription factor BATF as novel regulator of type I interferon production in plasmacytoid dendritic cells

Shafaqat Ali (1), Ritu Mann-Nüttel (1), Regine Dress (2), Patrick Petzsch (3), Karl Köhrer (3), Haifeng Chris Xu (4), Philipp Lang (4), Judith Alferink (5), and Stefanie Scheu (1)

(1) Institute of Medical Microbiology and Hospital Hygiene, University of Düsseldorf, Germany; (2) Institute of Systems Immunology, Center for Molecular Neurobiology Hamburg (ZMNH), University Medical Center Hamburg-Eppendorf, Germany; (3) Biological and Medical Research Center (BMFZ), University of Düsseldorf, Germany; (4) Department of Molecular Medicine II, University of Düsseldorf, Germany; (5) Department of Mental Health and Cells in Motion Interfaculty Centre, University of Münster, Germany

BATF (Basic leucine zipper transcription factor, ATF-like) plays a critical role in the haematopoiesis, differentiation, proliferation, metabolism, and effector function of lymphocytes in infection and immunity. In a recent transcriptome analyses we detected Batf as differentially expressed in interferon (IFN) β-producing plasmacytoid dendritic cells (pDCs). So far, no expression or function has been described for BATF in pDCs. In the present study, we characterized the implications of BATF expression in pDC functions. Using IFNβ/YFP reporter mice we found that BATF is highly expressed in IFNβ-producing splenic and bone marrow (BM) derived pDCs. Upon TLR9 activation, the maximum of Ifnb expression precedes the maximum of Batf expression. However, the expression of Batf is not dependent on IFNAR signaling in pDCs. In comparison to wild type (WT) littermates BM-derived pDCs from Batf-deficient mice produce increased amounts of IFNα and β at mRNA and protein levels after CpG stimulation. In line with the in vitro data, Batf-deficient mice show higher serum levels of type I IFN early after LCMV infection as compared to WT animals in vivo. We will present integrative multi-omics data that is currently being analysed and suggests molecular mechanisms underlying the BATF mediated modulation of type I IFN expression in pDCs.
Taken together our data point to a so far unrecognized function of BATF in modulating pDC dependent type I IFN responses. This suggests an important role for BATF in anti-infectious immune responses and type I IFN mediated autoimmunity.

ST-2

GPR183 dictates subtissular localisation of pulmonary CD301b+ conventional dendritic cells type 2 and instructs their survival via the TSLP – TSLP receptor axis

Lili Zhang (1) & Andreas Schlitzer (1)

(10 Quantitative Systems Biology, Life & Medical Sciences (LIMES) Institute, University of Bonn, 53115 Bonn, Germany

Regulatory mechanisms for the spatial distribution of non-lymphoid tissue-resident conventional dendritic cells (cDCs) remain unknown. However, cDCs are not randomly distributed across tissues, implying active regulation of intratissular placement of cDCs. Here we reveal a GPR183 instructed TSLP signalling axis-driven adventitial fibroblast : CD301b+ cDC2 survival circuit. We show that genetic ablation of GPR183 within the cDC lineage leads to a selective loss of lung-resident CD301b+ cDC2 as a function of stromal 7α,25-dihydroxycholesterol production, with no impact on DCpoesis. Confocal microscopy revealed a close association of CD301b+ cDC2 to PDGFRa+ fibroblasts within the adventitial region of the lung. Genetic ablation of TSLP receptor on cDCs revealed a adventitial fibroblast associated CD301b+ cDC2 niche fostering survival of lung-resident CD301b+ cDC2. These data have profound implication for the tissue specific functionalization of CD301b+ cDC 2 during homeostasis and disease.

ST-3

ETV3 and ETV6 enable monocyte differentiation into dendritic cells during inflammation by repressing interferon-stimulated genes

Javiera Villar (1), Adeline Cros (1), Alba De Juan (1), Pierre-Emmanuel Bonte (1), Colleen M Lau (2), Ioanna Tiniakou (2), Boris Reizis (2), Elodie Segura (1)*

(1) Institut Curie, PSL Research University, INSERM, U932, 26 rue d'Ulm, Paris, France (2) Department of Pathology, New York University Grossman School of Medicine, New York, NY 10016, USA

In inflamed tissues, monocytes differentiate into macrophages (mo-Mac) or dendritic cells (mo-DC). In chronic non-resolving inflammation, mo-DC are major drivers of pathogenic events. Manipulating monocyte differentiation would therefore represent an attractive therapeutic strategy. However, what regulates the balance of mo-DC versus mo-Mac fate commitment remains unclear. Here we show that the transcriptional repressors ETV3 and ETV6 control human monocyte differentiation into mo-DC. Mechanistically, we find that ETV3 and ETV6 repress mo-Mac development and inhibit the expression of interferon-stimulated genes. Moreover, we demonstrate that activation of STAT1 signaling favors mo-Mac differentiation. Mice deficient for ETV6 in monocytes showed spontaneous expression of interferon-stimulated genes, confirming that ETV6 regulates interferon responses in vivo. Furthermore, these mice display impaired mo-DC differentiation during peritonitis and ameliorated experimental autoimmune encephalomyelitis. Our findings elucidate molecular control of monocyte fate decision and identify ETV6 as a therapeutic target to redirect monocyte differentiation in inflammatory disorders.

ST-4

Breast cancer remotely remodel the hematopoietic niche favoring myelopoiesis and tumor growth.

Gerber-Ferder[1,2], Yoann Missolo-Koussou[2], Marine Dubois[2], Joëlle Veziers[3], Sylvain Baulande[4], Jason Cosgrove[5], Leila Perie[5], Eliane Piaggio[2], Julie Helft[2].

[1]Université de Paris, Institut Curie, INSERM U932, Paris, France; [2]Institut Curie, PSL Research University, INSERM U932, Paris, France; [3]Inserm, UMR 1229, Université de Nantes, ONIRIS, Nantes, France; [4]Institut Curie, PSL Research University, NGS Platform, 75005, Paris, France; [5]Institut Curie, PSL Research University, CNRS UMR168, 75005 Paris, France

Myeloid cell infiltration is a hallmark of solid cancers and often correlates with poor prognosis and disease severity. Myeloid cells (i.e neutrophils, monocytes and macrophages) play various roles in the immune resistance and evasion mechanisms of tumors. It is therefore crucial to decipher how myeloid cell generation (myelopoieisis) is regulated during cancer, paving the way for new therapeutic strategies.
Using flow cytometry, single cell and bulk RNA sequencing and imaging techniques, we have characterized the tumor-induced systemic changes impacting the bone marrow and myelopoiesis in an autochthonous breast cancer mouse model.
We found that breast tumors remotely impact hematopoietic stem cells (HSC) differentiation. We showed that HSC and early myeloid progenitors of tumor-bearing mice were transcriptionally and functionally primed towards myeloid cell differentiation. By screening soluble factors in the serum of tumor bearing animals, we found a repeated increase in several bone remodeling proteins, including the RANKL decoy receptor osteoprotegerin (OPG). Accordingly, tumor bearing animals had increased bone activity and elevated osteoblastic differentiation. We showed that OPG genetic inactivation in tumor cells inhibited tumor development and myeloid cell tumor infiltration *in vivo*.
Our data highlight a systemic remodeling of the bone marrow in both the hematopoietic and the stromal compartments, remotely controlled by the tumor. OPG blockade could therefore help normalize hematopoiesis during cancer development and prevent myeloid cell infiltration in the tumor-microenvironment.

ST-5

Spatial organization and prognostic impact of dendritic cell subsets in a large cohort of triple negative breast cancer patients

Elisa Gobbini (1,2), Margaux Hubert (1), Anne Claire Doffin (1), Justine Berthet (1), Bertrand Dubois (1), Christope Caux (1), Jenny Valladeau-Guilemond (1)

(1) Cancer Research Center Lyon, UMR INSERM 1052 CNRS 5286, Centre Léon Bérard, 28 rue Laennec, 69373 Lyon, France; (2) CHU Grenoble-Alpes, Avenue Maquis de Gresivaudan, 9870 La Tronche, France

Different dendritic cells (DC) subsets have been already identified in triple negative breast cancer (TNBC) by flow cytometry, RNAseq or by IHC. In human, in silico analysis only have been used to compare their impact on patient survival and their spatial organization and interaction with CD8 T cells haven't been explored yet in tumors.
Here, we set up a 7 colors immunofluorescence staining to precisely localized in a cohort of 70 chemotherapy naïve resected TNBC patients, pDC (plasmacytoid-DCs), cDC1 (conventional type 1 DCs), cDC2/LC (Langherans Cells) and mature DCs along with CD8+ T cells. We quantified by INFORM software and analyzed by an in-house bioinformatic pipeline their organization and their prognostic impact.
We showed for the first time, by our in situ approach, that pDC (plasmacytoid-DC), cDC1 (conventional type 1 DC) and mature DCs co-infiltrate TNBC along with CD8+ T cells and they were mostly located into the stroma compartment in contrast to LC found in the tumoral compartment. TNBC patients who were enriched in CD8+ T cells, cDC1, pDC or mature DCs in the stroma trended to have a longer overall survival. However, no DC subsets kept their prognostic value when looking they were localized into the tumor compartment. Distance analysis with CD8+ T cells suggested an intra-tumoral organization of DC subsets in a non-stochastic fashion. The bioinformatic pipeline allowed us to identify various DC aggregates containing different proportion of each DC subsets and CD8 T cells that may explain particular DC functions and the prognosis of patient.

Friday December 17th, 2021

SESSION III – DCs and macrophages in tissues

ST-6

Formation of the myeloid compartment in vivo: Revealing an unexpected role for the Arp2/3 complex.

Delgado, MG (1)*, Sanseau D (1), Maurin M (1), Da Silva N (1), Rivera C (1), Lacerda L (1), Piel M (2) & Lennon-Duménil AM (1)

(1) U932, Institut Curie, Paris, France. (2) CNRS UMR 144, Institut Pierre-Gilles de Gennes, Paris, France.

Arp2/3 is a seven-subunits complex that mediates branched actin network formation in eukaryotic cells. It has been shown that such reinforced actin network can play two roles in the migration of dendritic cells (DCs): (1) it helps protrusion formation for navigation in complex environments and (2) it nucleates actin around the DC nucleus for these cells to move within confined spaces. However, how lack of Arp2/3 impacts on the physiology of DCs in vivo is unknown. To address this question, we generated a mouse model deficient in the Arp2/3 actin-binding subunit ArpC4, and crossed it to Cd11c-CRE mice to obtain gene deletion in both DCs and macrophages. We found that in ArpC4 knock out (KO) cDC1s and cDC2s display impaired migration from skin to lymph nodes at steady-state, as well as upon inflammation. Remarkably, beyond this effect, we unexpectedly found that ArpC4flox/flox; Cd11c-CRE mice completely lack of Langerhans cells (LCs) as a result of the inability of LC embryonic precursors to colonize the skin early after birth. Using bone-marrow derived DCs as an in vitro model to understand this defect, we showed that impaired LC development, most likely results from defective coupling between cell migration and cell division. Strikingly, we observed that this defect is not only manifest in LCs but also in other populations of resident Cd11c+ macrophages; alveolar macrophages, which need to divide and migrate in order to colonize lung alveoli. These results indicate a novel unexpected role for the Arp2/3 complex in formation of the myeloid compartment in vivo.

ST-7

Microglia interactions with T cells in relapsing remitting EAE

Zhana Haimon (1) , Gal Frumer (1) , Jung-Seok Kim (1) , Rebecca Haffner (2) , Shifra Ben-Dor (2) , Ziv Porat (2) , Louise Chappell-Maor (1) , Sigalit Boura-Halfon (1) and Steffen Jung (1) †

Departments of (1) Immunology, (2) Veterinary Resources and (2) Life Sciences Core Facilities, Weizmann Institute of Science, Rehovot 76100, Israel.

Microglia, the parenchymal brain macrophages of the central nerve system (CNS), have emerged as critical players in brain development and homeostasis. Immune functions of these yolk sac-derived cells remain however less well defined. Here we investigated contributions of microglia as antigen presenting cells (APC) in a murine relapsing remitting (RR) multiple sclerosis paradigm, i.e. experimental autoimmune encephalitis (EAE) in C57BL/6 / SJL F1 hybrids. Fate mapping-assisted translatome profiling of the cells during the RR disease course revealed the potential of microglia to interact with T cells through antigen presentation, co-stimulation and -inhibition. Abundant microglia / T cell aggregates, as observed by histology and flow cytometry, supported the notion of functional interactions of these cells during remission, with a bias for T regulatory (Treg) cells. Finally, microglia-restricted MHC II deficiencies induced by H-2 or IFNγ receptor mutagenesis, while compatible with EAE onset, significantly affected the T cell and Treg compartment of the animals during remission. Collectively, these data establish a critical contribution of cognate interactions of antigen presenting microglia with T cells during autoimmune neurodegeneration.

ST-8

Early life interactions between commensal bacteria and dermal CD301b+ cDC2s facilitate long-term immune tolerance to the skin microbiome

Antonin Weckel (1), Julianne Riggs (1), Geil Merana (1), Jeanmarie Gonzalez (1), Joy Okoro (1), Miqdad Dariwhala (1), Yosuke Kumamoto (1), Tiffany Scharschmidt (1)

(1) University California San Francisco (UCSF)

Tolerance to commensal bacteria is critical for skin immune homeostasis. We have previously shown that tolerance to skin commensals is preferentially established in neonatal life and supported by generation of commensal-specific regulatory T cells (Tregs). Here, we set out to identify how dendritic cell (DC) interactions with bacteria in neonatal skin facilitate commensal-specific Treg formation. Colonization of neonatal mice with Staphylococcus epidermidis (SE) demonstrated that type 2 conventional DCs (cDC2s), particularly those expressing the C-type lectin CD301b, are the primary DC subset to phagocytose and traffic SE to the skin-draining lymph node. CITE-seq revealed that CD301b marks a subset of dermal cDC2s enriched for phagocytic and antigen presentation pathways. Notably, SE phagocytosis prompts neonatal CD301b+ cDC2s to increase expression of both maturation and regulatory molecules indicative of a mature regulatory DC (mregDC) program, previou sly linked to Treg generation. In vitro, using a SE-DC-T cell co-cultures, CD301b+ cDC2s preferentially drive commensal-specific CD4+ proliferation and Treg generation. Depleting this subset in neonatal Mgl2DTR mice prevented accumulation of SE-specific Tregs in vivo. After this transient neonatal depletion CD301b+ cDC2s, reduction of the SE-specific Treg compartment persists into adulthood, and adult re-exposure to SE causes heightened Th17-polarized skin inflammation. Lastly, we utilize a novel human skin explant system that enables bacterial colonization of human foreskin to demonstrate that SE colonization also causes human skin cDC2s to adopt an mregDC phenotype. Altogether, our results identify CD301b+ cDC2s and their polarization into mreg DCs as critical for the neonatal establishment of long-term tolerance to skin commensals.

ST-9

Yellow fever vaccination induces distinct and overlapping gene expression programs in human blood DC and monocyte subsets

Elena Winheim (1), Linus Rinke (1), Antionio Santos del Peral (3), Magdalena Zaucha (3), Tobias Straub (4), Simon Rothenfusser (3,5) , Anne B. Krug (1)

(1) Institute for Immunology, Biomedical Center, Ludwig Maximilian University of Munich, Planegg-Martinsried, Germany (2) Division of Infectious Diseases and Tropical Medicine, Ludwig Maximilian University of Munich, Munich, Germany (3) Division of Clinical Pharmacology, Department of Medicine IV, University Hospital, Ludwig Maximilian University of Munich, Munich, Germany (4) Core Facility Bioinformatics, Biomedical Center, LMU Munich; Munich, Germany (5) Unit Clinical Pharmacology (EKLiP), Helmholtz Zentrum München, German Research Center for Environmental Health (HMGU), Neuherberg, Germany

Yellow fever (YF) virus is a positive-strand RNA virus belonging to the flavivirus family. Vaccination with the live-attenuated vaccine strain (YF-17D) provides life-long protection against infection and is a unique model for studying the immune response to an acute self-limiting RNA virus infection in humans.
We investigated the response of blood dendritic cells (DC) and monocyte subsets to YF-17D in vivo by multi-dimensional flow cytometry and bulk RNA-sequencing of isolated DC and monocyte subsets before and at 3, 7, 14 and 28 days after vaccination to elucidate the early innate immune events which precede the rapid generation of adaptive immunity. We detected transiently increased expression of CD86, PD-L1 and Siglec1 on the surface of all DC (cDC1, cDC2, DC3, pDC, transitional (t)DCs) and monocyte subsets 7 days after vaccination. At the same time, all DC and monocyte populations upregulated a common Interferon-stimulated gene (ISG) signature, (e. g. OAS1, OAS3, RSAD2, IFIT3, IFIT1, EIF2AK2) marking a transient IFN response in the peripheral blood. The transcriptome response of DC3, which have been identified as a pro-inflammatory DC subset with a phenotype and transcriptional profile sharing features of monocytes and cDC2, was more similar to cDC2 than to monocytes. DC3 and cDC2 upregulated a shared set of genes associated with IFN signaling, antigen-processing and cross-presentation. Cell-type specific responses were also detected. Thus, the innate immune response to YF-17D vaccination is marked by concerted activation of all circulating DC and monocyte subpopulations with distinct and overlapping gene expression programs.

ST-10

Regulation of IRE1α, an ER stress sensor, in dendritic cells

Lucie MAISONNEUVE[1], Katrina PODSYPANINA[1], Eric CHEVET[2], Sophie JANSSENS[3] and Bénédicte MANOURY[1]

(1) Institut Necker Enfants Malades, INSERM U1151-CNRS UMR 8253, Université de Paris, Faculté de Médecine Necker, France
(2) UMR INSERM U1242, Centre de lutte contre le cancer Eugène Marquis, Rennes, Université de Rennes 1, France
(3) University of Ghent, VIB, Belgium

UNC93B1, a highly conserved 12-membrane spanning molecule residing in the endoplasmic reticulum (ER), has been identified as a key regulator in the trafficking[1] and folding[2] to endosomes of intracellular Toll-like receptors (TLRs) that detect microbial nucleic acids. Indeed, a mutation in the *Unc93b1* gene (3d mutation) results in inhibition of intracellular TLRs signalling in dendritic cells (DCs) and MHC class I antigen cross presentation[3]. IRE1α is one of the 3 sensor proteins of the Unfolded Protein Response (UPR) which is an adaptive response triggered upon disruption of endoplasmic reticulum (ER) protein homeostasis and whose function is to restore the altered functions of the ER. If ER homeostasis cannot be restored, the UPR then induces pro-apoptotic signals. Regulation of IRE1α activation is not well understood particularly in DCs where it plays a major role in MHC class I antigen cross presentation[4]. We provide evidence that UNC93B1 binds the luminal domain of IRE1α and regulates its function. In DCs bearing the 3d mutation, IRE1α activity is increased and cells are already primed to stress. Furthermore, inhibition of IRE1α activity in 3d DCs restores MHC class I antigen presentation. Altogether, our data highlight the essential role of UNC3B1 in regulating MHCI class I antigen presentation in DCs by controlling IRE1α activity.

1- Lee BL et al, 2013. *Elife* 19;2:e00291.
2- Pelka K et al, 2018. *Immunity*
3- Maschalidi S et al, 2017. *Nat Comm* 21;8(1):1640
4- Osorio F et al, 2014. *Nat Immunol*. 15(3):248-57

2.2. Selected POSTERS (P)

Thursday December 16th, 2021

P-1

The transcription factor BATF as novel regulator of type I interferon production in plasmacytoid dendritic cells

Shafaqat Ali (1), Ritu Mann-Nüttel (1), Regine Dress (2), Patrick Petzsch (3), Karl Köhrer (3), Haifeng Chris Xu (4), Philipp Lang (4), Judith Alferink (5), and Stefanie Scheu (1)

(1) Institute of Medical Microbiology and Hospital Hygiene, University of Düsseldorf, Germany; (2) Institute of Systems Immunology, Center for Molecular Neurobiology Hamburg (ZMNH), University Medical Center Hamburg-Eppendorf, Germany; (3) Biological and Medical Research Center (BMFZ), University of Düsseldorf, Germany; (4) Department of Molecular Medicine II, University of Düsseldorf, Germany; (5) Department of Mental Health and Cells in Motion Interfaculty Centre, University of Münster, Germany

BATF (Basic leucine zipper transcription factor, ATF-like) plays a critical role in the haematopoiesis, differentiation, proliferation, metabolism, and effector function of lymphocytes in infection and immunity. In a recent transcriptome analyses we detected Batf as differentially expressed in interferon (IFN) β-producing plasmacytoid dendritic cells (pDCs). So far, no expression or function has been described for BATF in pDCs. In the present study, we characterized the implications of BATF expression in pDC functions. Using IFNβ/YFP reporter mice we found that BATF is highly expressed in IFNβ-producing splenic and bone marrow (BM) derived pDCs. Upon TLR9 activation, the maximum of Ifnb expression precedes the maximum of Batf expression. However, the expression of Batf is not dependent on IFNAR signaling in pDCs. In comparison to wild type (WT) littermates BM-derived pDCs from Batf-deficient mice produce increased amounts of IFNα and β at mRNA and protein levels after CpG stimulation. In line with the in vitro data, Batf-deficient mice show higher serum levels of type I IFN early after LCMV infection as compared to WT animals in vivo. We will present integrative multi-omics data that is currently being analysed and suggests molecular mechanisms underlying the BATF mediated modulation of type I IFN expression in pDCs.
Taken together our data point to a so far unrecognized function of BATF in modulating pDC dependent type I IFN responses. This suggests an important role for BATF in anti-infectious immune responses and type I IFN mediated autoimmunity.

P-2

GPR183 dictates subtissular localisation of pulmonary CD301b+ conventional dendritic cells type 2 and instructs their survival via the TSLP – TSLP receptor axis

Lili Zhang (1) & Andreas Schlitzer (1)

(10 Quantitative Systems Biology, Life & Medical Sciences (LIMES) Institute, University of Bonn, 53115 Bonn, Germany

Regulatory mechanisms for the spatial distribution of non-lymphoid tissue-resident conventional dendritic cells (cDCs) remain unknown. However, cDCs are not randomly distributed across tissues, implying active regulation of intratissular placement of cDCs. Here we reveal a GPR183 instructed TSLP signalling axis-driven adventitial fibroblast : CD301b+ cDC2 survival circuit. We show that genetic ablation of GPR183 within the cDC lineage leads to a selective loss of lung-resident CD301b+ cDC2 as a function of stromal 7α,25-dihydroxycholesterol production, with no impact on DCpoesis. Confocal microscopy revealed a close association of CD301b+ cDC2 to PDGFRa+ fibroblasts within the adventitial region of the lung. Genetic ablation of TSLP receptor on cDCs revealed a adventitial fibroblast associated CD301b+ cDC2 niche fostering survival of lung-resident CD301b+ cDC2. These data have profound implication for the tissue specific functionalization of CD301b+ cDC 2 during homeostasis and disease.

P-3

ETV3 and ETV6 enable monocyte differentiation into dendritic cells during inflammation by repressing interferon-stimulated genes

Javiera Villar (1), Adeline Cros (1), Alba De Juan (1), Pierre-Emmanuel Bonte (1), Colleen M Lau (2), Ioanna Tiniakou (2), Boris Reizis (2), Elodie Segura (1)*

(1) Institut Curie, PSL Research University, INSERM, U932, 26 rue d'Ulm, Paris, France (2) Department of Pathology, New York University Grossman School of Medicine, New York, NY 10016, USA

In inflamed tissues, monocytes differentiate into macrophages (mo-Mac) or dendritic cells (mo-DC). In chronic non-resolving inflammation, mo-DC are major drivers of pathogenic events. Manipulating monocyte differentiation would therefore represent an attractive therapeutic strategy. However, what regulates the balance of mo-DC versus mo-Mac fate commitment remains unclear. Here we show that the transcriptional repressors ETV3 and ETV6 control human monocyte differentiation into mo-DC. Mechanistically, we find that ETV3 and ETV6 repress mo-Mac development and inhibit the expression of interferon-stimulated genes. Moreover, we demonstrate that activation of STAT1 signaling favors mo-Mac differentiation. Mice deficient for ETV6 in monocytes showed spontaneous expression of interferon-stimulated genes, confirming that ETV6 regulates interferon responses in vivo. Furthermore, these mice display impaired mo-DC differentiation during peritonitis and ameliorated experimental autoimmune encephalomyelitis. Our findings elucidate molecular control of monocyte fate decision and identify ETV6 as a therapeutic target to redirect monocyte differentiation in inflammatory disorders.

P-4

Highly coordinated spatiotemporal development of resident cDC1 in lymph nodes

Milas Ugur (1), Chloe Fenton (1), Reuben Jacob Labios (1), Wolfgang Kastenmueller (1)

(1) Institute of Systems Immunology, University of Wurzburg, Versbacherstr. 9, 97078, Wurzburg, Germany

Conventional dendritic cells (cDC) are essential players in adaptive immunity and form dense networks in both tissues and secondary lymphoid organs. Lymph nodes (LNs) represent a unique case of DC networks as they contain not only the ontogenic subsets cDC1 and cDC2 but also resident cDC that develop in the LN and migratory cDC that migrate to the LN from tissues. However, it is unclear how this network is maintained considering the relatively short half-lives and migrations of DCs. Focusing on cDC1s, we observed that majority of resident cDC1 cells were located at the periphery of the LN in contrast to the prevailing paradigm, which proposes that resident and migratory cDC1s are positioned at the paracortex together with T cells. Early precursors of resident cDC1s were found in medullary cords and required 3-4 days to become fully developed immature resident cDC1s. By using in situ and ex vivo local labelling via photoconversion as well as depletion and fate mapping strategies, we show that resident cDC1s develop around medullary cords and migrate towards paracortex as they develop and mature at steady state conditions. Furthermore, this process was accelerated during type I interferon-inducing infections as resident cDC1s upregulated CCR7 and migrated to the center of the paracortex within 24 hours. Together our data suggest a spatial model of DC developmental and network generation in which cDC precursors migrate and differentiate along defined paths and thereby generate a structured network.

P-5

Inositol requiring enzyme 1 (IRE1) is a regulator of apoptotic cell engulfment in cDC1s

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IRE1 is an endoplasmic reticulum (ER) resident protein that monitors the health status of the ER and initiates the unfolded protein response (UPR) upon ER stress. IRE1, a conserved endonuclease, cleaves Xbp1 mRNA and generates the transcription factor XBP1s. This leads to the induction of an adaptive gene program that restores ER homeostasis. IRE1 is typically active in secretory cells, in conditions of increased folding load.
We noticed that in vivo one subset of conventional DCs - cDC1s - displays constitutive IRE1 activity, and this in the absence of a canonical UPR response1,2. Despite activation of IRE1 and splicing of XBP1, we never observed any typical XBP1-dependent gene expression, and it remained enigmatic how and why the pathway was specifically active in cDC1s.
Guided by the outcome of a large-scale RNASeq approach, we found that activation of IRE1 in cDC1s is tightly associated with their unique capacity to engulf apoptotic cells. Recent data suggest that IRE1 can be activated by lipid bilayer stress such as cholesterol accumulation3 and we hypothesize that cholesterol influx during apoptotic cell uptake in cDC1s triggers IRE1. IRE1 plays an essential role in apoptotic cell engulfment and coordination of downstream metabolic pathways such as cholesterol efflux. IRE1 deficiency leads to a complete block in homeostatic cDC1 maturation, while cDC2 maturation and/or cholesterol homeostasis in cDC2s remained unaffected. This impedes dead cell derived antigen presentation by cDC1s and causes a loss in peripheral tolerance.
Our data add an unexpected player to the field of homeostatic DC maturation, they extend the role of IRE1 beyond its canonical function in the UPR and delineate the role of the ER as an upstream organelle sensing apoptotic cell engulfment.

1 Osorio, Nat Imm, 2014
2 Tavernier, Nat Cell Biol 2017
3 Halbleib, Mol Cell, 2017

P-6

Advanced Ly6D+ Siglec H+ lymphoid precursors contribute to conventional dendritic cells via a Ly6D+ Siglec H+ Zbtb46+ intermediate stage

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Plasmacytoid and conventional dendritic cells (pDC, and cDC) are generated from distinct lymphoid and myeloid progenitor cells in murine bone marrow. Despite the early separation of pDC and cDC lineages, cells in the CD11c+ Siglec-H+ CCR9lo pDC-like precursor fraction are able to differentiate into both pDCs and cDCs. Single-cell transcriptomics and high-dimensional flow cytometry combined with cell fate analysis revealed the heterogeneity and commitment of subsets in this compartment. CD11c+ Siglec H+ Ly6Dhi Zbtb46- cells contained CCR9lo B220hi cells which were immediate pDC precursors and CCR9lo B220lo cells which still generated pDCs and cDCs in vitro and in vivo. These lo-lo cells generated pDCs by acquiring B220 and CCR9 expression without cell division. At the same time a fraction of these lo-lo cells rapidly upregulated Zbtb46 expression and passed through an intermediary Zbtb46+ Ly6D+ stage before acquiring cDC phenotype after cell division. Zbt b46+ Ly6D+ intermediary cells isolated from primary bone marrow cells also generated exclusively cDCs. Type I IFN stimulation limited cDC output and promoted pDC output from the Ly6Dhi Zbtb46- lo-lo precursors by arresting cDC-primed cells in the Zbtb46+ Ly6D+ intermediary stage and preventing expansion and differentiation into cDCs. Thus, generation of cDCs from late stage Siglec H+ Ly6Dhi Zbtb46- pDC-biased precursors is modulated by type I IFN.

P-7

PTK7 tyrosine kinase receptor in cancer epithelial – immune cell interaction in the tumor microenvironment

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Protein Tyrosine Kinase-7 (PTK7) is a co-receptor of Wnt pathway, involved during embryonic development and in adult tissue homeostasis1. It is overexpressed in many cancers, which is associated with poor prognosis2. PTK7 was also shown to maintain a pool of hematopoietic stem cells and progenitors3. Recently, PTK7 expression was observed in dendritic cells (DCs)4. DCs are crucial to capture and process antigens at the periphery. Upon antigen encounter DCs can maturate and migrate towards the draining lymph node to prime antigen-specific naïve T cells. Thus, DC play a central role in initiating the cancer-immunity cycle5. Here, we investigate for the first time the expression and role of PTK7 in DCs to further understand its implication in cancer.

Aims:
1- To identify the PTK7+ immune cells and determine their transcriptional profile
2- To define the phenotypic maturation of dendritic cells expressing PTK7
3- To determine the role of PTK7 on dendritic cells functions

1- To identify the PTK7+ immune cells and determine their transcriptional profile
We observed that PTK7 was expressed in a subset of migratory DCs (cDC2) from cutaneous lymph nodes (cLN) and skin of WT mice at steady state. In addition to cDC2s, PTK7 was also observed in macrophages and monocytes in a melanoma mouse model. To complete these data, we have recently performed a pilot CITE-seq experiment to build a cartography on the expression of PTK7 in the whole immune compartment of cLN, and subsequently in skin, tumor and tumor-draining lymph node.
2- To define the phenotypic maturation of dendritic cells expressing PTK7
In order, to evaluate the link between PTK7 expression and the maturation of DCs, we sorted primary PTK7+ and PTK7- migratory cDC2 from cLN. Then, we stimulated them with the TLR ligands: LPS and R848 to induce a strong activation of DCs. We observed that activated PTK7+ cDC2 had a higher activation profile (increased expression of CCR7, CD40, CD80

and PDL-1) compared to activated PTK7- cDC2. This may suggest that PTK7 may have a role in antigen presentation of T cells.

3- To determine the role of PTK7 on dendritic cells functions

To test the role of PTK7 in antigen presentation of T cells, we are currently setting two ex-vivo antigenic presentation assays, based on the classical OT-I/II and pmel models. To do so, we will sort migratory cDC2 PTK7- and PTK7+ and put them separately in co-culture with naïve CD4+ or CD8+ T cells depending on the model of the antigen presentation assay described before. As read out we will measure proliferation, polarization and/or cytolytic activity of T cells to compare PTK7+ from PTK7- co-cultures.

Another feature of DCs, is their ability to migrate from the periphery to the draining lymph node. To test whether the expression of PTK7 by DCs influences their migratory capacities, we will use microfabricated devices that mimic the conditions of migration of dendritic cells in tissues(microchannels and 3D collagen matrix)6. We will compare the migration capacity between PTK7-DC and PTK7+DC in spontaneous and directed migration (i.e. to chemokines, growth factors, to wnt ligands).

So far, we have shown for the first time that PTK7 is expressed in a subset of DC, monocytes and macrophages in mice. We will now determine its function at the steady and in cancer. This study will add new knowledges on the biology of PTK7 in immune cells and help to clarify its role in cancer to improve current drugs targeting PTK7.

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P-8

Select hyperactivating NLRP3 ligands enhance the TH1- and TH17-inducing potential of human type 2 conventional dendritic cells

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The detection of microorganisms and danger signals by pattern recognition receptors on dendritic cells (DCs) and the consequent formation of inflammasomes are pivotal for initiating protective immune responses. Typically, the activation of inflammasomes leads to IL-1β secretion accompanied by pyroptotic cell death. However, dependent on the cell type and the inflammasome ligands used, some cells can survive inflammasome activation and exist in a state of hyperactivation (defined by IL-1β secretion from living cells along with other pro-inflammatory cytokines). Here, we report that the conventional type 2 DC (cDC2) subset is the major human DC subset that is transcriptionally and functionally able to induce inflammasome formation and enter a state of hyperactivation. When cDC2 were stimulated with ligands that relatively weakly activated the inflammasome, the cells did not enter pyroptosis but instead secreted IL-12 family cytokines together with IL-1β . Hyperactivated cDC2 induced prominent T helper type 1 (TH1) and TH17 responses that were superior to those seen in response to Toll-like receptor (TLR) stimulation alone or to stronger, classical pyroptosis-inducing inflammasome ligands. These findings not only define the human cDC2 subpopulation as a prime target for the treatment of inflammasome-dependent inflammatory diseases but may also enable new approaches for adjuvant and vaccine development.

P-9

Tolerogenic dendritic cell maturation revisited: some old concepts and new tricks

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Depending on how an antigen is perceived, dendritic cells (DCs) mature in an immunogenic or tolerogenic manner, safeguarding the balance between immunity and tolerance. Which signals drive homeostatic tolerogenic maturation are still poorly understood. Here we demonstrate that engulfment of apoptotic cells drives maturation of conventional DCs in the spleen. The process can be mimicked by engulfment of lipid nanoparticles, is marked by intracellular accumulation of cholesterol, and highly unique to type 1 cDCs. CITE-Seq studies reveal distinct gene signatures related to cholesterol efflux, metabolic adaptation to efferocytosis and IFN-I signaling, at key transition points in maturing cDC1s. Thus, homeostatic cDC1 maturation is not a stochastic process, but instructed by apoptotic cell uptake and concomitant changes in cellular cholesterol metabolism. Moreover, LNPs can provide a powerful tool to induce tolerogenic DC maturation in vivo.

P-10

Epithelial Colonization by Gut Dendritic Cells promotes their Functional Diversification

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Dendritic cells (DCs) patrol tissues and transport antigens to lymph nodes to initiate adaptive immune responses. Within tissues, DCs constitute a complex cell population composed of distinct subsets that can exhibit different activation states and functions. How tissue-specific cues orchestrate DC diversification remains elusive. Here, we show that the small intestine included two pools of cDC2s originating from common preDC precursors: (1) lamina propria (LP) CD103+CD11b+ cDC2s that were mature-like pro-inflammatory cells and (2) intraepithelial cDC2s that exhibited an immature-like phenotype as well as tolerogenic properties. These phenotypes resulted from the action of food-derived retinoic acid (ATRA), which enhanced actomyosin contractility and promoted LP cDC2 transmigration into the epithelium. There, cDC2s were imprinted by environmental cues including ATRA itself and the mucus component Muc2. Hence, by reaching distinct sub-tissular niches, DCs can exist as immature and mature cells within the same tissue, revealing an additional mechanism of DC functional diversification.

P-11

Exploring membrane contact sites in DCs antigen cross presentation

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Antigen (Ag) cross-presentation (XPT) by dendritic cells (DCs) is an essential mechanism for initiating adaptive immune responses, in which exogenous or mutated proteins are presented on MHC-I molecules, eliciting cytotoxic CD8+ T cell responses. However, its molecular mechanisms are poorly understood.
DC-based vaccines exploiting the XPT ability are promising anti-cancer immunotherapy strategies in mouse models but only 30% effective in humans. This is due, partially, to the use of Mo-DCs, a DC subtype poor at XPT, together with the gap of knowledge on XPT mechanisms.
XPT is potentiated by phagocytosis and requires endoplasmic reticulum (ER) proteins, but their connection remains unclear.
Membrane contact sites (MCS) are regions of close appositions between the ER and other organelles, playing important roles in localized signaling, lipid transfer and membrane trafficking.
Previous work in our laboratory discovered the existence of MCS between ER and phagosomes. Furthermore, it was demonstrated that these MCS regulate localized store operated calcium entry signaling (SOCE). DCs are full of MCS, and MCS frequency correlates with XPT activity (Nunes-Hasler 2017)
Sec22b, a SNARE protein of the ER-Golgi-intermediate-compartment (ERGIC), was described to increase XPT and phagolysosome fusion (PLF), and later found to act as non-fusogenic tether of ER-plasma membrane MCS.
Previous work in our laboratory showed that Sec22b also mediates ER-phagosome MCS and regulates phagosome lipids, moderating PLF and impacting phagosomal maturation. However, the impact on XPT was not explored.
This project aims to explore these molecular pathways and mechanisms in order to enhance XPT, and potentially improve cancer immunotherapy formulations.

P-12

Breast cancer remotely remodel the hematopoietic niche favoring myelopoiesis and tumor growth.

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Myeloid cell infiltration is a hallmark of solid cancers and often correlates with poor prognosis and disease severity. Myeloid cells (i.e neutrophils, monocytes and macrophages) play various roles in the immune resistance and evasion mechanisms of tumors. It is therefore crucial to decipher how myeloid cell generation (myelopoieisis) is regulated during cancer, paving the way for new therapeutic strategies.
Using flow cytometry, single cell and bulk RNA sequencing and imaging techniques, we have characterized the tumor-induced systemic changes impacting the bone marrow and myelopoiesis in an autochthonous breast cancer mouse model.
We found that breast tumors remotely impact hematopoietic stem cells (HSC) differentiation. We showed that HSC and early myeloid progenitors of tumor-bearing mice were transcriptionally and functionally primed towards myeloid cell differentiation. By screening soluble factors in the serum of tumor bearing animals, we found a repeated increase in several bone remodeling proteins, including the RANKL decoy receptor osteoprotegerin (OPG). Accordingly, tumor bearing animals had increased bone activity and elevated osteoblastic differentiation. We showed that OPG genetic inactivation in tumor cells inhibited tumor development and myeloid cell tumor infiltration *in vivo*.
Our data highlight a systemic remodeling of the bone marrow in both the hematopoietic and the stromal compartments, remotely controlled by the tumor. OPG blockade could therefore help normalize hematopoiesis during cancer development and prevent myeloid cell infiltration in the tumor-microenvironment.

P-13

Spatial organization and prognostic impact of dendritic cell subsets in a large cohort of triple negative breast cancer patients

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Different dendritic cells (DC) subsets have been already identified in triple negative breast cancer (TNBC) by flow cytometry, RNAseq or by IHC. In human, in silico analysis only have been used to compare their impact on patient survival and their spatial organization and interaction with CD8 T cells haven't been explored yet in tumors.
Here, we set up a 7 colors immunofluorescence staining to precisely localized in a cohort of 70 chemotherapy naïve resected TNBC patients, pDC (plasmacytoid-DCs), cDC1 (conventional type 1 DCs), cDC2/LC (Langherans Cells) and mature DCs along with CD8+ T cells. We quantified by INFORM software and analyzed by an in-house bioinformatic pipeline their organization and their prognostic impact.
We showed for the first time, by our in situ approach, that pDC (plasmacytoid-DC), cDC1 (conventional type 1 DC) and mature DCs co-infiltrate TNBC along with CD8+ T cells and they were mostly located into the stroma compartment in contrast to LC found in the tumoral compartment. TNBC patients who were enriched in CD8+ T cells, cDC1, pDC or mature DCs in the stroma trended to have a longer overall survival. However, no DC subsets kept their prognostic value when looking they were localized into the tumor compartment. Distance analysis with CD8+ T cells suggested an intra-tumoral organization of DC subsets in a non-stochastic fashion. The bioinformatic pipeline allowed us to identify various DC aggregates containing different proportion of each DC subsets and CD8 T cells that may explain particular DC functions and the prognosis of patient.

P-14

Identification of immune suppressive lipid-associated macrophages involved in resistance to immunotherapy in triple negative breast cancer.

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Tumor-associated macrophages (TAM) play a detrimental role in triple negative breast cancer (TNBC). Nevertheless, their in-depth characterization and interactions with stromal cells, such as the cancer-associated fibroblast (CAFs), are lacking. We identify at the single-cell level a monocyte-derived-STAB1+TREM2high lipid-associated macrophage (LAM) subpopulation that bears immune suppressive features and is implicated in resistance to immune checkpoint blockade (ICB). Genetic depletion of mouse monocyte-derived-STAB1+TREM2high LAMs in TNBC-Trem2-/- mice partially controls the growth of TNBC tumors via T- and NK- anti-tumor activity. Cell-to-cell interaction modeling and in vitro assays demonstrate a key role for the inflammatory CXCL12-CXCR4 axis in CAF-myeloid cell crosstalk and recruitment of monocytes at tumor site. Indeed, co-culture in vitro of TNBC-derived FAP+CAFs induces reprogramming of blood monocytes towards STAB1+TREM2high immunosuppressiv e LAM phenotype. Altogether, we propose an inflammation model, whereby monocytes – recruited to the tumor via the inflammatory CAF-driven CXCL12-CXCR4 axis – acquire pro-tumorigenic LAM capacities supporting an immunosuppressive microenvironment amenable to therapeutic targeting.

P-15

Tumor-derived extracellular vesicles uptake by immune cells

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Tumor-derived extracellular vesicles (TD-EVs) have been linked to malignant transformation as they contain oncoproteins, RNA and DNA fragments that induce malignant phenotypic changes in the recipient cells. The different subtypes of TD-EVs can modulate diverse immune responses, both suppressing or activating the immune system. This work aims to discern the role of EVs in immune regulation and to determine which immune cells preferentially uptake the TD-EVs. In order to study the uptake by the different immune cell types in human PBMCs, two EV sources have been evaluated: MDA-MB-231 and Jurkat compared to fluorescent beads. We have identified the CD14+ population as the major uptaking cell type. In addition, incubation with TD-EVs resulted in the appearance of an FSC low CD14+ population with phosphatidylserine exposure. Further evaluation will be carried out in the CD14+ population to determine the variations at the phenotypic level. Understanding the aspects and mechanisms involved in the EVs and immune system interactions will provide significant insights into immune modulation by cancer cells and the role of EVs in the tumor microenvironment.

P-16

Study of DNase1L3 in the regulation of anti-tumor immune responses

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Detection of tumor-derived DNA (tDNA) by dendritic cells (DCs) plays a crucial role in activation of anti-tumor immunity by stimulating the production of type I interferons (IFN-I). IFN-I is associated with improved patients' outcomes and better efficacy of immunotherapies. In addition, chemotherapies and radiotherapy boost anti-tumor responses by increasing IFN-I production induced by tDNA. We have characterized a nuclease produced by DCs called DNASE1L3 that digests DNA released by dying cells and thus limits self-DNA abundance and its immunostimulatory potential. However, it remains unknown whether DNASE1L3 may be involved in the regulation of tDNA-induced anti-tumor immune responses. Given the expression profile of DNASE1L3 and its property to regulate the levels of extracellular cell free DNA, we aimed to characterize the impact of DNASE1L3 deficiency on cancer progression and responsiveness to chemotherapies, radiotherapy and immunotherapy.

Our preliminary results show that Dnase1l3 deficiency didn't directly affect the growth of either spontaneous or transplantable tumors, or the tumor immune cell infiltrate. Though, the therapeutic efficacy of the chemotherapies was strongly reduced in Dnase1l3 deficient mice. Thus, DNASE1L3 may somehow process tDNA to enhance its immunostimulatory potential. Further studies are needed, particularly of the mechanisms of action of DNASE1L3 in the regulation of anti-tumor immune responses induced by immunogenic therapies. Characterizing DNASE1L3 function in cancer may contribute to the development of novel therapeutic strategies to boost anti-tumor immunity and the efficacy of current therapies.

P-17

Role of Galectin-9 in the induction of tolerogenic dendritic cells by Nasopharyngeal Carcinoma exosomes

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Nasopharyngeal Carcinoma (NPC) is characterized by an immunosuppressive microenvironment dominated by regulatory T lymphocytes and tumor-derived exosomes (Exo-NPC). Preliminary results have shown that Exo-NPC carrying Galectin-9 (Exo-NPC-Gal9+) induce dendritic cells with tolerogenic properties (tDC). In this context, our objective is to assess the role of Galectin 9 (Gal-9) in these induction mechanisms by evaluating the role of exogenous and exosomale Gal-9. For that, we have isolated and characterized tumor-derived exosomes. Then we generated dendritic cells (DC) from human monocytes with recombinant Gal-9S (DC-Gal9) or Exo-NPC-Gal9+ (DC-Exo-NPC-Gal9+). The state of maturation of the DCs was validated at the phenotypic (flow cytometry) and functional (ELISA, MLR) level. Preliminary results show that DC-Gal9 and DC-Exo-NPC-Gal9+ express maturation markers similarly to maturation controls (mDC). DC-Gal9 also exhibit a cytokine secretion profile identica l to mDC. Nevertheless, our first results suggest that DC-Gal9 and DC-Exo-NPC-Gal9+ possess immunosuppressive properties by inducing a decrease of LTCD3+ proliferation. Then, we evaluated the effect of blocking recombinant Gal-9 on the maturation and suppressor function of DCs, by the use of an anti-Gal-9 antibody [1G3 clone]. The blocking of Gal-9 seems to inhibit the suppressive function of DC-Gal9 by restoring LTCD3+ proliferation and be able to inhibit the suppressive function of Exo-NPC-Gal9+ by restoring autologous PBMCs proliferation. These results show that recombinant Gal-9 and Exo-Gal9+ don’t impact DC maturation but seems to play a role in the induction of DC with tolerogenic properties. Interestingly, the use of an antibody targeting Gal-9 seems to reverse this immunosuppression, leading to promising therapeutic prospects.

P-18

Decipher the early immune surveillance pathways in dendritic cells during breast cancer development using a transplantable pre-neoplastic organoid model.

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While tumor immune evasion mechanisms are now well characterized in mammary tumors, the very early events implicated in the immune sensing of preneoplastic cells remain poorly understood due to the lack of human biological samples and appropriate preclinical mouse model. Given the important role of dendritic cells (DC) and interferon (IFN) pathways in the initiation of anti-tumor immune response, we hypothesize they could play a central role in the anti-tumor immune surveillance. We have previously demonstrated that cDC infiltrate human breast tumors and are associated, with their production of IFN-III, to a good prognosis. Using a spontaneous mammary tumor model in mice (BLG-Cre, BRCA1f/f, p53+/-) which recapitulates the different stages of development, we demonstrated that all DC subsets are present from the hyperplasia stage, but, the proportion of cDC1 and cDC2 decreases during tumor progression while pDC remain stable. Next, we have developed an inn ovative organoid model of preneoplastic cells from the spontaneous mouse mammary tumor model. To determine the specific role of DC subsets and/or IFN pathways in the tumor immune surveillance, we will determine and characterize the tumorigenic potential of these preneoplastic organoids orthotopically injected into recipient mice deficient for DC and/or IFN pathways. By exploiting a unique mouse model to study early TNBC immune surveillance, our original project will help to understand the cellular and molecular mechanisms involved in the immune surveillance of preneoplastic cells and ultimately to identify new therapeutic targets promoting anti-tumoral functions of DC and IFNs, in advanced tumors resistant to conventional immunotherapies.

P-19

IFN-III produced by type 1 conventional dendritic cells (cDC1) induces plasmacytoid Dendritic Cells (pDCs) survival and activation in human tumors

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Dendritic cells (DC) play a key role in the anti-tumoral immune responses. Conventional Dendritic Cell 1 (cDC1) is one DC subset which plays an important role by performing antigen (Ag) presentation and T cells recruitment. In this context, we have previously shown that cDC1 produce high level of type III interferons (IFN-III). Nevertheless, the precise role of IFN-III produced by cDC1 in the tumor microenvironment (TME) is still not known.

Here, we show that intra-tumoral plasmacytoid Dendritic Cells (pDCs) are the tumor infiltrating immune cells that best respond to IFN-III. We demonstrate that the survival of pDCs from PBMCs and the TME is increased after 24h treatment with IFN-III, along with their expression of PD-L1 and ICOS-L. Moreover, IFN-III stimulation of pDCs induced an intermediate activation phenotype based on CD80/CD86, BDCA2, CD123, HLA-DR, PD-L1, ICOS-L expression compared to a stimulation with type-I interferons (IFN-I). We also performed RNA-sequencing of IFN-III treated pDCs and observed 1582 differentially expressed genes (DEG) compared to non-treated pDC. In addition, we did confirm the intermediate activation induced by IFN-III compared to IFN-I thanks to this RNA-sequencing. Pathways upregulated are currently analyzed. Finally, thanks to a 7 colors immunofluorescence staining of the different infiltrating DC subsets performed on 70 TNBC patients, we were also highlight a particular pDC– cDC1 cr oss-talk in situ.

In conclusion, our team was able to describe the impact of IFN-III produced by cDC1 on pDCs survival and activation in the TME.

P-20

Boosting type1 DCs to expand T cell responses in mismatch repair deficient experimental lung tumors

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Tumor neoantigens arising in mismatch repair deficient tumors are critical targets of the host antitumor immune response and correlates with the efficacy of immunotherapy. Recent experimental studies to decipher the extent and quality of the neoantigen specific T cell responses still relied in large part on ectopic expression of surrogate neoantigens. To overcome these limitations, we generated an hypermutated KrasG12D/+, p53-/- transplantable lung tumor model (KP) to faithfully recapitulates the multiplicity and variety of neoantigens generated spontaneously in vivo. To this goal we genetically inactivated Mlh1, an enzyme implicated in repairing DNA mismatches. This resulted in generation of 24 private neoantigens, exclusively expressed in mutant cells and absent from the original line. Growth of the hypermutated KP line (KPneo) induced T cell activation and was controlled in immune competent host, indicating acquired immunogenicity. Delayed tumor growt h was partially lost upon CD8 depletion and in type-1 DCs deficient hosts, unveiling a critical role for cross-presentation of neoantigens in mediating tumor control. Interestingly, administration of a combined therapy including Flt3L, TLR3 agonist, and PD-L1 had no impact on the original KP line but induced a robust T cell activation and significant growth delay in KPneo tumors. Ongoing experiments aim to identify immunogenic epitopes and to examine how DC and T cell therapy, respectively, contribute to the intensity and diversity of the T cell response. Altogether, these results illustrate generation and validation of a novel preclinical tumor model carrying multiple bona-fide neoantigens, suitable to test combinatorial immunotherapies.

P-21

cDC1 and cDC2 co-operate in CD40 agonist response while suppressive immune microenvironments and lack of antigens subvert efficacy

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Background: Activating antigen-presenting cells using an agonistic αCD40 antibody has shown to inhibit cancer progression in several preclinical tumour models, but clinical benefit was observed only in a small fraction of patients. Hence, understanding the cancer cell-intrinsic and microenvironmental determinants of αCD40 therapy response are crucial to identify responsive patient populations and design efficient combination treatments.
Methods: To investigate the mechanism underlying αCD40 treatment we used the B16F10 melanoma and Lewis lung carcinoma (LLC) models. We used genetic mouse models (Xcr1-DTR) and antibodies (αCSF1R, αCD20) to identify which immune cell populations mediate antitumour immunity in response to αCD40. Single-cell RNA sequencing allowed us to identify macrophage populations that could support resistance to αCD40 therapy. Finally, we investigated potential synergistic therapies including specific depletion of suppressive immune populations, increased abundance of stimulatory immune populations, and immunogenic cell death-inducing chemotherapy to sensitise resistant tumours to αCD40 therapy.
Results: We identified that the therapeutic efficacy of αCD40 in responsive B16F10 tumours relied on the presence of cDC1s prior to therapy, however cDC1s were dispensable after αCD40 administration. In response to αCD40 the abundance of CCR7+ cDCs, potentially derived from cDC2s increased, thereby further activating antitumour CD8+ T cells. LLC tumours, characterised by a high abundance of macrophages, were resistant to αCD40 therapy. Combining αCD40 therapy with macrophage depletion led to tumour growth inhibition only in immunogenic LLC tumours expressing OVA, indicating that αCD40/αCSF1R therapy requires the presence of neoantigens. Accordingly, treatment with immunogenic cell-death inducing chemotherapy sensitised non-immunogenic LLC tumours to αCD40/αCSF1R therapy.
Conclusions: These results suggest that αCD40 efficacy relies on pre-existing cDC1-primed CD8+ T cells, that are potentially re-activated by cDC2s, and combination of macrophage depletion and immunogenic chemotherapy could re-sensitise αCD40-resistant macrophage-enriched tumours.

P-22

Nasopharyngeal Carcinoma Exosomes Modulate the Function of Human Dendritic Cells and Favour their Recruitment

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Nasopharyngeal Carcinoma (NPC) is characterized by a large prevalence of regulatory T cells and the production of tumor-derived exosomes (ExoNPC) with immunosuppressive properties. We previously showed that ExoNPC favour the suppressive activity and recruitment of human Tregs via the CCL20 chemokine, thus contributing to NPC immune escape. Our objective here is to demonstrate that ExoNPC could alter monocyte-derived dendritic cell (DCs) maturation and promote tolerogenic DCs (tDCs). Moreover, we aim to (i) define the metabolic status of DCs induced by NPC exosomes (DCExoNPC), and (ii) highlight the chemoattractive properties of ExoNPC and the potential involvement of CCL20. The maturation status of DCs cultured with ExoNPC was evaluated both at a phenotypical and functional level. Phenotypically, we studied the expression of maturation markers (flow cytometry) and the metabolism status using Seahorse® technology (Oxygen consumption rate measurements) an d observed that DCExoNPC exhibit a mature phenotype with a metabolic state similar to control mDCs. Functionally, DCExoNPC decreased the production of pro-inflammatory cytokines (IL-6 and Il-12p70) and decreased the proliferation rate of total CD3 T lymphocytes. The study of IDO1 and tryptophan metabolites indicates that after differentiation, DCExoNPC exhibit a high Kynurenine/Tryptophan ratio and IDO expression compared to DC controls. Finally, chemoattractive potential of ExoNPC on DCs was analyzed and suggests that ExoNPC could preferentially attract DCExoNPC compare to mDC but not in a CCL20-dependant manner. Taken together, our results describe the major role of ExoNPC in promoting immune tolerance within the NPC microenvironment and so identify potential new anti-tumoral therapeutic targets.

Friday December 17th, 2021

P-23

Formation of the myeloid compartment in vivo: Revealing an unexpected role for the Arp2/3 complex.

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Arp2/3 is a seven-subunits complex that mediates branched actin network formation in eukaryotic cells. It has been shown that such reinforced actin network can play two roles in the migration of dendritic cells (DCs): (1) it helps protrusion formation for navigation in complex environments and (2) it nucleates actin around the DC nucleus for these cells to move within confined spaces. However, how lack of Arp2/3 impacts on the physiology of DCs in vivo is unknown. To address this question, we generated a mouse model deficient in the Arp2/3 actin-binding subunit ArpC4, and crossed it to Cd11c-CRE mice to obtain gene deletion in both DCs and macrophages. We found that in ArpC4 knock out (KO) cDC1s and cDC2s display impaired migration from skin to lymph nodes at steady-state, as well as upon inflammation. Remarkably, beyond this effect, we unexpectedly found that ArpC4flox/flox; Cd11c-CRE mice completely lack of Langerhans cells (LCs) as a result of the inability of LC embryonic precursors to colonize the skin early after birth. Using bone-marrow derived DCs as an in vitro model to understand this defect, we showed that impaired LC development, most likely results from defective coupling between cell migration and cell division. Strikingly, we observed that this defect is not only manifest in LCs but also in other populations of resident Cd11c+ macrophages; alveolar macrophages, which need to divide and migrate in order to colonize lung alveoli. These results indicate a novel unexpected role for the Arp2/3 complex in formation of the myeloid compartment in vivo.

P-24

Microglia interactions with T cells in relapsing remitting EAE

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Microglia, the parenchymal brain macrophages of the central nerve system (CNS), have emerged as critical players in brain development and homeostasis. Immune functions of these yolk sac-derived cells remain however less well defined. Here we investigated contributions of microglia as antigen presenting cells (APC) in a murine relapsing remitting (RR) multiple sclerosis paradigm, i.e. experimental autoimmune encephalitis (EAE) in C57BL/6 / SJL F1 hybrids. Fate mapping-assisted translatome profiling of the cells during the RR disease course revealed the potential of microglia to interact with T cells through antigen presentation, co-stimulation and -inhibition. Abundant microglia / T cell aggregates, as observed by histology and flow cytometry, supported the notion of functional interactions of these cells during remission, with a bias for T regulatory (Treg) cells. Finally, microglia-restricted MHC II deficiencies induced by H-2 or IFNγ receptor mutagenesis, while compatible with EAE onset, significantly affected the T cell and Treg compartment of the animals during remission. Collectively, these data establish a critical contribution of cognate interactions of antigen presenting microglia with T cells during autoimmune neurodegeneration.

P-25

Early life interactions between commensal bacteria and dermal CD301b+ cDC2s facilitate long-term immune tolerance to the skin microbiome

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Tolerance to commensal bacteria is critical for skin immune homeostasis. We have previously shown that tolerance to skin commensals is preferentially established in neonatal life and supported by generation of commensal-specific regulatory T cells (Tregs). Here, we set out to identify how dendritic cell (DC) interactions with bacteria in neonatal skin facilitate commensal-specific Treg formation. Colonization of neonatal mice with Staphylococcus epidermidis (SE) demonstrated that type 2 conventional DCs (cDC2s), particularly those expressing the C-type lectin CD301b, are the primary DC subset to phagocytose and traffic SE to the skin-draining lymph node. CITE-seq revealed that CD301b marks a subset of dermal cDC2s enriched for phagocytic and antigen presentation pathways. Notably, SE phagocytosis prompts neonatal CD301b+ cDC2s to increase expression of both maturation and regulatory molecules indicative of a mature regulatory DC (mregDC) program, previou sly linked to Treg generation. In vitro, using a SE-DC-T cell co-cultures, CD301b+ cDC2s preferentially drive commensal-specific CD4+ proliferation and Treg generation. Depleting this subset in neonatal Mgl2DTR mice prevented accumulation of SE-specific Tregs in vivo. After this transient neonatal depletion CD301b+ cDC2s, reduction of the SE-specific Treg compartment persists into adulthood, and adult re-exposure to SE causes heightened Th17-polarized skin inflammation. Lastly, we utilize a novel human skin explant system that enables bacterial colonization of human foreskin to demonstrate that SE colonization also causes human skin cDC2s to adopt an mregDC phenotype. Altogether, our results identify CD301b+ cDC2s and their polarization into mreg DCs as critical for the neonatal establishment of long-term tolerance to skin commensals.

P-26

Fine-tuning of IL4-induced immune responses by Aryl Hydrocarbon Receptor

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IL4 orchestrates key aspects of immune responses in allergy, helminth infections and tissue repair. The tight regulation of this process is essential to avoid inappropriate responses leading to immuno-pathologies. However, how IL4-induced responses are regulated at the molecular level remains poorly understood. Using transcriptomic analysis on human monocytes, we demonstrate that signaling of IL4-receptor and of the transcription factor Aryl Hydrocarbon Receptor (AhR) synergize for the induction of a cluster of the IL4-response genes. In particular, we find that AhR controls the metabolic switch induced in monocytes by IL4 exposure. Furthermore, we show that the IL4/AhR synergy also occurs in human B cells, T cells and macrophages, and in vivo in mouse macrophages. To address the role of AhR in the regulation of the tissue repair program in macrophages, we analyze cutaneous wound healing in mice deficient for AhR in macrophages (LysM*AhR mice). Deficient mice have delayed wound closure, and deficient macrophages display impaired secretion of repair molecules. We also address the response of LysM*AhR mice to helminth infection with the filaria L. sigmodontis. Deficient mice show increased inflammation and decreased expression of molecules involved in resolution of inflammation. Collectively, our results demonstrate that AhR fine-tunes the IL4 response program in immune cells, and modulates the tissue repair function of macrophages.

P-27

Heterogeneity and function of conventional dendritic cells from early to adult life

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Conventional dendritic cells (cDCs) in early life exhibit qualitative differences compared to adult life, including lower expression of costimulatory molecules and an intrinsic Th2 bias, especially at barrier organs. As a result, cDCs in early life are often considered under-developed or functionally immature.
We have revisited cDC development and function in early life and found that during the neonatal period cDC2 exhibit a distinct hematopoietic origin, and, like other myeloid and lymphoid cells, develop in waves. Ontogenetically distinct cDC2 in early life are transcriptionally and functionally similar. cDC2 in early and adult life, however, are exposed to distinct cytokine environments that shape their transcriptional profile, their ability to sense pathogens, secrete cytokines and polarize T cells. Importantly and in contrast to current dogma, despite exhibiting age-dependent differences in cell function, cDC2 in early life are functionally competent to induce T cell responses.
Because cDC1 and cDC2 have distinct ability to drive immunity, we have profiled the cDC compartment of mouse spleen at different ages using single cell RNA sequencing. These data indicate that both cDC subsets are subject to dynamic environmentally imprinted changes within the first weeks of life. Using phenotypic, functional and genetic analyses we are currently investigating the consequences of these changes for immunity.

P-28

Dietary ligands of Aryl Hydrocarbon Receptor modulate the severity of cutaneous allergic responses via the control of Langerhans cell migration

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Emerging evidence indicates that nutritional compounds can affect immune responses. In particular, dietary metabolites that activate the Aryl Hydrocarbon Receptor (AhR) have been shown to play a major role in intestinal immune cell homeostasis, and at a distant site during neuro-inflammation. Whether dietary AhR ligands also play a role in other pathological contexts remains unclear. Here we addressed the impact of dietary AhR ligands in allergic responses, by analyzing mice fed with a synthetic diet deprived of AhR ligands or the same diet enriched with Indole-3-Carbinol (I3C), a natural AhR ligand precursor. In a model of cutaneous papain-induced allergy, we found that mice fed with the synthetic diet have increased Th2 responses compared to mice fed on the I3C diet. This phenomenon was independent of AhR activation in T cells. However, we found that Langerhans cell migration to the lymph nodes upon papain exposure was severely impaired in mice fed with the synthetic diet, leading to exacerbated T cell responses. Using transcriptomic analysis, we showed that dietary AhR ligands modulate the level of inflammation driven by keratinocytes, including molecules involved in the cross-talk with Langerhans cells. Finally, we addressed the impact of dietary AhR ligands in an asthma model after skin sensitization. Our results identify a role for dietary AhR ligands in the modulation of allergic responses via the control of Langerhans cell migration.

P-29

Fate mapping reveal dual ontogeny of disease-associated microglia and disease inflammatory macrophages in ageing and neurodegeneration

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Brain macrophage populations include parenchymal microglia, meningeal macrophages, choroid plexus perivascular macrophages and recruited monocyte-derived cells. Together they control brain development and homeostasis, but are also implicated in the pathogenesis of ageing, autoimmunity and neurodegeneration. Studies using single-cell RNA sequencing and mass cytometry have yet to fully resolve the phenotypes, localizations and functions of these populations in these contexts. Here, we generated an indexed-sorted murine brain myeloid single-cell dataset including embryonic and adult stages, and combined it with published datasets in order to conclusively delineate macrophage populations. This integration of 6 single-cell RNA sequencing dataset composed in total of 188437 cells allowed us to generate a macrophage map from embryonic to aged and neurodegenerative brain. This map as well as fate mapping mice model, bulk RNA sequencing, cytometry, immunofluoresence microscopy revealed two ontogenetically and functionally different cell lineages within the disease-associated microglia (DAM) population: embryonically-derived physiological TREM2-dependent DAM exhibiting fetal-like reprogramming in neurodegenerative disease; and a pathological monocyte-derived TREM2-expressing disease inflammatory macrophages (DIMs) that accumulate in the brain during ageing and neurodegeneration in mice and human. These data clarify brain myeloid cell heterogeneity in development, homeostasis and disease and identify new molecular and cellular targets in neuroinflammation.

P-30

Sensory neuron-derived TAFA4 promotes dermal macrophages tissue repair functions

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Tissue-resident macrophages have a key role in tissue repair, but the precise molecular mechanisms that regulate the balance between inflammatory and pro-repair macrophages remain poorly understood. Here we demonstrate a major role for sensory neurons in promoting the tissue-repair function of dermal macrophages. In a sunburn-like model of skin damage in mice, the conditional ablation of sensory neurons expressing the Gαi-interacting protein (GINIP) results in defective tissue regeneration and dermal fibrosis. Elucidation of the underlying molecular mechanisms revealed a crucial role for the neuropeptide TAFA4, which is produced in the skin by C-low threshold mechanoreceptors, a subset of GINIP+ neurons. In vivo studies in Tafa4-deficient mice revealed that TAFA4 promotes the production of IL-10 by dermal macrophages after UV-induced skin damage. This TAFA4–IL-10 axis also ensures the survival and maintenance of IL-10+TIM4+ dermal macrophages, reducin g skin inflammation and promoting tissue regeneration. These results reveal a new neuroimmune regulatory pathway driven by sensory neurons, promoting anti-inflammatory properties of tissue-resident macrophages, and preventing tissue fibrosis after injury. Our findings could lead to new therapeutic perspectives for other inflammatory skin disorders and tissue regenerative medicine.

P-31

Resident macrophage subsets differentially impact bladder immunity to uropathogen infection

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Urinary tract infections (UTI) are the second most common infection, impacting nearly 30% of all adults. Over 80% of UTI are caused by uropathogenic E. coli (UPEC), which induces a robust innate inflammatory response, followed by a non-sterilizing adaptive immune response to a second or challenge infection. Previously, we showed that the nonsterilizing immune response initiated by the host is due in part to the presence of bladder-resident macrophages. More recently, our studies reveal that two macrophage populations reside in the naive bladder, with distinct phenotypes, locations, and transcriptomes. Using RNA sequencing, fluorescent imaging, and flow cytometry, we observed that one macrophage subset is in close contact with the urothelium, in the lamina propria, and dies rapidly after infection. The second subset resides primarily in the detrusor muscle and acquires more bacteria during infection compared to the lamina propria subset. In the course of UTI, these macrophage subsets are replaced by infiltrating monocytes, which leads to distinct transcriptional profiles in the subsets after resolution of infection compared to their naïve counterparts. Finally, depletion of these subsets before a second UTI leads to a lower bacterial burden concomitant with an increase in Th1-biased effector cell infiltration. These findings contribute to our understanding of how immunity is regulated in response to UTI, potentially highlighting pathway to target for development of new therapeutic strategies to prevent or treat UTI.

P-32

Epidermal maintenance of Langerhans cells relies on autophagy-regulated lipid metabolism

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Macroautophagy (often-named autophagy), a catabolic process regulated by several autophagy-related (Atg) genes, prevents accumulation of harmful cytoplasmic components and mobilizes energetic reserves in long-lived and self-renewing cells. Autophagy deficiency affects antigen presentation in conventional DCs (cDCs) without impacting survival. However, no study addressed the role of autophagy in epidermal Langerhans cells (LCs), which are endowed with proliferative capacities and an extended lifespan. Here, we reveal that both Atg5 or Atg7 deletion in LCs leads to their gradual depletion from the epidermis. Transcriptional analysis of Atg5-deficient LCs showed dysregulation of lipid metabolism pathways. Autophagy-incompetent LCs accumulated neutral lipids within cytoplasm compartments and, despite increased mitochondrial respiratory capacity, they were unable to support ATP production by beta-oxidation. Therefore, autophagy represents a critical regulator for lipid storage and metabolism in LCs, requested for the maintenance of their epidermal immunosurveillance network.

P-33

Human tonsils contain macrophage subsets distinct by their transcriptome, ontogeny and function

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In the mouse, macrophages are a heterogenous population whose identity and function are imprinted by their tissue of residence. By contrast, the heterogeneity of human tissue macrophages remains poorly characterized. Here, we analyzed human tonsil macrophages. Using scRNA-seq, we identified three macrophage subsets, that we validated by flow cytometry. Transcriptomic analysis of purified macrophage subsets revealed distinct transcriptional profiles and ontogeny. While CD36high macrophages displayed evidence of monocyte signature, CD36low macrophages were actively cycling and showed an embryonic macrophage progenitor signature. Using a series of ex vivo assays, we found that CD36high macrophages are specialized in the production of Activin A and the stimulation of T follicular helper cells. To analyze the cross-talk between tonsil macrophages and their micro-environment, we used computational tools to predict ligand-receptor interactions between stromal c ells and each macrophage subset. We showed that CD36high macrophages are primed to respond to TNFa produced by stromal cells, enabling the production of Activin A. Our results provide new insight into the heterogeneity of human lymphoid organ macrophages and extent to humans the concept of macrophage niche.

P-34

Tracking DC-T cell interactions in vivo by proximity dependent enzymatic labeling

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Interaction between dendritic cells (DCs) and T cells is crucial for T cell response. Indeed, molecular messages exchanged in the context of this interaction strongly contribute to define T cell fate. To dissect DC-T cell interactions in vivo, we developed a novel approach, called u-LIPSTIC (universal LIPSTIC), which allows to mark and retrieve interaction partners based on proximity dependent enzymatic labeling. Using this strategy, we characterized the dynamics of antigen dependent and independent interactions between DCs and CD4+ T cells. Our results indicate that DCs-T cell communication occurs in two different ways: initially, in a cognate antigen-driven response, followed by a progressively increasing antigen-independent interaction between cells. We are currently investigating the differential role of these interactions in defining DCs state in vivo.

P-35

Implication of alveolar macrophages migration in lung homeostasis

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Every system and tissue in the human body relies on oxygen. The lungs are the primary organ of the respiratory system in humans and other animals such as mice. When we inhale, we bring dioxygen to every cell in our body and with it comes energy1. And when we exhale, we get rid of the carbon dioxide that is toxic to our body. Dioxygen is brought in through the nose, goes through the trachea, then the bronchi and into bronchioles to finally arrive into small sacs called alveoli. The exchange of gas happens through these alveoli2. They are connected to the vessels that will take up the gas and distribute it to other body tissues. The problem is that when we inhale oxygen, we also inhale the microbes from our environment. Surprisingly, the lungs are mainly microbe-free with no chronic inflammation3. This is where immune cells come in.

Key immune cells in charge of patrolling lung alveoli are Alveolar Macrophages (AM). They internalize inhaled microbes. This can occur by phagocytosis, for example for inhaled bacteria, or by macropinocytosis, which allows uptake of smaller particles such as viruses. Microbe internalization at steady state by AMs is essential to maintain lung homeostasis as it prevents the recruitment and activation of inflammatory cells, such as monocytes and neutrophils, in response to microbe inhalation. Nonetheless, such tolerogenic function of AMs must be tightly tuned as, in case of infection, inflammatory cells must be recruited for adaptive immune responses to be launched and the infection cleared. Understanding how AMs limit lung inflammation without compromising lung immunity, in other words, how AMs control the balance between immunity and tolerance, is therefore essential.

Interestingly, it has been shown that the number of AMs is reduced as compared to the number of alveoli. So, a fair question to ask is how do AMs manage to survey all alveoli in order to limit the entry of microbes? A putative answer to this question has been proposed by a recent study showing that, in the mouse lungs, AMs can move from one alveolus to another, suggesting that only few of them might indeed be required to patrol the lung environment4. To patrol alveoli, AMs must (i) follow the thin fluid films of pulmonary surfactant covering the alveolus wall, and (ii) cross the small holes located in between these structures, which are referred to as pores of Kohn (see figure 1.B)5,6,7. The size of these pores vary from 0.8um to 15um depending on the specie8, implying that AMs, whose diameter is superior, must deform in order to migrate through these structures. Remarkably, such migration events were indeed observed by lung intravital imaging4. These results were unexpected as, in g eneral, mammalian tissue-resident macrophages had been rather considered as adhesive non-migratory cells. The mechanisms underlying AM migration in alveoli and through pores of kohn had therefore not been addressed, nor the impact of migration on the immune-surveillance properties of these cells, i.e. their ability to control the balance between lung immunity and tolerance.

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Yellow fever vaccination induces distinct and overlapping gene expression programs in human blood DC and monocyte subsets

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Yellow fever (YF) virus is a positive-strand RNA virus belonging to the flavivirus family. Vaccination with the live-attenuated vaccine strain (YF-17D) provides life-long protection against infection and is a unique model for studying the immune response to an acute self-limiting RNA virus infection in humans.
We investigated the response of blood dendritic cells (DC) and monocyte subsets to YF-17D in vivo by multi-dimensional flow cytometry and bulk RNA-sequencing of isolated DC and monocyte subsets before and at 3, 7, 14 and 28 days after vaccination to elucidate the early innate immune events which precede the rapid generation of adaptive immunity. We detected transiently increased expression of CD86, PD-L1 and Siglec1 on the surface of all DC (cDC1, cDC2, DC3, pDC, transitional (t)DCs) and monocyte subsets 7 days after vaccination. At the same time, all DC and monocyte populations upregulated a common Interferon-stimulated gene (ISG) signature, (e. g. OAS1, OAS3, RSAD2, IFIT3, IFIT1, EIF2AK2) marking a transient IFN response in the peripheral blood. The transcriptome response of DC3, which have been identified as a pro-inflammatory DC subset with a phenotype and transcriptional profile sharing features of monocytes and cDC2, was more similar to cDC2 than to monocytes. DC3 and cDC2 upregulated a shared set of genes associated with IFN signaling, antigen-processing and cross-presentation. Cell-type specific responses were also detected. Thus, the innate immune response to YF-17D vaccination is marked by concerted activation of all circulating DC and monocyte subpopulations with distinct and overlapping gene expression programs.

P-37

Regulation of IRE1α, an ER stress sensor, in dendritic cells

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UNC93B1, a highly conserved 12-membrane spanning molecule residing in the endoplasmic reticulum (ER), has been identified as a key regulator in the trafficking[1] and folding[2] to endosomes of intracellular Toll-like receptors (TLRs) that detect microbial nucleic acids. Indeed, a mutation in the *Unc93b1* gene (3d mutation) results in inhibition of intracellular TLRs signalling in dendritic cells (DCs) and MHC class I antigen cross presentation[3]. IRE1α is one of the 3 sensor proteins of the Unfolded Protein Response (UPR) which is an adaptive response triggered upon disruption of endoplasmic reticulum (ER) protein homeostasis and whose function is to restore the altered functions of the ER. If ER homeostasis cannot be restored, the UPR then induces pro-apoptotic signals. Regulation of IRE1α activation is not well understood particularly in DCs where it plays a major role in MHC class I antigen cross presentation[4]. We provide evidence that UNC93B1 binds the luminal domain of IRE1α and regulates its function. In DCs bearing the 3d mutation, IRE1α activity is increased and cells are already primed to stress. Furthermore, inhibition of IRE1α activity in 3d DCs restores MHC class I antigen presentation. Altogether, our data highlight the essential role of UNC3B1 in regulating MHCI class I antigen presentation in DCs by controlling IRE1α activity.

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P-38

Single-cell RNA-Seq analysis reveals dual sensing of HIV-1 in blood Axl+ dendritic cells

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Dendritic cells (DC) are heterogeneous and play a dual role regarding HIV-1. They elicit anti-viral immunity but are also key in the establishment and spreading of the infection. Recently, high dimensional analyses revealed the presence of a new DC subset in human blood, named Axl+DC (or Axl+DC or AS DC).
We previously showed that constitutive expression of Siglec-1 on Axl+DC grant them with unique capacities to bind, replicate, and transmit HIV-1. This prompted us to evaluate Axl+DC immune response to HIV-1.
We show that Axl+DC generate a broad and intense response to HIV-1 exposure. Single cell RNAseq allows us to accurately quantify viral transcripts at the single cell level, and to discriminate cells that have only fused with the virus from cells productively infected. scRNAseq analysis further revealed that HIV-1 induced two main transcriptional programs in different Axl+DC; a NF-KB-mediated program that led to DC maturation and efficient antigen-specific CD4+T cell activation, and a program mediated by STAT1/2 that activated type I IFN and an ISG response. Finally, cells producing new viruses exhibit a higher ISG response that may result from post-integration sensing mechanisms.
Our results suggest that the dual response observed in Axl+DC exposed to HIV-1 probably results from different sensing mechanisms involving STING and TLRs, rather than heterogeneity of the Axl+DC population. Axl+DC exposed to HIV-1 possess a higher capacity to promote antigen driven expansion and IL2 secretion of naïve CD4+ T cells. Axl+DC therefore stands out among blood DC populations for its multifaced relationship with HIV-1.

P-39

A single cell landscape of human dendritic cells in health and disease

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Dendritic cells (DC) are professional antigen presenting cells and comprise a growing number of cell subsets. They have until recently included plasmacytoid DC (pDC) that are highly specialized in type I interferon production, but these later develop from lymphoid progenitors and do not efficiently present antigens, contrary to conventional DCs (cDC) that arise from pre-DC. While cDC were initially classified into cross-presenting cDC1 and CD4 T cell-activating cDC2, these later were recently split into DC2 and DC3 (also called cDC2A and cDC2B, respectively). Furthermore, following their activation, both cDC1 and DC2/3 become “mature DCs enriched in immunoregulatory molecules” [mregDC also called migratory DC (migrDC), LAMP3 DC or DC3 by certain groups]. All these DC subsets are now routinely defined in published single cell RNA sequencing (scRNAseq) datasets using various nomenclatures which is overwhelming the field. Here, through the integration of in house and published scRNAseq studies, we demonstrate that spleen cDC2A comprise CD5+ DC2 and a subset of spleen-specific LTB+ DC2, while cDC2B correspond to CD5- DC3. We next integrated 38,293 DC from 13 tissues across 41 datasets to generate a DC single-cell RNA compendium (DC-VERSE). While the ratio of “inflammatory DC3”/DC2 was increased in most tumours, IFN-primed and CD207+ DC2 accumulated in the 11 analysed cancers. Furthermore, CD207+ DC2 accumulated only in non-T cell infiltrated breast tumours. This observation paves the way to the investigation of the potential role of CD207+ DC2 in the inhibition of T cell infiltration in human tumours, a hallmark of tumour progression.

P-40

Natural killer cells license type 1 conventional DC for accelerated cross-priming of antiviral CD8 T cells

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We previously demonstrated that efficient natural killer (NK) cell responses early during murine cytomegalovirus (MCMV) infection accelerate the induction of anti-viral CD8+ T cell responses (Robbins et al. PLoS Pathog. 2007. doi: 10.1371/journal.ppat.0030123). Type 1 conventional dendritic cells (cDC1) excel in CD8+ T cell cross-priming. We contributed to show that, in a conserved manner in mice and humans, cDC1 specifically express the chemokine receptor Xcr1, whose ligand Xcl1 is selectively expressed by cytotoxic lymphocytes including NK and CD8+ T cells (Crozat et al. J Exp Med. 2010. doi: 10.1084/jem.20100223). Here, we investigated whether and how the Xcr1/Xcl1 signaling axis contributed to the cross-talk between NK cells and cDC1 during MCMV infection in a manner promoting downstream adaptive immunity. This was achieved by combining a unique collection of reporter and knockout mice with kinetics quantitative confocal microscopy analyses and functional assays. We showed that, early after systemic MCMV infection, splenic cDC1 clustered with activated NK cells in the marginal zone. This XCR1-dependent repositioning of cDC1 allowed them to deliver IL-12 and IL-15 locally to enhance NK cell responses. In turn, NK cells delivered granulocyte-macrophage colony-stimulating factor to cDC1, leading to their upregulation of CCR7 and their consecutive migration into the T cell zone. This XCR1- and CCR7-dependent licensing of cDC1 then accelerated antiviral CD8+ T cell responses. The combined perturbation of antiviral NK and CD8+ T cell responses occurring in Xcr1-KO mice strongly compromised virus control. This study revealed a novel mechanism through which cDC1 bridge innate and adaptive immunity.

P-41

Differential response of human myeloid and plasmacytoid dendritic cells to Saccharomyces cerevisiae

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Saccharomyces cerevisiae is a commensal yeast colonizer of mucosal surfaces. The role of S. cerevisiae has been largely characterized in monocyte derived dendritic cells, where yeast cells induce production of inflammatory cytokines through the interaction with mannose receptors, chitin receptors, DC SIGN, and dectin1. However, the response of blood circulating dendritic cells (DC) to S. cerevisiae has never been investigated. Among blood DC, myeloid DC (mDC) are producers of inflammatory cytokines, while plasmacytoid dendritic cells (pDC) are a specialized population producing large amount of interferon (IFN)-α, which is involved in antiviral immune response. Here, we report that both human DC subsets are able to sense laboratory and natural strains of S. cerevisiae. In particular, mDC express activation markers, produce IL-6, and promote Thelper 17 polarization in response to yeasts, behaving similarly to monocyte derived DC, previously described. I nterestingly, pDC sense fungal nucleic acids, which interact with Toll Like Receptor (TLR) 7 and 9 leading to generation of P1-pDC (PD-L1+CD80–) subset, characterized by the production of IFN-α and the polarization of CD4 naïve T cells towards a Th profile producing IL-10. These results highlight a novel role of pDCs in response to commensal S.cerevisiae, and this interaction could be important for the regulation of the host microbiota-immune system balance.

P-42

The p84/p110g complex of PI3Kg regulates NOX2 assembly and cross-presentation of immune complexes

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Antibody-mediated cross-presentation is known to elicit strong CD8+ T cell responses, particularly relevant in cancer therapy. Anti-tumor antibodies kill rapidly cancer cells by antibody-dependent cellular cytotoxicity (ADCC), but the long-term immune control of tumors is CD8+ T cell-dependent and relies on anti-tumor antibody-mediated cross-presentation. The molecular mechanisms that allow poor cross-presenting cells, such as type 2 dendritic cells (DCs) and monocyte-derived DCs (moDCs), to efficiently cross present antigens are not well understood. Here we demonstrate that the enzymatic activity of p84/p110γ complex of PI3Kγ regulates the assembly of the NADPH oxidase NOX2 and the ROS production in murine splenic type 2 DCs and bone marrow derived DCs, enhancing thus the cross-presentation of immune complexes by these cells. Our results suggest that the combination of anti-tumor monoclonal antibodies and PI3Kγ inhibitors might block the antibody- mediated cross-presentation of tumor antigens and should be avoided in clinical therapy of cancers.

P-43

PreDC subsets characterization among the cynomolgus macaque model and study of their dynamics during a SIV / HIV infection

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Dendritic cells include several discrete populations that play an important role in the shaping of immune responses leading to viral control or persistence. In this regard, previous reports show that HIV infection induces dysregulation of dendritic cells.
Moreover, recent in vitro studies demonstrated that a new subset called pre-DCs is highly prone to HIV infection. However, the interplay between pre-DCs and HIV infection is not characterized in vivo, yet.
Here, we investigated the dynamics of dendritic cell subsets with a focus on pre-DCs during the course of HIV infection. For this purpose, we used a macaque model of infection by SIVmac251 strain and developed a flow cytometry strategy to simultaneously identify pre-DCs and other DC subsets in this specie. Thanks to this approach we could characterized the dynamics of pre-DCs in blood and tissues in early and late phase of SIV infection. Moreover, we investigated the impact of anti-retroviral treatments on the dynamics of pre-DCs. Overall, our data should bring a better understanding of the role of dendritic cells in the pathophysiology of HIV infection

P-44

Investigating the effect of obesity-induced acute and chronic inflammation on dendritic cell development and function

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Conventional dendritic cells (cDCs) are professional antigen-presenting cells and a crucial interface between innate and adaptive immunity. cDC subsets (cDC1 and cDC2) development and function are influenced by external factors present within their local microenvironment. However, how acute and chronic inflammation modulate the development and function of the cDC compartment in the context of diet-induced obesity remain largely elusive.

Here, in order to study the impact of high fat diet-induced inflammation on DC development and function overtime, we first analysed the effect of high-fat diet feeding for 3 and 8 weeks on murine mature cDC and DC progenitors frequency in different tissues. We found that acute and chronic exposition to HFD result in a profound loss of XCR1+ cDC1s in the bone marrow, spleen and adipose tissue. Within the bone marrow, the frequency of XCR1+ cDC1 committed pre-DCs was significantly reduced, whereas an increase of the common dendritic cell progenitor was detected as early as 3 weeks of HFD exposure, indicating a rewiring of cDC1 development in response to short-term HFD feeding. Subsequently chronic exposure to HFD resulted in a marked downregulation of pre-cDC1 proliferation and IRF8 expression, resulting in a loss of lineage commitment.

These data shed light on an obesity-induced regulatory mechanism affecting cDC1 development and function, which has to be studied further in regards to pathology progression. Further studies will define the transcriptional and functional features of cDC1s and their progenitors in bone marrow and peripheral adipose tissue and their role during acute and chronic obesity-induced inflammation.