PROGRAM & ABSTRACT BOOK

French Dendritic Cell Society biennial meeting - 2023

" Development, metabolism and function of dendritic cells and macrophages in health and disease "

December 7th – 8th 2023, Université de Bordeaux Pey Perland, France

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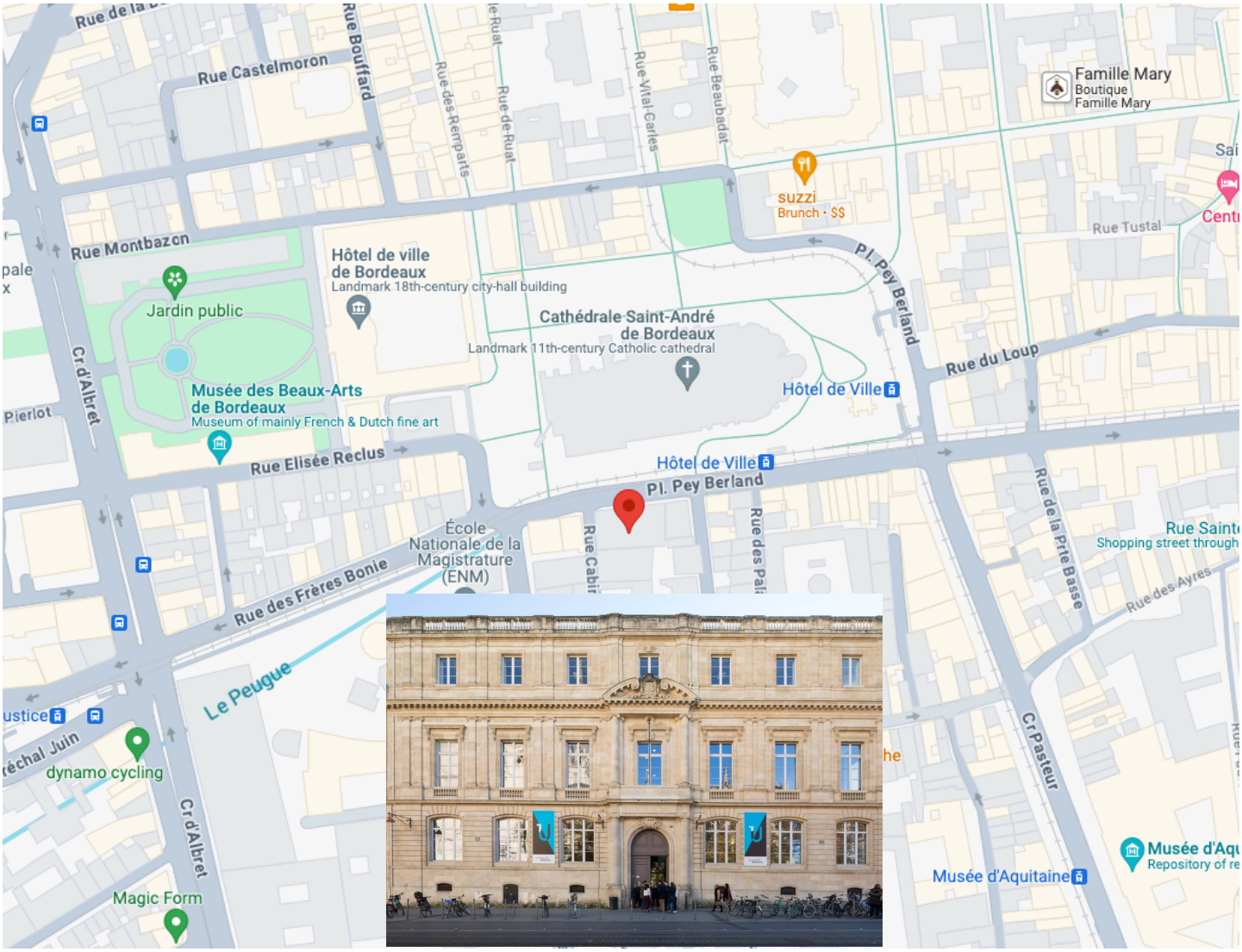
VENUE

The biennial French DC Society meeting of 2023 will take place in the heart of Bordeaux

Location : Université de Bordeaux Pey Berland

Address : 35 Place Pey Berland, 33000 Bordeaux

How to come: Bordeaux is a small city so you can easily walk from your hotel to the conference venue. You can also take the Tramway A or B and stop at the station Hotel de Ville



PROGRAM

Thursday December 7th, 2023

8:30 **Opening of the registration desk**

9:00 **Welcome address** *Bénédicte Manoury*

SESSION I – DCs and macrophages in anti-tumor immunity
Chaired by Julie Helft & Paula Michea-Veloso

9:15-9:40 **Caetano Reis e Sousa *(The Francis Crick Institute, London, UK)***
Development and function of dendritic cells

9:40-10:05 **Stefani Spranger *(Massachusetts Institute of Technology, Cambridge, USA)***
Not all T cell responses are equal: how dendritic cells shape anti-tumor immunity

10:05-11:05 **Selected oral presentations (1st part)**

10:05. Emmanouil Aerakis *(BSCR Vari, Greece)*

Interferon-induced lysosomal membrane permeabilization and death cause cDC1-deserts in tumors

10:20. Maria Koufaki (*CR UK, Manchester, UK*)

Functionally decoding the enigmatic "mregDCs": guardians or saboteurs of tumour immunity?

10:35-11:00  Coffee Break

11:00-11:30 **Selected oral presentations (2nd part)**

11:00 Pauline Santa *(Immunoconcept, Bordeaux, France)*

Functions of DNASE1L3 in the regulation on anti-tumor immune responses.

11:15 Guillaume Darrasse-Jeze (*i3, Pitié Salpêtrière, Paris, France*)

FLT3L-dependent dendritic cells control tumor immunity by modulating Treg and NK cell homeostasis

11:30-11:55 **Massimiliano Mazzone *(VIB KU Center for Cancer Biology, Leuven, Belgium)***
Harnessing tumor metabolism to overcome immunosuppression: A novel way to enhance the success of cancer immunotherapy

11:55-12:20 **Mikaël Pittet *(School of Medicine, Geneva, Switzerland)***
Microenvironmental coordination in human cancers

12:20-13:05 Lunch talks by Sponsors

- **12:20-12:35 William Amoyal** (VIZGEN)
  Highly multiplexed, multi-omic tissue mapping at single-cell resolution with the MERSCOPE spatial biology platform
- **12:35-12:50 Ali Tebbi** (AKOYA Biosciences)
  Scaling up deep Spatial Phenotyping with a novel Omics approach
- **12:50-13:05 Thibaud Metzger** (NanoString)
  Beyond the Slide: Revolutionizing Spatial Profiling with NanoString Technology

13:05-15:00 LUNCH and Poster viewing

SESSION II – Multi-omics approaches of DC and macrophage development
Chaired by Florent Ginhoux & Jérôme Martin

15:00-15:25 **Matthew Collin *(Translational and Clinical Research Institute, Newcastle, UK)***
Macrophages and dendritic cells in human graft versus host disease

15:25-15:50 **Shalin Naik *(The Walter and Eliza Hall Institute of Medical Research, Australia)***
The development of DCs in steady-state and emergency

15:50-16:35 **Selected oral presentations**

15:50. Thomas Laurent *(CR2TI, Nantes, France)*
Combinatorial analysis of MNP molecular programs in Crohn disease uncovers inflammatory states associated with anti-TNF resistance

16:05. Nathan Vaudiau *(Institut Pasteur, Paris, France)*
Distinct migratory dendritic cell subsets cooperate to promote tissue-resident memory $CD8^+$T cells specification in tumor-draining lymph nodes

16:20. Cécile Piot *(Francis Crick Institute, London, UK)*
Heterogeneity and spatiotemporal dynamics of tumour cDC1s

16:35-17:00 

Coffee Break

17:00-17:25 **Julie Giraud *(ImmunoConcEpT, Bordeaux, France)***
TREM1+ CD163+ Myeloid Cells are Potent Immunosuppressive Cells and Associate with Poor Clinical Factors in Human Hepatocellular Carcinoma

17:25-17:50 
Ido Amit *(Weizmann Institute of Science, Rehovot, Israël)*
The power of ONE: Immunology in the age of spatial and single cell genomics

18:00 
Cocktail

Friday December 8th, 2023

8:30 Opening of the registration desk

SESSION III – Role of metabolism in the function of DCs and macrophages
Chaired by Johan Garaude & Fabien Blanchet

9:00-9:25 **Rafael Argüello *(Centre d'Immunologie de Marseille-Luminy, Marseille, France)***
DC subsets and their metabolic dependencies: You are what you eat? or You eat what you are?

9:25-9:50 **Sophia Maschalidi *(VIB Center for Inflammation Research, Ghent, Belgium)***
Dendritic cells and Solute Carrier Transporters in apoptotic cell clearance and tissue repair

9:50-10:35 **Selected oral presentations**

9:50. Vincent Flacher *(I^2CT, IBMC, Strasbourg, France)*

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

10:05. Stefanie Wculek *(IRB, Barcelona, Spain)*

Mitochondrial metabolism regulates the immunogenic responsiveness of dendritic cells

10:20 Nathalie Bendriss-Vermare *(CRCL, Lyon, France)*

Conventional Dendritic cells drive the generation of polyfunctional and antitumor ST2+ NK cells

10:35-11:00  Coffee Break

11:00-11:25 **Evanna Mills (*Dana Farber Cancer Institute, Boston, USA)***
Metabolite signaling in macrophages during health and disease

11:25-11:50 **Giulia Pasqual (*Università degli Studi di Padova, Padova, Italy)***
Dendritic cell-T cell interactions in immunity and tolerance

11:50-12:20 Lunch talks by Sponsors

- **11:50-12:05** **Anis Larbi** (Beckman Coulter)
  Extracellular vesicles (EVs) in the immunobiology of dendritic cells and macrophages

- **12:05-12:20** **Olivier Jaen** (Cytek)
  Analysis of Dendritic Cell subsets by Spectral Flow Cytometry and Imaging

12:20-14:15 LUNCH and Poster viewing

14:15-14:30 **CFCD General Assembly**

SESSION IV – Tissue-specific functions of DCs and macrophages
Chaired by Helena Paidassi & Vanja Sisirak

14:30-14:55 **Ruth Franklin *(Harvard Stem Cell Institute, Boston, USA)***
Regulation of inflammatory responses by macrophages in the lung

14:55-15:20 **Bart Lambrecht *(VIB Center for Inflammation Research, Ghent, Belgium)***
Biology of DC's and macrophages in the lung.

15:20-16:05 **<u>Selected oral presentations</u>**

15:20. Sébastien This *(GCI, Montreal, Canada & CIRI, Lyon France)*

Back dans les bacs: rehabilitating the contribution of cDCs subsets in the maintenance of mucosal tolerance

15:35. Fabiola Osorio *(ICBM, Santiago, Chile)*

Control of intestinal Th17 homeostasis by the unfolded protein response sensor IRE1 in myeloid cells

15:50. Katrina Podsypanina *(INEM, Paris, France)*

Toll-like receptor 9 dependent dendritic cells-NK cooperation in anti-tumor immune control

16:05-16:30  Coffee Break

16:30-16:55	**Ana-Maria Lennon *(Institut Curie, Paris, France)*** *Dendritic Cell Migration: from basics to application*
16:55-17:20	**Camille Bigenwald *(Institut Gustave Roussy, Villejuif, France)*** *Senolysis in Histiocytosis*
17:20	**Award ceremony and concluding remarks**
17:40	**End of the meeting**

SHORT TALK ABSTRACTS

SESSION I – DCs and macrophages in anti-tumor immunity

Short Talk - 1

Interferon-induced lysosomal membrane permeabilization and death cause cDC1- deserts in tumors.

Emmanouil Aerakis[1,2], M. Alvanou[1], D. Koumadorakis[1], A. Chatzigeorgiou[1], M. Matthaiakaki-Panagiotaki[1], A. Galaras[3,4], P. Hatzis[3], D. Kerdidani[1,5], M. Makridakis[6], A. Vlachou[6], B. Malissen[7], S. Henri[7], M. Merad[8,9,10,11] and M. Tsoumakidou[1,2].

[1]. Institute of Bioinnovation, BSRC Alexander Fleming, Vari, Greece. [2]. Department of Physiology, Medical School, National and Kapodistrian University of Athens, Greece. [3]. Institute for Fundamental Biomedical Research (IFBR), Biomedical Sciences Research Center "Alexander Fleming". [4]. Department of Biochemistry and Biotechnology, University of Thessaly, Larissa Greece. [5]. Immune Regulation Laboratory, Center of Basic Research, Biomedical Research Foundation Academy of Athens, 11527 Athens, Greece. [6]. Center of Systems Biology, Biomedical Research Foundation, Academy of Athens, 15527 Athens, Greece. [7]. Centre d'Immunologie de Marseille-Luminy, Aix Marseille Université, INSERM, CNRS, Marseille, France. [8]. The Precision Immunology Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [9]. The Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [10]. Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [11].Human Immune Monitoring Center, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

T cell immunity requires antigen capture by conventional dendritic cells (cDCs), digestion and transfer to draining lymph nodes for presentation to antigen-inexperienced T cells. cDCs type I excel as cancer-antigen presenting cells, due to their ability prime both CD8 and CD4 T cells. In tumor tissues cDCs1 become particularly scarce and this restricts anti-tumour immunity, immunotherapy responses and patient survival. Tumor cDC1 paucity is not fully understood and no specific treatment currently exists. Here, we find that type I interferons (IFN) induce lysosomal stress, lysosomal membrane permeabilization (LMP) and lysosomal-dependent cell death (LDCD) in cDCs1. Two parallel pathways downstream of IFNAR1 converged to induce cDC1 LDCD. Up-regulation of expression of lysosomal genes driven by Stat1 and Irf7 enhanced the proteolytic activity of lysosomes, while IFN-inducible guanylate binding protein-2 (GBP-2) accumulated in the membrane of the stressed lysosomes, leading to LMP, proteolytic enzyme release and death. Protease inhibition or GBP-2 repression rescued cDCs1 from LDCD and boosted their anti-tumor efficacy. GBPs are amongst the most abundant IFN induced genes and known to form toxic pores in pathogen containing vacuoles and pathogen membranes. GBP-2-driven LMP is likely due to the ability of GBP-2 to form pores on the lysosomes of cDC1s. We anticipate our findings to be a starting point for more rational cDC1-directed immunotherapies. For instance, protease inhibition, GBP-2 downregulation or induced expression of LMP repair machinery may boost cDC1 efficacy in adoptive cell therapies or their use as live vaccines.

Short Talk - 2

Functionally decoding the enigmatic "mregDCs": guardians or saboteurs of tumour immunity?

Maria A. Koufaki[1], Eduardo Bonavita[1], Agrin Moeini[1], Eimear Flanagan[1], Charlotte R. Bell[1], Natalia Moncaut[2] and Santiago Zelenay[1]

1. Cancer Inflammation and Immunity, Cancer Research UK Manchester Institute, University of Manchester, Manchester, UK 2. Genome Editing and Mouse Models, Cancer Research UK Manchester Institute, University of Manchester, Manchester, UK

Conventional dendritic cells (cDCs) constitute crucial orchestrators of adaptive immune response against tumours. Recent single cell transcriptional analyses have unraveled a cluster of tumour infiltrating cDCs which cannot be classified to the previously described cDC1 and cDC2 subsets. Their transcriptional profile suggests they represent an activated state of cDCs (referred to herein as actDCs a.k.a. mregDCs, mDCs, or cDC3s) with putative opposing T cell stimulating or inhibitory roles. Due to their intratumoural scarcity and the lack of appropriate experimental tools, the actual contribution of actDCs to tumour immunity remains unknown. Here, we show that actDCs, transcriptionally and phenotypically reminiscent of their tumour-infiltrating in vivo counterparts, can be found within bone marrow-derived cDC cultures in vitro, especially following co-culture with cancer cells. Furthermore, using intersectional genetics we here describe new genetically-engineered mouse models to conditionally and selectively label, isolate or deplete actDCs. Using these novel tools, we show that actDCs derived from both cDC1s and cDC2s similarly and efficiently crosspresent cancer cell-associated antigens but, unexpectedly, actDC2s display a markedly superior ability to drive effector CD8 T cell-differentiation. Crucially, we demonstrate that specific and acute depletion of actDCs impairs T cell priming and tumour eradication in vivo. Overall, our work formally establishes an essential, non-redundant contribution of actDCs to T cell-mediated, anti-cancer immunity.

Short Talk - 3

Functions of DNASE1L3 in the regulation on anti-tumor immune responses.

Pauline Santa[1], Aliki Vasilakou[1,2], Séverine Loizon[1], Anne Garreau[1], Anaïs Roubertie[1], Dorothée Duluc[1] and Vanja Sisirak[1]

[1]. Immunoconcept, CNRS UMR 5164, Bordeaux University, Bordeaux 33000, France [2]. Cancer Biology Graduate Program, UB Grad 2.0, University of Bordeaux

Tumor DNA (tDNA) is crucial in the induction of anti-tumor immunity, by stimulating dendritic cells' (DCs) production of type I interferons, which subsequently activate tumor eliminating CD8 T lymphocytes. Chemotherapy (CT)/radiotherapy (RT) promote the release of tDNA and consequently "boost"" anti-tumor immunity. However, the mechanisms involved in the regulation of the immunostimulatory potential of tDNA remain poorly understood. The endonuclease DNASE1 Like 3 (DNASE1L3) which is produced by DCs, is known to degrade DNA released by dead and dying cells, limiting its ability to activate aberrant immune responses. Given its DC-restricted expression and function in DNA digestion, we investigated the role of DNASE1L3 in cancer. We observed that Dnase1l3 deficiency did not affect mammary tumor growth in spontaneous or transplantable tumor models. However, the absence of DNASE1L3 inhibited the therapeutic efficacy of CTs in both tumor models. We next showed that DNASE1L3 specifically fragments tDNA released by tumor cells in response to CTs and that this fragmented tDNA is more efficient than the unfragmented one to induce human DCs activation in vitro. Finally, murine DCs that were deficient for Dnase1l3 were shown to be impaired in their ability to produce inflammatory cytokines in response to DNA triggering TLR9 stimulation. Altogether, our work suggests that DNASE1L3 is not only disposing of DNA originating from dying cells. In the context of cancer DNASE1L3 likely processes tDNA released upon CT to make it amenable to stimulate anti-tumor immunity. Therefore, DNASE1L3 may be used in conjunction to preexisting cancer therapies to enhance their efficacy.

Short Talk – 4

FLT3L-dependent dendritic cells control tumor immunity by modulating Treg and NK cell homeostasis

Paul Régnier,[1,2,3] Mathias Vetillard,[4,5] Adèle Bansard,[1,6] Eméranne Pierre,[6] Xinyue Li,[2] Nicolas Cagnard,[7] Emmanuel L. Gautier,[8] Pierre Guermonprez,[4,5] Bénédicte Manoury,[1] Katrina Podsypanina,[1,9] and **Guillaume Darrasse-Jèze**[**1**,2,6]

[1] Institut Necker Enfants Malades, INSERM U1151, CNRS UMR-8253, UniversitéParis Cité, Paris, France , [2]Sorbonne Université, INSERM, UMR_S959, Immunology-Immunopathology-Immunotherapy, Paris, France, [3]AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Department of Internal Medicine and Clinical Immunology, DMU3ID, Paris, France [4]Université de Paris Cité, Centre for Inflammation Research, INSERM U1149, CNRS ERL8252, Paris, France, [5]Dendritic Cells and Adaptive Immunity Unit, Institut Pasteur, Paris, France, [6]Université Paris Cité, Faculté de Médecine, Paris, France, [7]Structure Fédérative de Recherche Necker, Université Paris Descartes, Paris, France
[8]Inserm, UMR_S1166, Sorbonne Université, Hôpital Pitié-Salpêtrière, Paris, France
[9]Institut Curie, PSL Research University, CNRS, Sorbonne Université, UMR3664, Paris, France

FLT3-L-dependent classical dendritic cells (cDCs) recruit anti-tumor and tumor-protecting lymphocytes. We evaluated cancer growth in mice with low, normal or high levels of cDCs. Paradoxically, both low or high numbers of cDCs improve survival in mice with melanoma. In low cDC context tumors are restrained by the adaptive immune system through influx of activated effector T cells and decrease of Tregs and NK cells. High cDC numbers favor the innate anti-tumor response, with massive recruitment of activated NK cells, despite high Treg infiltration. Anti CTLA-4 but not anti PD-1 therapy synergizes with FLT3-L therapy in the cDC^{Hi} but has no effect in the cDC^{Lo} context, hinting that, in this model, Tregs and not Teffs are the main target of CTLA-4 ICBT. Combination of cDC boost and Treg depletion dramatically improves survival of tumor-bearing mice. We then examined Flt3-L, cDC1, cDC2 & pDCs gene signatures in the biopsies of untreated cancer patients with 42 types of cancer and identified a beneficial, adverse or paradoxical effect of these signatures in 35 types of human cancers. Thus, transcriptomic data confirm the paradoxical effect of cDC levels on survival in several human tumor types. cDC^{Hi}-$Treg^{Lo}$ state in such patients predicts best survival. These results have important implications for improving immunotherapy in specific types of cancer.

SESSION II – Multi-omics approaches of DC and macrophage development

Short Talk - 5

Combinatorial analysis of MNP molecular programs in Crohn disease uncovers inflammatory states associated with anti-TNF resistance

Thomas Laurent (1), Ephraim Kenigsberg(2), Aurélie Joussaume(1), Gaëlle Bériou(1), Monika Mykhaylyshyn(1), Nicolas Chapelle(1), Lucas Brusselle(1), Camille Lécuroux(1), Laurence Delbos(1), Jeremie Poschmann(1), Cynthia Fourgeux(1), Arnaud Bourreille(3), Catherine Le Berre(3), Caroline Trang(3), Theo Soude(3), Juliette Podevin(3), Jean Francois Mosnier(4), Cécile Girard(4), Miriam Merad(2), Samarth Hedge(2), Clotilde Hennequin(2), Jessica Le Berichel(2), Saurabh Mehandru(2), Pablo Canales-Herrerias(2) and Jérôme Martin(1)

(1) CHU Nantes, Nantes Université, INSERM, Center for Research in Transplantation and Translational Immunology, UMR 1064, ITUN5, F-44000 Nantes, France. (2) Precision Institute of Immunology, Icahn School of Medicine at Mount Sinai, New York, NY, USA. (3) CHU Nantes, Institut des Maladies de l'Appareil Digestif, F-44000 Nantes, France. (4) Service Anatomie et Cytologie Pathologiques, CHU Nantes, F-44000 Nantes, France.

Crohn's Disease (CD) is a disabling inflammatory bowel disease (IBD) associated with severe complications like fibrostenosis, often leading to surgical resection. Anti-TNF therapy has transformed CD clinical care but up to 40% of patients never respond. Using single-cell technologies, we previously described a cellular response enriched in inflamed CD ileums of a subset of patients associated with anti-TNF resistance. We named it GIMATS (IgG plasma cells, Inflammatory Mononuclear phagocytes, Activated T cells and Stromal cells) and predicted dysregulated actions of monocytes/macrophages as central drivers. While current evidences indicate that CD pathogenic macrophages could emerge from recently infiltrated blood monocytes, their nature, diversity and distribution remain unclear. To resolve their molecular heterogeneity, we FACS-sorted total MNP populations and analyzed by single-cell RNA sequencing the MNP-enriched suspensions from inflamed mucosa of 7 patients with stricturing ileum. Using Metacells-2 and gene module-based analyses, we characterized 17 gene programs, defining 10 molecular subgroups of monocytes/macrophages with distinct inflammatory and metabolic features. Applying these programs to a larger cohort, we characterized a molecular state uniquely defining monocyte-like cells in GIMATShigh patients. Using TF motif enrichment analyses and pathway activity inference, we predicted GIMATS-associated monocytes could be targeted by upadacitinib, a JAK inhibitor recently approved, to treat active CD patients with inadequate response to TNF blockers. We supported this hypothesis by exposing blood classical monocytes to conditions predicted to drive GIMATS-associated inflammatory monocytes. In summary, our data identify a unique molecular state of monocyte-like cells enriched in GIMATShigh patients, which is targetable with JAK inhibitors.

Short Talk - 6

Distinct migratory dendritic cell subsets cooperate to promote tissue-resident memory CD8+T cells specification in tumor-draining lymph nodes.

Nathan Vaudiau[1,2,3,*], Pierre Bourdely[3,4,*], Agathe Ok[3,4], Roberto Savoldelli[3], Yohan Gerber-Ferder[4], Mathias Vétillard[1,2,3], Louise Gorline[1,2,3], Fillipe Luiz Rosa do Carmo[1,2,3], Aurélie Semervil[1,2,3,4], Guillaume Darrasse-Jèze[5], Emmanuel L Gautier[6], Marc Dalod[7], Eric Tartour[8], Loredana Saveanu[3], Kairbaan M Hodivala-Dilke[9], Klaas P J M Van Gisbergen[10], Julie Helft[4], Federica Benvenuti[11], Pierre Guermonprez[1,2,3]

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Tumor infiltration by CD8+ T cells endowed with a tissue-resident phenotype (TRM) is generally associated to favorable outcomes in human tumors. Here, we delineate the requirements for migratory dendritic cells (DCs) during the activation of tissue-resident and effector cells within tumor-draining lymph nodes. We identify a discrete subset of activated CXCR6+CD103+ CD8+ T cells aligning with TRM in the lymph nodes. These cells are generated in tumor-draining lymph nodes in a process dependent on both XCR1+ migratory DC1s and IRF4-dependent migratory DC2s. By contrast, the wide majority of PD1- effector and PD1+TIM3+ exhausted cells solely rely on XCR1+ DC1s for their activation. Mechanistically, IRF4-dependent DC2s are less efficient at driving T cell proliferation but maintain CD103 expression during T cell activation, thereby enabling TRM specification. Altogether, these findings highlight the fine tuning of T cell fate and memory formation by the cooperation of multiple DCs subsets within draining lymph nodes.

Short Talk - 7

Heterogeneity and spatiotemporal dynamics of tumour cDC1s

Cécile Piot[1], Mariana Pereira da Costa[1], Carlos M. Minutti[1], Mary Green[2], Jonathan Lim[1], Evangelos Giampazolias[1], Michael Buck[1], Ana Cardoso[1], Bruno Frederico[1], Neil Rogers[1], and Caetano Reis e Sousa[1]

[1]Immunobiology laboratory, Francis Crick Institute, London NW1 1AT, UK. [2] Experimental Histopathology, Francis Crick Institute, London NW1 1AT, UK

Cytotoxic CD8+ T cells are central to the anti-tumour response, but their activity depends on adequate priming by type 1 conventional dendritic cells (cDC1s) in lymph nodes (LNs). Recent studies have shown that cDC1s play additional roles within the tumour itself, suggesting that different spatiotemporal behaviours of cDC1s are important for anti-tumour immunity. Using the CytoMAP image analysis pipeline to identify patterns of tissue organization, we find that cDC1s localize in two main regions during anti-cancer responses: at the tumour border, where they cluster with CD8+ T cells, and in the stroma where they make sparse clusters. Spatiotemporal analysis of intra-tumoural cDC1s by inducible lineage tracing showed that some cDC1s remain in tumours long-term and localise preferentially at the tumour border. We performed scRNAseq to uncover how cDC1 heterogeneity in tumours links with these spatiotemporal features. We identified two distinct states of cD C1 activation, marked by increased Cxcl9 or Il12b/Ccr7 expression respectively. Using RNAscope, we found that that Cxcl9 and Il12b cDC1s mapped to different regions of the tumour, accumulating preferentially at the tumour parenchyma and in the stroma, respectively. Finally, IL- 12p40/CCR7 but not CXCL9 cDC1s were associated with antigen uptake and activation of the noncanonical NF-kB pathway, while IFNGR signalling was necessary for CXCL9 expression by cDC1s. Further understanding of these cDC1 activation programs and spatiotemporal dynamics will help uncover the roles of cDC1s in cancer.

SESSION III – Role of metabolism in the function of DCs and macrophages

Short Talk - 8

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

Florent Arbogast[1,2#], Raquel Sal-Carro[1#], Wacym Boufenghour[1], Quentin Frenger[2,9], Delphine Bouis[1], Louise Filippi De La Palavesa[1], Jean-Daniel Fauny[1], Olivier Griso[4], Hélène Puccio[4], Rebecca Fima[5], Thierry Huby[5], Emmanuel L. Gautier[5], Anne Molitor[3], Raphaël Carapito[3,6], Seiamak Bahram[3,6], Nikolaus Romani[7], Björn E. Clausen[8], Benjamin Voisin[1], Christopher G. Mueller[1], Frédéric Gros[1,2,9*], **Vincent Flacher**[1*]

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Macroautophagy (often-named autophagy), a catabolic process involving autophagy-related (Atg) genes, prevents accumulation of harmful cytoplasmic components and mobilizes energy reserves in long-lived and self-renewing cells. Autophagy deficiency affects antigen presentation in conventional dendritic cells (DCs) without impacting their survival. However, previous studies did not address epidermal Langerhans cells (LCs), a proliferating skin DC subset with extended lifespan. Here, we demonstrate that deletion of either Atg5 or Atg7 in LCs leads to their gradual depletion. ATG5- deficient LCs showed metabolic dysregulation and accumulated neutral lipids. Despite increased mitochondrial respiratory capacity, they were unable to process lipids, eventually leading them to ferroptosis. Metabolically impaired LCs upregulated proinflammatory transcripts and showed decreased expression of neuronal interaction receptors, in line with a reduction of epidermal nerves upon LC depletion. Altogether, autophagy represents a critical regulator of lipid storage and metabolism in LCs, allowing their maintenance in the epidermis.

Short Talk - 9

Mitochondrial metabolism regulates the immunogenic responsiveness of dendritic cells

Stefanie K. Wculek[1,2 †*], Ignacio Heras-Murillo1[†], Diego Mañanes[1], Annalaura Mastrangelo[1], Josep Calafell[3], Navdeep S. Chandel[4], Esteban Ballestar[3,5], David Sancho[1*]

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Dendritic cells (DCs) are antigen presenting cells that control T cell responses upon immunogenic activation. This is characterized by a metabolic reprogramming of DCs to elevate glycolysis. However, the relevance of mitochondrial metabolism and the electron transport chain (ETC) for the activation of conventional type 1 and 2 DC subsets (cDC1 and 2) is poorly defined. Here, we revealed that the immunogenic stimulation of cDC1s causes a rapid and sustained increase in their oxygen consumption, which is absent in cDC2s. To investigate the importance of this, we generated mice deficient for a component of the ETC complex III specifically in DCs. Indeed, the upregulation of costimulatory molecules, migratory capacity, ability to generate a T cell effector response and control cancer growth was notably impaired in complex III-deficient versus control splenic cDC1s upon activation, but only mildly affected in cDC2s. Mechanistically, the immunogenic functions of complex III-deficient splenic cDC1s were rescued by re-establishing electron flow through the ETC, but not ATP production, via transgenic expression of alternative oxidase in vivo. This suggests that a metabolic deregulation and not a bioenergetic crisis underlies the functional impairment of complex III-deficient cDC1s. In line, loss of complex III in splenic cDC1s, but not cDC2s, led to DNA hyp ermethylation in binding regions of the transcription factor PU.1, which is regulating DC activation. This caused impaired expression of early stimulus-induced genes in complex IIIdeficient cDC1s already in the steady state and hampered their timely immunogenic responsiveness. In conclusion, our findings manifest the importance and DC subset-specific requirement of a functional mitochondrial metabolism for the immunogenic activity of natural DCs.

Short Talk - 10

Conventional Dendritic cells drive the generation of polyfunctional and antitumor ST2+ NK cells

Valentin Picant[1], Lara Revol-Bauz[1], Yamila Rocca[1], Anaïs Eberhardt[1], Aurélien Voissière[1], Dominique Poujol[1], Christophe Caux[1], **Nathalie Bendriss-Vermare**[1]

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Natural Killer cells (NKs) have been described as key players in cancer immunosurveillance. NKs and dendritic cells (DCs) engage in an intercellular crosstalk to coordinate adaptive immunity against cancer that has been linked to clinical responses to anti-PD-1 in melanoma patients. We previously demonstrated the importance of NKs/DCs crosstalk in the generation of efficient immune responses (Perrot et al, 2010; Deauvieau et al, 2015). More recently, we identified a novel and unique subset of polyfunctional NKs with increased secretory, cytotoxic, and proliferative abilities and expressing IL- 33 receptor (IL-33R/ST2) that exhibit potent antitumor activity (Eberhardt et al, under revision). In vitro, we showed that the activation of PBMC or NKs/DCs coculture with TLR3 (polyIC, pIC) and TLR7/8 (R848) agonists resulted in a DC-dependent generation of ST2+ NKs that produce huge amounts of IFN-g in response to IL-33. This induction was dependent on IL-12/IL-1 8/IFN-b/TNF-a produced by conventional DCs, while plasmacytoid DCs were not involved. Using in vivo tumor models, we observed an antitumor activity of R848+pIC treatment in wild type mice that was lost in IL33KO mice, highlighting a role for endogenous IL-33 in therapeutic response to TLR3/7/8 agonists. Furthermore, exogenous IL-33 was able to synergize with TLR3/7/8 agonists to limit tumor development when used in combination. We are currently deciphering the role of DCs and NKs in the antitumor activity of TLR agonists. Thus, our work identifies the NKs/DCs crosstalk to empower NKs with polyfunctionality and antitumor immune activity in response to IL-33, opening on novel immunotherapies targeting innate cells

SESSION IV – Tissue-specific functions of DCs and macrophages

Short Talk – 11

Back dans les bacs: rehabilitating the contribution of cDCs subsets in the maintenance of mucosal tolerance

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Peripherally induced regulatory T cells (pTreg) in the intestinal mucosa are critical in maintaining tolerance to the commensal flora. We and other have previously shown that pTreg differentiation is critically dependent on activation of latent TGFβ by αvβ8 integrin on CD11c-expressing antigen presenting cells (APC). Conventional dendritic cells (cDC) have long been thought to be the primary APCs of pTreg induction in the gut via αvβ8-mediated TGFβ activation. However, this prevailing concept was recently challenged by three groups. In their models, they excluded a role for cDC in the development of commensal-specific pTreg and shed light on new RORγt-expressing APC subsets in the development of commensal-specific pTreg, once again via αvβ8-mediated TGFβ activation. However, cDC remain the most abundant APCs in the gut, and while we do not challenge the importance of RORγt+APCs, our results put the DCs back into the picture. Here, thanks to mouse models that allow selective targeting of cDC subsets (and not RORγt+APC as confirmed by lineage tracing), we show that deletion of β8 integrin into either cDC1 or cDC2 is sufficient to decrease gut pTreg at homeostasis. αvβ8-expression by cDC1 or cDC2 is also required for optimal induction of dietary-specific pTreg. Finally, while cDC1- or cDC2- specific β8KO mice develop normally, mice lacking β8 integrin in both cDC1 and cDC2 develop late-onset ulcerative colitis. Thus, contrary to the recent exclusion of cDCs by aforementioned studies, our results instead show that αvβ8 expressing cDC1, -cDC2 and -RORγt+APCs play hand in hand in instructing tolerance at mucosal surfaces.

Short Talk – 12

Control of intestinal Th17 homeostasis by the unfolded protein response sensor IRE1 in myeloid cells

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The intestinal immune system comprises a broad diversity of cell types performing specialized functions. Perturbations in this equilibrium are associated with the development of gastrointestinal diseases. In this context, the sensor IRE1 of the unfolded protein response (UPR) and its associated transcription factor XBP1s can regulate the development and survival of several types of myeloid cell subsets, including intestinal type 1 conventional dendritic cells (cDC1s). However, it is not known whether IRE1 activity emerging from intestinal DCs and myeloid cell subtypes can contribute to shaping adaptive immunity in the gut. Here, by using conditional knock-out mice lacking the RNase domain of IRE1 in CD11c+ cells (IRE1truncDC mice), we discover that expression of the UPR sensor in this lineage regulates the homeostasis of intestinal Th17 cells. IRE1truncDC mice display a selective accumulation of Th17 cells in the small intestine lamina propria (siLP) accompanied by increased neutrophil infiltration, increased goblet cell numbers, epithelial enlargement, and antimicrobial peptides. Notably, siLP Th17 accumulation is dependent on IRE1 RNase activity but independent of XBP1s transcriptional activity in CD11c+ cells. On a mechanistic level, we found that siLP cDCs from IRE1truncDC mice produce higher levels of IL-6 and express lower amounts of retinoic acid biosynthetic enzymes. compared to control counterparts, which may contribute to promoting Th17 polarization. In conclusion, we uncovered a novel regulatory mechanism regulating Th17 homeostasis in the intestine, which depends on IRE1 RNase activity CD11c -expressing cells.

Short Talk – 13

Toll-like receptor 9 dependent dendritic cells-NK cooperation in anti-tumor immune control

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The release of sterile Danger-Associated Molecular Pattern signals (DAMPs) by a tumor promotes activation of tissue dendritic cells (DCs) via Toll-Like Receptors (TLRs) and their migration to tumor draining lymph nodes (LNs) to present tumor antigens to T lymphocytes and to recruit NK cells. While DAMPs are important for efficient anti-tumor immune response, different danger signals may have inhibitory effect on each other. We show that Toll like receptor 9 (TLR9) signaling in tumor associated dendritic cells (DCs) is tolerogenic in contrast to what is observed in systemic TLR9 deficient mice. Indeed, mice with DC-restricted loss of TLR9 display slower melanoma growth and extended survival. TLR9-deficient DCs migrate less to the draining lymph nodes and accumulate in the tumor where they recruit NK cells. Anti-tumor effect is dependent on NK cells, as their depletion severely accelerated tumor growth in mice lacking the expression of TLR9 in DCs but not in control mice. We also show that with tumor progression a small, but non-negligible proportion of tumor associated T and NK cells acquire TLR9 expression. Altogether our results suggest that TLR9 signal is required for DC migration, but not for DC maturation, while TLR9 signal in lymphocytes might promote anti-tumor immune response.

POSTER ABSTRACTS

Thursday December 7th, 2023

Poster – 1

Interferon-induced lysosomal membrane permeabilization and death cause cDC1- deserts in tumors.

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T cell immunity requires antigen capture by conventional dendritic cells (cDCs), digestion and transfer to draining lymph nodes for presentation to antigen-inexperienced T cells. cDCs type I excel as cancer-antigen presenting cells, due to their ability prime both CD8 and CD4 T cells. In tumor tissues cDCs1 become particularly scarce and this restricts anti-tumour immunity, immunotherapy responses and patient survival. Tumor cDC1 paucity is not fully understood and no specific treatment currently exists. Here, we find that type I interferons (IFN) induce lysosomal stress, lysosomal membrane permeabilization (LMP) and lysosomal-dependent cell death (LDCD) in cDCs1. Two parallel pathways downstream of IFNAR1 converged to induce cDC1 LDCD. Up-regulation of expression of lysosomal genes driven by Stat1 and Irf7 enhanced the proteolytic activity of lysosomes, while IFN-inducible guanylate binding protein-2 (GBP-2) accumulated in the membrane of the stressed lysosomes, leading to LMP, proteolytic enzyme release and death. Protease inhibition or GBP-2 repression rescued cDCs1 from LDCD and boosted their anti-tumor efficacy. GBPs are amongst the most abundant IFN induced genes and known to form toxic pores in pathogencontaining vacuoles and pathogen membranes. GBP-2-driven LMP is likely due to the ability of GBP-2 to form pores on the lysosomes of cDC1s. We anticipate our findings to be a starting point for more rational cDC1-directed immunotherapies. For instance, protease inhibition, GBP-2 downregulation or induced expression of LMP repair machinery may boost cDC1 efficacy in adoptive cell therapies or their use as live vaccines

Poster – 2

The healing power of emotions: Brain-to-skin neuroimmune axis heals a Sunburn

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Most skin diseases share chronic inflammatory and healing disorders, which can lead to long-term disabilities and social stigmatization. The nervous system is emerging as a major regulator of skin disorders and beyond a fundamental aspect, the identification of skin neuroimmune regulatory mechanisms is crucial for the development of innovative therapeutic approaches. We have recently shown, in a model of sunburn, that GINIP+ sensory neurons innervating the skin, promoted repair functions of dermal macrophages (MΦ) (Hoeffel et al., Nature 2021). Our current work also revealed the involvement of the hypothalamo-pituitary-adrenal (HPA) neuroendocrine axis leading to the systemic release of endogenous Glucocorticoids (GC) into the bloodstream upon skin UV injury. Thus, we are currently characterizing the regulation of the skin repair process, locally by GINIP+ neurons and systemically by endogenous GC. We recently highlighted that dermal resident TIM4+ MΦ were embedded in a peri-vascular niche and their local regulation by GINIP+ neurons actively controlled neutrophil infiltration upon skin injury. In parallel, we observed a key role of endogenous GC during the skin healing process; First by promoting the expansion of myeloid precursors in the bone marrow and second, by regulating monocyte-derived inflammatory MΦ functions once infiltrating the skin lesion. We are looking forward to decipher these mechanisms at the molecular level by transcriptomic, metabolic and functional approaches. Understanding these neuro-immune pathways could improve the therapeutical management of many skin disorders and our results identifies new targets to design innovative strategies for personalized medicine.

Poster – 3

Conventional Dendritic cells drive the generation of polyfunctional and antitumor ST2+ NK cells

Valentin Picant[1], Lara Revol-Bauz[1], Yamila Rocca[1], Anaïs Eberhardt[1], Aurélien Voissière[1], Dominique Poujol[1], Christophe Caux[1], **Nathalie Bendriss-Vermare**[1]

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Natural Killer cells (NKs) have been described as key players in cancer immunosurveillance. NKs and dendritic cells (DCs) engage in an intercellular crosstalk to coordinate adaptive immunity against cancer that has been linked to clinical responses to anti-PD-1 in melanoma patients. We previously demonstrated the importance of NKs/DCs crosstalk in the generation of efficient immune responses (Perrot et al, 2010; Deauvieau et al, 2015). More recently, we identified a novel and unique subset of polyfunctional NKs with increased secretory, cytotoxic, and proliferative abilities and expressing IL- 33 receptor (IL-33R/ST2) that exhibit potent antitumor activity (Eberhardt et al, under revision). In vitro, we showed that the activation of PBMC or NKs/DCs coculture with TLR3 (polyIC, pIC) and TLR7/8 (R848) agonists resulted in a DC-dependent generation of ST2+ NKs that produce huge amounts of IFN-g in response to IL-33. This induction was dependent on IL-12/IL-1 8/IFN-b/TNF-a produced by conventional DCs, while plasmacytoid DCs were not involved. Using in vivo tumor models, we observed an antitumor activity of R848+pIC treatment in wild type mice that was lost in IL33KO mice, highlighting a role for endogenous IL-33 in therapeutic response to TLR3/7/8 agonists. Furthermore, exogenous IL-33 was able to synergize with TLR3/7/8 agonists to limit tumor development when used in combination. We are currently deciphering the role of DCs and NKs in the antitumor activity of TLR agonists. Thus, our work identifies the NKs/DCs crosstalk to empower NKs with polyfunctionality and antitumor immune activity in response to IL-33, opening on novel immunotherapies targeting innate cells.

Poster – 4

Immunosuppressive Myeloid Cells foster Cancer Stem Cell emergence

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Cells of myeloid origin represent major components of the complex immunosuppressive tumor microenvironment. These myeloid cells such as tumor-associated macrophages (TAM), neutrophils and so-called "myeloid-derived suppressor cells" (MDSC) among others have been widely described for their immunosuppressive properties and their ability to inhibit anti-tumor immune responses. They thus represent major obstacles for efficient immunotherapeutic approaches. However, beyond this cardinal immunosuppressive function, MDSC are also endowed with a broad array of "nonimmunological" tumor-promoting functions. Indeed, accumulating evidences has demonstrated that these cells can directly promote primary tumor cell survival and proliferation and promote local tissue invasion among others. Importantly, MDSC play a key role in the preparation of the premetastatic niches before the arrival of cancer cells, thus contributing to the preparation of the "soil" for seeding by metastatic tumor cells. Evidence has also emerged that tumor-induced immunosuppressive myeloid cells may impact cancer stem cells (CSC), a subpopulation of cancer cells within the tumor, defined by self-renewal, asymmetrical division and differentiation properties, giving rise to more or less differentiated cells composing the tumor mass. Using 3-D tumor sphere formation assays we demonstrate that human monocyte-derived suppressor cells (HuMoSC, a surrogate to study MDSC in vitro) are endowed with the capability to promote stemness features in breast cancer cells in a contact-dependent manner and that this interaction involves TGF-β receptors. Moreover, our data provide insights into the ability of mouse-derived MDSC along with myeloid cells isolated for breast tumor bearing patients to increase tumor sphere formation.

Poster – 5

Colon-resident macrophages work together to ensure epithelial integrity

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The colon is the main site of absorption while displaying the higher abundance of microbes (bacteria, archea, fungi and so on), therefore, epithelial cells need to tightly regulate the amount of water they absorb in order not to get intoxicated. Our lab previously demonstrated that, in the distal colon, this relies on the detection of fungal products by resident macrophages (mΦ) displaying balloon-like protrusions (BLPs). They will instruct epithelial cells to stop water absorption, protecting them from fungi-induced cell death (Chikina et al., 2020). Using Talin-depleted mΦ, I could observe that BLP formation was focal adhesion-independent. Still, BLPs had a strikingly different morphology, suggesting that Talin could be involved in the function of BLPs. Then, I wanted to investigate the involvement of actin in this phenomenon. In ARPC4 depleted mΦ, I could see a decrease in BLP numbers and a decrease response to osmotic choc as well as a tissue leakage. Finally, I asked if myosin-dependent contractility was regulating BLP formation and indeed, in a MyosinIIA KO in mΦ, I could observe an increased number of BLPs. This suggests that at steady-state, myosinIIA might be required for the retraction of the BLP. Overall, this data suggests that BLPs do not behave as classical protrusions but still rely on the actomyosin contractility for their regulation. Using live imaging, I could observe that BLP mΦ communicate at distance by echanging material and that they seem to form a network that could protect the epithelium at the tissue-scale.

Poster – 6

Unraveling the functions of tissue resident macrophages in maintaining oral mucosa homeostasis

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Commensal microbes are key in maintaining tissue homeostasis by protecting the barrier tissues against injury and infection. However, these microbes can also be harmful so a strict control by the host immune system is crucial. Among commensal microorganisms, fungi are increasingly being recognized as common members of the microbiota on mucosal surfaces, including the oral cavity where the tongue acts as a reservoir for the fungus. This organ is seeded by various immune populations presumably involved in the homeostasis establishment. This include Tissue-Resident Macrophages (TRMs), a subset of immune cells inhabiting all mammalian tissues and involved in both the tissue immunosurveillance and homeostasis. TRMs maintenance in the tissue is regulated by local niches which provide trophic factors indispensable for their survival such as colony stimulating factor 1 (CSF1) or the interleukin 34 (IL-34). Deletion of the trophic factor specifically from a macro phage niche represent a novel strategy to depletes indirectly and permanently a macrophage subset. Studying the establishment/regulation of the homeostatic balance between the host and C. albicans is challenging because the fungal strain used in experimental models do not establish persistent colonization in mice and is rapidly cleared after inoculation. Here, we propose to use a model of long-term oral C. albicans colonization contrasting from traditional infection models by its longevity, independence of antibiotic/immunosuppressant treatment and lack of inflammation. By combining this model with the niche concept, we will investigate the role of TRMs during the establishment of a stable homeostasis between the host and the fungus.

Poster – 7

FLT3L-dependent Dendritic Cells Control Tumor Immunity by Modulating Treg and NK

Paul Régnier, Mathias Vetillard, Adèle Bansard, Eméranne Pierre, Xinyue Li, Nicolas Cagnard, Emmanuel L Gautier, Pierre Guermonprez, Bénédicte Manoury, Katrina Podsypanina, **Guillaume Darrasse-Jeze**

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FLT3-L-dependent classical dendritic cells (cDCs) recruit anti-tumor and tumor-protecting lymphocytes. We evaluated cancer growth in mice with low, normal or high levels of cDCs. Paradoxically, both low or high number of cDCs improve survival in mice with melanoma. In low cDC context tumors are restrained by the adaptive immune system through influx of effector T cells and depletion of Tregs and NKcells. High cDC numbers favor the innate anti-tumor response, with massive recruitment of activated NKcells, despite high Treg infiltration. Anti CTLA-4 but not anti PD-1 therapy synergizes with FLT3-L therapy in the cDCHi but not in the cDCLo context. Combination of cDC boost and Treg depletion dramatically improves survival of tumor-bearing mice. We then examined Flt3-L, cDC1, cDC2 & pDCs gene signatures in the biopsies of untreated cancer patients with 42 types of cancer and identified a beneficial, adverse or paradoxical effect of these signatures in 35 types of human cancers. Thus, transcriptomic data confirm the paradoxical effect of cDC levels on survival in several human tumor types. cDCHi-TregLo state in such patients predicts best survival. These results have important implications for improving immunotherapy in specific types of cancer.

Poster – 8

Enhancing Phagocyte and Adaptive Immune Cell Infiltration in Wounded Skin

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The skin serves as our primary defense against external insults, including physical injuries that can disrupt its integrity. Swift restoration of the skin barrier post-injury is crucial to prevent infections and preserve quality of life. A crucial step in wound healing is the removal of apoptotic cells present at the injury site, typically performed by phagocytes as part of an anti-inflammatory process called efferocytosis. We have previously shown that genetic deletion of the cystine-glutamate antiporter SLC7A11 enhances the ex vivo efferocytic capacity of dendritic cells (DCs), specifically dermal cDC1s. In vivo, pharmacological inhibition of SLC7A11 accelerates wound closure in mice and reduces the number of apoptotic cells that remain at the wound site. Altogether, this suggests a previously overlooked role for DC-mediated efferocytosis in tissue repair. However, the impact of efferocytosis on dermal DCs' transcriptional and functional aspects, their management of high apoptotic cell levels, migration to lymph nodes, antigen presentation, and T cell activation, remains unexplored. We have recently demonstrated enhanced infiltration of innate and adaptive immune cells during the early stages of wound healing upon local inhibition of SLC7A11, raising the question whether and how immune cell crosstalk during the early inflammatory stages of wound healing affects tissue restoration later on. Using a combination of transgenic mice, in vivo immunological and efferocytosis assays, and single-cell transcriptomic analyses of wounded skin of transgenic mice, we seek to uncover how dermal DC-mediated cell clearance contributes to cutaneous wound healing across phases.

Poster – 9

A Glucocorticoid-dependent axis controls dendritic cell migration through calcium homeostasis and response modulation.

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Dendritic cell (DC) migration is essential for their function, which is partly controlled by a glucocorticoid-response protein, the Glucocorticoid-Induced Leucine Zipper (GILZ). We previously showed that deleting this transcriptional and signaling regulator in DCs promotes their actin dependent antigen macropinocytosis while limiting their migration from skin to draining lymph nodes. Here, we aimed to determine which mechanisms are involved in GILZ-dependent control of DC migration. First, using bone marrow-derived DCs (BMDCs) derived from CD11c-GILZ-KO mice, in which GILZ is deleted in CD11c+ cells selectively, we confirmed that GILZ promotes immature DC motricity while limiting their macropinocytic activity, and favors activated BMDC chemotaxis towards CCL21. Second, using fluorometry, confocal microscopy, and flow cytometry approaches, we showed that GILZ-KO BMDCs present defects in both their basal levels of cytosolic calcium and store-operated calci um entry due to a reprogramming of the expression and localization of calcium release activated channels. The latter is associated with alterations in the localization of the serum and glucocorticoid-regulated kinase 1, a related glucocorticoid-response protein. Our results shed light on a glucocorticoid-dependent axis that controls DC migration at the motricity level through calcium homeostasis and response modulation. Considering glucocorticoids' role in regulating circadian rhythmicity, we are investigating whether this axis contributes to the circadian modulation of DC migration.

Poster – 10

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

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Macroautophagy (often-named autophagy), a catabolic process involving autophagy-related (Atg) genes, prevents accumulation of harmful cytoplasmic components and mobilizes energy reserves in long-lived and self-renewing cells. Autophagy deficiency affects antigen presentation in conventional dendritic cells (DCs) without impacting their survival. However, previous studies did not address epidermal Langerhans cells (LCs), a proliferating skin DC subset with extended lifespan. Here, we demonstrate that deletion of either Atg5 or Atg7 in LCs leads to their gradual depletion. ATG5- deficient LCs showed metabolic dysregulation and accumulated neutral lipids. Despite increased mitochondrial respiratory capacity, they were unable to process lipids, eventually leading them to ferroptosis. Metabolically impaired LCs upregulated proinflammatory transcripts and showed decreased expression of neuronal interaction receptors, in line with a reduction of epidermal nerves upon LC depletion. Altogether, autophagy represents a critical regulator of lipid storage and metabolism in LCs, allowing their maintenance in the epidermis.

Poster – 11

UNC93B1 controls the activation of STING 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 and folding 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). We have shown that UNC93B1, but not the 3d mutant, also binds the Ca2+ sensor stromal interaction molecule 1 or STIM1 in the ER, an association essential for antigen cross presentation and controls the activation of Inositol requiring enzyme 1- alpha (IRE1a), the major ER stress sensor. STING or stimulator of interferon genes is a protein critical for type I interferon response to pathogens containing DNA and its abnormal activation is associated to inflammation and autoimmunity. We observe that in DCs, which express high levels of UNC93B1, UNC93B1 interacts with STING. In addition, we find that the absence of UNC93B1, or the expression of the 3d mutant in DCs, leads to defective ER-to-Golgi trafficking of STING (necessary for STING to signal), and a significant decrease in type I interferon secretion. Altogether, these results suggest that UNC93B1 controls STING activation in DCs.

Poster – 12

Dendritic cells orchestrate their epigenetic marks and nuclear architecture during efferocytosis.

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Billions of cells dying in our body, under normal or disease conditions, are cleared out by phagocytes in order to maintain tissue homeostasis. This process is referred to as efferocytosis and phagocytes like dendritic cells (DCs) internalize antigens to present them to cells of adaptive immune system. Upon efferocytosis, phagocytes undergo epigenetic changes. Although epigenetic changes of DCs have been investigated mainly in the contexts of tumor immune microenvironment or during DC activation and maturation, the epigenetic and architectural changes in DC nucleus during efferocytosis have not been addressed. Both of these processes are known to play roles in multiple cellular aspects of the immune cells such as their motility and their antigen presentation capacity. To shed light on the nuclear landscape of DCs upon efferocytosis, we performed an RNAseq of primary bone-marrow derived DCs engulfing apoptotic cells. Our analysis revealed that 115 genes related to epigenetic changes and chromatin modifications are differentially expressed upon efferocytosis. More specifically, 20 genes were related to histone acetylation, 14 with histone methylation and 9 genes with histone phosphorylation or ubiquitination. Considering that regulating the efferocytic capacity of DCs holds great potential in disease treatments, we have benefitted from inhibition of. some of the aforementioned genes, affecting H3 methylation and acetylation. Interestingly, our results show that pharmacological inhibition of the activity of the respective epigenetic processes affect efferocytic capacity of DC. Notably, H3K4metylation changes, driven by inhibition of MLL complex components, not only affect apoptotic cell clearance but also phagosome maturation in DCs. Changes in H3K4metylation also affects the efferocytic capacity of bone marrow derived macrophages supporting further the notion that effective efferocytosis might be dictated by the epigenetic state of phagocytes.

Poster – 13

Cytosolic phospholipase A2: Orchestrating communication between macrophages and epithelial cells in the distal colon

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The colon, an organ with the most substantial microbial load across the digestive tract, must efficiently absorb water for tissue hydration and feces solidification. How does the colon manage water absorption without being intoxicated by microbiota-derived metabolites? We've previously unraveled a protective mechanism of water absorption regulation and epithelial cell protection that involves a subset of macrophages enriched in the distal colon. These macrophages form balloon-like protrusions (BLPs) nestled between epithelial cells. BLP+ macrophages can sense fungal toxins in absorbed water and instruct epithelial cells to stop absorption in case of an overload. This prevents fungal-induced cell damage and maintains the intestinal barrier's integrity (Chikina et al. 2020). Our aim is to identify the molecular mechanisms behind BLP macrophages' function. We explored the role of prostaglandin E2 (PGE2), an arachidonic acid derivative produced by macrophages. We. found that intrarectal infusion of PGE2 decreased number of BLPs and reduced water absorption by colonic epithelial cells. Additionally, macrophages lacking the enzyme cytosolic phospholipase A2 (cPLA2), responsible for AA production, showed decreased numbers and aberrant morphology BLPs. Mice with conditional cPLA2 knockout in myeloid cells exhibited increased water absorption in the presence of fungi-derived metabolites compared to wild-type mice. This highlights the critical role of cPLA2 in the dialogue between BLP-macrophages and epithelial cells, maintaining intestinal barrier integrity and colon homeostasis.

Poster – 14

Functionally decoding the enigmatic "mregDCs": guardians or saboteurs of tumour immunity?

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Conventional dendritic cells (cDCs) constitute crucial orchestrators of adaptive immune response against tumours. Recent single cell transcriptional analyses have unraveled a cluster of tumour infiltrating cDCs which cannot be classified to the previously described cDC1 and cDC2 subsets. Their transcriptional profile suggests they represent an activated state of cDCs (referred to herein as actDCs a.k.a. mregDCs, mDCs, or cDC3s) with putative opposing T cell stimulating or inhibitory roles. Due to their intratumoural scarcity and the lack of appropriate experimental tools, the actual contribution of actDCs to tumour immunity remains unknown. Here, we show that actDCs, transcriptionally and phenotypically reminiscent of their tumour-infiltrating in vivo counterparts, can be found within bone marrow-derived cDC cultures in vitro, especially following co-culture with cancer cells. Furthermore, using intersectional genetics we here describe new genetically-engineered mouse models to conditionally and selectively label, isolate or deplete actDCs. Using these novel tools, we show that actDCs derived from both cDC1s and cDC2s similarly and efficiently crosspresent cancer cell-associated antigens but, unexpectedly, actDC2s display a markedly superior ability to drive effector CD8 T cell-differentiation. Crucially, we demonstrate that specific and acute depletion of actDCs impairs T cell priming and tumour eradication in vivo. Overall, our work formally establishes an essential, non-redundant contribution of actDCs to T cell-mediated, anti-cancer immunity.

Poster – 15

Combinatorial analysis of MNP molecular programs in Crohn disease uncovers inflammatory states associated with anti-TNF resistance

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Crohn's Disease (CD) is a disabling inflammatory bowel disease (IBD) associated with severe complications like fibrostenosis, often leading to surgical resection. Anti-TNF therapy has transformed CD clinical care but up to 40% of patients never respond. Using single-cell technologies, we previously described a cellular response enriched in inflamed CD ileums of a subset of patients associated with anti-TNF resistance. We named it GIMATS (IgG plasma cells, Inflammatory Mononuclear phagocytes, Activated T cells and Stromal cells) and predicted dysregulated actions of monocytes/macrophages as central drivers. While current evidences indicate that CD pathogenic macrophages could emerge from recently infiltrated blood monocytes, their nature, diversity and distribution remain unclear. To resolve their molecular heterogeneity, we FACS-sorted total MNP populations and analyzed by single-cell RNA sequencing the MNP-enriched suspensions from inflamed mucosa of 7 patients with stricturing ileum. Using Metacells-2 and gene module-based analyses, we characterized 17 gene programs, defining 10 molecular subgroups of monocytes/macrophages with distinct inflammatory and metabolic features. Applying these programs to a larger cohort, we characterized a molecular state uniquely defining monocyte-like cells in GIMATShigh patients. Using TF motif enrichment analyses and pathway activity inference, we predicted GIMATS-associated monocytes could be targeted by upadacitinib, a JAK inhibitor recently approved, to treat active CD patients with inadequate response to TNF blockers. We supported this hypothesis by exposing blood classical monocytes to conditions predicted to drive GIMATS-associated inflammatory monocytes. In summary, our data identify a unique molecular state of monocyte-like cells enriched in GIMATShigh patients, which is targetable with JAK inhibitors.

Poster – 16

Role(s) of DC subsets in maternal-fetal immune tolerance

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The fetus is the equivalent of a semi-allogeneic transplant but is not rejected by the mother. Regulatory T lymphocytes (Tregs) and uterine NK cells (uNKs) are essential for the establishment of this fetal-maternal tolerance and successful implantation respectively. Despite that the homeostasis of these two populations is regulated by dendritic cells (DCs) little is known about the role of DCs at the maternofetal interface in normal or pathological pregnancy. We studied the DC composition ofthe uterus in pregnant or non-pregnant mice, and show that uterus-resident DCs increase during normal pregnancy, but not in a multiple miscarriage mouse model. Artificial increase of cDCs in this model protect the fetuses from maternal immune rejection. DCs appear to influence the success of allogeneic pregnancies.

Poster – 17

Distinct ontogenetic lineages dictate cDC2 heterogeneity

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Conventional dendritic cells (cDCs) comprise functionally and phenotypically diverse populations, including cDC1s and cDC2s. The latter population has been variously sub-divided into Klf4- dependent cDC2s, Notch-dependent cDC2s, T-bet+ cDC2As and T-bet– cDC2Bs but it is unclear how all these sub-types are interrelated and to what degree they represent cell states or cell subsets. All cDCs derive from bone marrow progenitors, called pre-cDCs, that leave via the circulation to colonise peripheral tissues. Here, we report the identification of distinct mouse pre-cDC2 subsets that give rise to cDC2As or cDC2Bs and account for the generation of previously-described cDC2 subtypes. We show that a SiglecH+ pre-cDC2A population in the bone marrow gives rise to SiglecHCD8α+ pre-cDC2As in tissues, which differentiate into T-bet+ cDC2As. In contrast, a SiglecH- fraction of pre-cDCs in the bone marrow and periphery mostly generates T-bet– cDC2Bs, a lineage mar ked by expression of LysM. Our results reveal that cDC2A vs cDC2B fate specification occurs in the bone marrow and suggest that cDC2 subsets are ontogenetically-determined lineages rather than cell states imposed by the peripheral tissue environment.

Poster – 18

MMMTV-R26Met mouse model as a reliable preclinical tool to harness myeloid cells in triple negative breast cancer

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In human, Triple-negative breast cancer (TNBC) is considered as an immunologically heterogeneous cancer. Divergences in immune profiles have been linked to prognosis, immunotherapeutic responses in TNBC. However, causal effects and mechanistic functions of different immune cells remains unclear in clinical settings. Preclinical researchers need robust and representative TNBC models to overcome these limitations. We developed a series of immunocompetent syngeneic transplant models by sequential orthotopic transplantation of tumors coming from MMTV-R26Met, a murine model of breast cancer in which Met, a tyrosine-kinase receptor, was increased in the mammary-gland. Previous studies showed that MMTV-R26Met mice develop spontaneous tumors, exclusively TNBC, which recapitulate the human heterogeneity of circuits and crosstalk between cancer and immune cells. Here we propose to identify and quantify the immune (lymphoid and myeloid) cell infiltration of MMTV-R26Met primary tumors and their derived syngeneic transplants by spectral flow-cytometry
with a 18-colors panel. Our preliminary results show that MMTV-R26Met primary tumors are preferentially enriched in myeloid cells (dendritic cells, neutrophils, macrophages). Notably, primary tumors are characterized by significant conventional dendritic cells (DC) infiltrate, especially cDC2. Longitudinal resampling of syngeneic transplants showed that the growth rate, spleen weight and frequency of the most abundant myeloid population of each tumor was independent of the number of transplantation passages. The stability observed in the MMTV-R26Met syngeneic transplants suggests that these syngeneic models of orthotopic tumor transplantation in immunocompetent provide an adequate experimental approach to harness inter-patient heterogeneity of the myeloid compartment in TNBC.

Poster – 19

GPNMB controls immune cell infiltration and tumor development in breast cancer

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Abstract :
Glycoprotein non-metastatic B (GPNMB) is a type 1 transmembrane glycoprotein. Within the immune compartment GPNMB has been shown to act as a negative regulator of an adaptive immune response and of the inflammatory macrophages. In tumors, we found that GPNMB protein is specifically expressed on monocyte-derived macrophages infiltrating human breast cancer. Here we propose to explore the mechanisms by which GPNMB regulate tumor immunity. Preliminary data from the lab show that GPNMB expressing-macrophages are an abundant immune cell population in breast tumors. Thus, we hypothesize that GPNMB expression on TAMs may participate to the suppression of immune response and the promotion of tumor growth. Therefore, we performed in vivo and ex vivo approaches to define the role of GPNMB in shaping the pro-tumoral phenotype of macrophages in breast cancer. Using Gpnmb-deficient mice, we found that GPNMB deficiency induces a significant decrease in tumor growth of a luminal B type breast cancer cell line (E0771). In addition, GPNMB deficiency leads to the alteration in myeloid and lymphoid compartments of tumor-infiltrated cells. In particular, we found significantly lower infiltration by macrophages, monocytes and neutrophils in Gpnmb-deficient mice. In the lymphoid compartment, we found decreased percentage of CD4+ tissue-resident memory and regulatory T cells. Altogether, these data suggest that GPNMB may control the development and immunity of the E0771 breast cancer model. Further experiments are required to define the main immune cell subtype causing these changes and to develop therapeutic strategy to modulate GPNMB function.

Poster – 20

Lung adenocarcinoma co-opt and expand a subset of poorly mature dendritic cells enriched in epithelium-associated and tissue residency features.

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Abstract: Dendritic cells (DCs) are sentinel cells of the immune system controlling the development of adaptive immunity. Depending on the cues instructing their terminal differentiation, DCs can either spark the onset of adaptive immune responses or tolerance. Immune suppression is a hallmark of cancer, with multiple mechanisms orchestrated by tumors-derived factors precluding the development of efficacious adaptive immunity, T cell responses in particular. Here, we have decided to characterize DC populations and their maturation stage within the KP model of lung adenocarcinoma. Using single-cell RNA sequencing, we found that tumor infiltrating DCs underwent a tumor-induced expansion and changed in composition. Specifically, CD11b+ type 2/3 DCs increased as compared to IRF8+XCR1+ type 1 DCs. Also, tumor induced expression of the E-cadherin ligand alpha-E integrin CD103 on CD11b+ DCs, together with multiple other epithelium-associated features. CD103+CD11b+ DCs express a transcriptional program associated to tissue residency including negative regulators of migration. Mechanistically, we show that tumor-induced CD103+CD11b+ DCs are dependent on tumor-derived CSF2 and rely on the IRF4 transcription factor activated downstream CSF2R signaling. As compared to CD103-CD11b+ DCs, CD103+CD11b+ are less prone to engage in terminal differentiation tracked by the upregulation of CCR7 which supports migration and antigen delivery to tumor-draining lymph nodes. Altogether, these results highlight a tumor-activated program that reshapes the phenotype of type-2 dendritic cells to promote tissue-resident features at the expense of terminal differentiation and migration. The outcome of these modifications for T cell responses is being investigated using genetic tools enabling the depletion of CD11b+CD103+ epithelium-associated DCs.

Poster – 21

Engineering the stromal compartment of solid tumors activates dendritic cell infiltration and spark anti-tumor immunity.

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Conventional type 1 dendritic cells (cDC1) cDC1 infiltration in TME and tumor to lymph node trafficking correlates with favourable outcomes and clinical responses to immunotherapy. Depletion studies in preclinical models have revealed that cDC1s play a key role in both CD8 + T-cell activation within the tumor microenvironment (TME). FLT3L is a growth factor for cDC1s supporting their differentiation from HSCs, terminal differentiation within tissues, proliferation and survival. Here, we present a new immunotherapeutic approach based on local delivery of FLT3L within solid tumors. We show that intra-tumoral engraftment of autologous engineered mesenchymal stromal cells engineered to express membrane bound FLT3L (eMSC-FLT3L) efficiently stimulate cDC1 infiltration when combined with the TLR3 agonist poly(I:C) or anti-CD40 agonistic antibodies. By contrast, eMSC-FLT3L alone are inefficient at triggering intra-tumoral DC expansion. We show poly(I:C) synergiz e with eMSC-FLT3L by providing on CXCR3 and CCR5 chemokine receptors. Also, this procedure, promote trafficking of cDC1s to lymph nodes and the cross priming of tumor-specific CD8+ T cells. As a result, eMSC-FLT3L+poly(I:C) therapy induces T, NK and DC1-dependent tumor regression and reduces lung metastasis. Furthermore, this immunotherapeutic approach circumvents resistance to CTLA4 and PD1 blockade in the hard-to-treat B16 melanoma model. Altogether, these data support the immunotherapeutic potential of intra-tumoral engraftment of engineered, autologous mesenchymal stromal cells acting as local “factories” for the local delivery of specific cues supporting anti-tumor immunity.

Friday December 8th, 2023

Poster – 22

Heterogeneity of mononuclear phagocytes subsets in the tumour microenvironment

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Mononuclear phagocytes (MNPs), including dendritic cells, monocytes, and macrophages, play vital roles in antimicrobial defence, homeostasis, and immune regulation. However, the field lacks a standardised nomenclature despite numerous studies characterising MNPs in different tissues and conditions. To address this, we integrated 41 datasets from 13 tissues, resulting in the creation of the MNP-VERSE. Subsequently, we divided it into the MoMac-VERSE and DC-VERSE. The MoMac-VERSE analysis revealed three tumour-associated macrophage populations: proliferating cells, TREM2+ macrophages, and a novel subset called IL4I1+CD274(PD-L1)+IDO1+ immune-suppressive macrophages (IL4I1_Macs). The differentiation of IL4I1_Macs was induced by IFNg from CD8+ T cells and CD40L-expressing CD4+ T. Flow cytometry validated these IL4I1_Macs in liver and lung cancer. Using the DC-VERSE, we characterised dendritic cell subsets and "states" across tissues. In the 11 neo-plastic tissues studied, we found the presence of CD207+ DC2/3 cells resembling Langerhans cell histiocytosis (LCH) cells, which specifically expressed CD1a. The expansion of CD207+ DC2/3 cells inversely correlated with T cell clonality and tumour-resident CD8+ memory T cells but positively correlated with terminally differentiated exhausted CD8+ T cells within tumours. This signature of CD207+ DC2/3 cells was associated with lower patient survival in patients who got ICB treatment. Spatial transcriptomic and immune-histo fluorescence analyses confirmed the accumulation of CD207+ DC within tumour nests (glands) of lung adenocarcinoma. These findings contribute to a better understanding of the heterogeneity of MNP subsets, including their functions, states, and localisation within the tumour microenvironment.

Poster – 23

Macrophage adhesion to cancer cells favors early tumor growth

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Although considerable knowledge has been accumulated on the mechanisms underlying the various pro-tumorigenic activities of macrophages, it remains unclear whether the physical forces exerted by macrophages can contribute to early tumor development. Here, we have set up an in vitro system to monitor tumor spheroid growth in 3 dimensions by real-time microscopy, which recapitulates the positive effect on tumor growth of alveolar macrophages, but not monocytes. Using the data generated, we construct a physical model of 3D spheroid growth using a semi-continuous particlebased approach to treat proliferating cancer cells and interacting macrophages. The model fits well with experiments in the absence or presence of macrophages. When KP cells are grown alone, they form a single aggregate that tightens and shrinks over time, limiting access to nutrients for cells in the center. Both approaches show that adding macrophages rapidly induces the nucleation of cancer cells into multiple aggregates, favoring their access to nutrients. The presence of macrophages also slows fusion between aggregates, eventually coalescing into a single, larger tumor spheroid. The model predicts adhesion forces between tumor cells and macrophages are essential for the observed pro-tumor effect. Among integrins potentially involved, CD11c is expressed by alveolar macrophages but not monocytes. Adding an anti-CD11c blocking antibody decreases adhesion forces between macrophages and tumor cells, prevents spheroid nucleation, and impairs spheroid growth. Our results reveal that physical interaction via CD11c between tissue-resident macrophages and cancer cells contributes to the pro-tumor effect of macrophages. Targeting this interaction may represent a new therapeutical approach to hinder early tumor and metastasis development

Poster – 24

Cell-intrinsic factors modulate the dynamics of SARS-CoV-2 spread and plasmacytoid dendritic cell responses

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Type I and III interferons (IFN-I/III) are pivotal antiviral mediators against SARS-CoV-2: a robust IFNI/III response associates with viral clearance, whereas their weak and/or late production leads to severe COVID-19. Plasmacytoid dendritic cells (pDCs) are immune cells specialized in early production of extremely high levels of IFN-I/III. As opposed to SARS-CoV-2-mediated inhibition of the innate response within infected-epithelial cells, we recently demonstrated that pDCs physically detect viral particles from SARS-CoV-2-infected cells resulting in a robust antiviral response. The regulation of the targeting of viral elements to pDC sensor is a still-uncovered question that we addressed. The establishment of a novel fluorescence assay allowed us to assess the dynamics and regulation of viral spread to neighboring cells. Indeed, we showed that SARS-CoV-2 spread is restricted by the inhibition of specific cell-intrinsic factors, known as critical for S ARS-CoV-2 entry, or interestingly pDC IFN-I/III response. Through a genome-scale RNAseq analysis, we then defined which similar cell-intrinsic factors could be expressed by pDCs and hence involved in the regulation of viral elements targeting to pDCs, leading to an indirect control of viral spread. Functional study of the selected factors showed that members of ADAM (a disintegrin and metalloproteinase) and MMP (matrix metalloproteinases) families as well as furin protease control viral spread, acting on viral entry in epithelial cells just as pDC activation. Together our results uncovered entry processes involved in pDC sensing and control of SARS-CoV-2 spread, and offers an innovative framework to study pDC-detection of other viruses.

Poster – 25

Control of intestinal Th17 homeostasis by the unfolded protein response sensor IRE1 in myeloid cells

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The intestinal immune system comprises a broad diversity of cell types performing specialized functions. Perturbations in this equilibrium are associated with the development of gastrointestinal diseases. In this context, the sensor IRE1 of the unfolded protein response (UPR) and its associated transcription factor XBP1s can regulate the development and survival of several types of myeloid cell subsets, including intestinal type 1 conventional dendritic cells (cDC1s). However, it is not known whether IRE1 activity emerging from intestinal DCs and myeloid cell subtypes can contribute to shaping adaptive immunity in the gut. Here, by using conditional knock-out mice lacking the RNase domain of IRE1 in CD11c+ cells (IRE1truncDC mice), we discover that expression of the UPR sensor in this lineage regulates the homeostasis of intestinal Th17 cells. IRE1truncDC mice display a selective accumulation of Th17 cells in the small intestine lamina propria (siLP) accompanied by increased neutrophil infiltration, increased goblet cell numbers, epithelial enlargement, and antimicrobial peptides. Notably, siLP Th17 accumulation is dependent on IRE1 RNase activity but independent of XBP1s transcriptional activity in CD11c+ cells. On a mechanistic level, we found that siLP cDCs from IRE1truncDC mice produce higher levels of IL-6 and express lower amounts of retinoic acid biosynthetic enzymes. compared to control counterparts, which may contribute to promoting Th17 polarization. In conclusion, we uncovered a novel regulatory mechanism regulating Th17 homeostasis in the intestine, which depends on IRE1 RNase activity CD11c -expressing cells.

Poster – 26

New Extracellular Mode of B-Cell Activation Through Exosome-Free Release of Antigen by Dendritic cells

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Dendritic cells (DCs) are antigen-presenting cells (APCs), which sample antigen (Ag) in the periphery and migrate to the lymph node (LN) where they activate T cells. Previously, we showed that DCs were able to store Ag and to release it not degraded into the extracellular medium. B-cell activation is still considered to be predominantly promoted through Ag transfer by APCs upon cell-to-cell contact. Alternative modes of B-cell activation, involving release of Ag by DCs remain however not investigated. Using subcutaneous delivery of Ag-loaded DCs in vivo, and co-culture in vitro, we aimed to visualize Ag trafficking by DCs to the LN; to investigate the modalities of Ag transfer and B cell activation by DCs; and to probe the role of exosomes (Exo) in Ag release and its regulation. DCs are peripheral transporters of Ag to the LN-B cell zone and potent B-cell activators in vivo and in vitro. We highlight a novel extracellular mode of B-cell activation by DCs by showing that Ag release by DCs is sufficient to efficiently induce early B-cell activation through the transcription factor NFkB/cRel. Strikingly, this mechanism consists in an Exo-free release of native Ag, contrasting with the well-established Exo-dependent extracellular T-cell activation by DCs. Interestingly, while chloroquine enhances Ag release by preventing degradation, the glucocorticoid Dexamethasone inhibits Ag release and B-cell activation by DCs. Thus, our study provides new mechanistic insights into the modes of Ag delivery for B-cell activation by DCs and a promising approach of drug modulation of the DC-elicited Ag-dependent B-cell responses.

Poster – 27

The response of plasmacytoid dendritic cells to Endoplasmic Reticulum stress: the effect on STING response

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Several perturbations, such as viral infections or dysregulation of calcium homeostasis, can disrupt proper functioning of the endoplasmic reticulum (ER) and induce accumulation of misfolded or unfolded proteins. This initiates an Unfolded Protein Response (UPR) through three signaling branches, IRE1, PERK and ATF6. Plasmacytoid Dendritic Cells (pDC) are a subset of Dendritic Cells (DC) associated with antiviral immunity due to their capacity to quickly produce copious amounts of IFN-I, for which they possess a large ER. Stimulator of Interferon Genes (STING) is an ER resident protein important for responses against viral or self-cytosolic DNA. However, the impact of ER stress on STING signaling remains unknown. In this work we aim to characterize the impact of rapid and persistent ER stress responses in STING signaling in pDC. For that, CAL-1 cells, a pDC cell line, were stimulated with an inducer of ER stress, thapsigargin, for different time-points, and STING signaling stimulated with cGAMP. Thapsigargin treatment rapidly decreased protein synthesis and induced phosphorylation of PERK and eIF2α. Two hours after stimulation, the levels of protein synthesis were reestablished, but there was sustained phosphorylation of PERK and increased expression GADD34. Interestingly, both acute and prolonged ER stress induced a delay in shutdown of STING response culminating in increased IFN-I production. These results will contribute to understand the influence of ER stress in innate immune responses.

Poster – 28

Heterogeneity and spatiotemporal dynamics of tumour cDC1s

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Cytotoxic CD8+ T cells are central to the anti-tumour response, but their activity depends on adequate priming by type 1 conventional dendritic cells (cDC1s) in lymph nodes (LNs). Recent studies have shown that cDC1s play additional roles within the tumour itself, suggesting that different spatiotemporal behaviours of cDC1s are important for anti-tumour immunity. Using the CytoMAP image analysis pipeline to identify patterns of tissue organization, we find that cDC1s localize in two main regions during anti-cancer responses: at the tumour border, where they cluster with CD8+ T cells, and in the stroma where they make sparse clusters. Spatiotemporal analysis of intra-tumoural cDC1s by inducible lineage tracing showed that some cDC1s remain in tumours long-term and localise preferentially at the tumour border. We performed scRNAseq to uncover how cDC1 heterogeneity in tumours links with these spatiotemporal features. We identified two distinct states of cD C1 activation, marked by increased Cxcl9 or Il12b/Ccr7 expression respectively. Using RNAscope, we found that that Cxcl9 and Il12b cDC1s mapped to different regions of the tumour, accumulating preferentially at the tumour parenchyma and in the stroma, respectively. Finally, IL- 12p40/CCR7 but not CXCL9 cDC1s were associated with antigen uptake and activation of the noncanonical NF-kB pathway, while IFNGR signalling was necessary for CXCL9 expression by cDC1s. Further understanding of these cDC1 activation programs and spatiotemporal dynamics will help uncover the roles of cDC1s in cancer.

Poster – 29

Toll-like receptor 9 dependent dendritic cells-NK cooperation in anti-tumor immune control

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The release of sterile Danger-Associated Molecular Pattern signals (DAMPs) by a tumor promotes activation of tissue dendritic cells (DCs) via Toll-Like Receptors (TLRs) and their migration to tumor draining lymph nodes (LNs) to present tumor antigens to T lymphocytes and to recruit NK cells. While DAMPs are important for efficient anti-tumor immune response, different danger signals may have inhibitory effect on each other. We show that Toll like receptor 9 (TLR9) signaling in tumor associated dendritic cells (DCs) is tolerogenic in contrast to what is observed in systemic TLR9 deficient mice. Indeed, mice with DC-restricted loss of TLR9 display slower melanoma growth and extended survival. TLR9-deficient DCs migrate less to the draining lymph nodes and accumulate in the tumor where they recruit NK cells. Anti-tumor effect is dependent on NK cells, as their depletion severely accelerated tumor growth in mice lacking the expression of TLR9 in DCs but not in control mice. We also show that with tumor progression a small, but non-negligible proportion of tumor associated T and NK cells acquire TLR9 expression. Altogether our results suggest that TLR9 signal is required for DC migration, but not for DC maturation, while TLR9 signal in lymphocytes might promote anti-tumor immune response.

Poster – 30

DNASE1L3 deficiency exacerbates obesity-mediated inflammation and metabolic syndome

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Obesity is one of the greatest health challenge of the 21st century. The development of obesity associated metabolic syndrome and health complications are attributed to chronic low-grade inflammation in metabolic tissues. Recently, cell-free self-DNA (cfDNA), which accumulates in obese individuals, was shown to activate innate immune responses and to contribute to such inflammation. DNASE1L3 is an endonuclease selectively produced by macrophages and dendritic cells and regulates cfDNA levels and their immunostimulatory potential, however its function in the regulation of obesity-mediated inflammation remains unknown. To evaluate the function of DNASE1L3 in obesity, we fed Dnase1l3-/- and controls mice with a normal and high fat diet (HFD). The deficiency of Dnase1l3 exacerbated weight gain, the development of metabolic syndrome, induced hepatic steatosis, and increased pro-inflammatory macrophages levels in adipose tissue. Conversely, adenoviral supplementation of DNASE1L3 in control mice exposed to HFD led to an improvement in hepatic pathology and reduced the overall inflammation in metabolic tissues. Finally, we observed that obese patients display elevated levels of cfDNA that correlated to disease severity. This elevation of cfDNA in obese patients wasn't due to the downregulation of DNASE1L3 expression but rather to the diminution of global circulatory DNASE activity. Thus, our results indicate that DNASE1L3 plays an important role in the regulation of obesity mediated inflammation and metabolic syndrome in vivo. Obesity impacts DNASE1L3 activity in patients and may contribute to elevated levels of cfDNA and disease severity. Therefore, restoring DNASE1L3 function in obesity could represent a novel therapeutic approach to ameliorate obese patients' outcome.

Poster – 31

TL1A and IL-18 synergy promote GM-CSF dependent thymic emergency myelopoiesis

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The current model of hematopoiesis only resolves the thymus around the production and establishment of the peripheral T cell pool. However, the role and in situ development of other immune subsets have been overlooked. We aim to demonstrate that during TL1A and IL-18-induced inflammation, the thymus is capable of producing other cell types such as neutrophils, monocytes, and macrophages, and that these potentially have other functions aside from supporting T cell development as scavengers. Here we have proven that ex vivo and in vivo treatment with TL1A and IL-18 results in acute thymic atrophy by a massive loss of DN4 and DP T cells and alterations in the thymic morphology. By electron microscopy, flow cytometry, and single-cell we demonstrate that neutrophils are developing inside the thymus. We have characterized different subsets according to their nuclear morphology and gene expression. Our fate-mapping studies using the Rag1-Cre Rosa26-YFP fate-map ping model suggest that neutrophils and T cells share a common progenitor while monocytes/macrophages do not. \ Furthermore, we show that thymic-derived neutrophils are functional and are capable of forming extracellular neutrophils traps (NETs) compared to benchmark peritoneal neutrophils. We found that the expansion of thymic neutrophils is GM-CSF dependent by using Csf2rb KO mice. Additionally, we identified DR3+ and IL-18Rα+ expressing subsets of ILCs and gdT cells as the cellular source GM-CSF. Lastly, in vivo treatment with TL1A+IL-18 lead to emergency granulopoiesis and an increase of neutrophils in all the organs investigated, including the thymus.

Poster – 32

IFN-III primes pDCs for TLR7 activation and antagonizes TGF-β mediated-immune suppression in tumors

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Dendritic cells subpopulations orchestrating innate and adaptive immunity during malignancy and tumor progression. Conventional (cDC1, cDC2) and plasmacytoid dendritic cells (pDCs) subset have specialized function in tumors and can be modulated by the tumor microenvironment (TME) Interferons are central players in anti-viral and anti-tumor immune responses through their direct effects on infected or tumor cells, but also on immune cells. While the positive impact of Type I Interferons (IFN-I, produced by pDCs) on cancer development is well understood, the role of type III interferons (IFN-III) produced by the cDC1 in the TME remains unclear. After demonstrating in previous studies that IFN-III produced by cDC1 was associated with good prognosis in breast cancer, we used flow cytometry and RNA sequencing analysis to demonstrate that plasmacytoid dendritic cells (pDCs) strongly respond to IFN III in blood and tumors. We observed in blood that IFN-III increases TLR7 expression and signaling in pDCs, enhancing their capacity to respond to TLR7 agonist. Indeed, IFN-III-treated pDCs show an impressive increase in IFN-α production upon TLR7 activation. As TGF-β is involved in the inhibition of IFN-α by pDCs in the TME, we finally demonstrated that IFNIII can prevent the inhibition of pDCs induced by the TME or by TGF-B by restoring their IFN-α production. Our findings indicate that targeting tumor-associated pDC with a combination of IFN-III and TLR7-L to restore their IFN-α production might be a strategy to induce antitumor immunity.

Poster – 33

Functions of DNASE1L3 in the regulation on anti-tumor immune responses.

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Tumor DNA (tDNA) is crucial in the induction of anti-tumor immunity, by stimulating dendritic cells' (DCs) production of type I interferons, which subsequently activate tumor eliminating CD8 T lymphocytes. Chemotherapy (CT)/radiotherapy (RT) promote the release of tDNA and consequently "boost"" anti-tumor immunity. However, the mechanisms involved in the regulation of the immunostimulatory potential of tDNA remain poorly understood. The endonuclease DNASE1 Like 3 (DNASE1L3) which is produced by DCs, is known to degrade DNA released by dead and dying cells, limiting its ability to activate aberrant immune responses. Given its DC-restricted expression and function in DNA digestion, we investigated the role of DNASE1L3 in cancer. We observed that Dnase1l3 deficiency did not affect mammary tumor growth in spontaneous or transplantable tumor models. However, the absence of DNASE1L3 inhibited the therapeutic efficacy of CTs in both tumor models. We next showed that DNASE1L3 specifically fragments tDNA released by tumor cells in response to CTs and that this fragmented tDNA is more efficient than the unfragmented one to induce human DCs activation in vitro. Finally, murine DCs that were deficient for Dnase1l3 were shown to be impaired in their ability to produce inflammatory cytokines in response to DNA triggering TLR9 stimulation. Altogether, our work suggests that DNASE1L3 is not only disposing of DNA originating from dying cells. In the context of cancer DNASE1L3 likely processes tDNA released upon CT to make it amenable to stimulate anti-tumor immunity. Therefore, DNASE1L3 may be used in conjunction to preexisting cancer therapies to enhance their efficacy.

Poster – 34

Analysis of in vivo antigen presentation by cDC subsets.

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Conventional dendritic cells (cDCs) are antigen presenting cells (APCs) that prime naïve CD8 and CD4 T cells for expansion and differentiation. cDCs process antigens for loading peptides onto major histocompatibility class (MHC) -I and MHC-II molecules to prime CD8 and CD4 T cells respectively. In vivo analysis of cDC1-deficient mice indicated that type 1 cDCs (cDC1) prime CD8 T cells in response to cell-associated antigens through cross-presentation. In vivo analysis also indicated that cDC2 subsets dependent on Notch2 or KLF4 prime TH17 or TH2 responses in response to specific pathogens. However, some studies show that cDC2 and monocyte-derived DCs (MoDCs) can also cross-present antigens to CD8 T cells in vitro. To test their role in cross-presentation in vivo, we compared cDC1-deficient (Irf8 +32-/-) and cDC2-deficient (Zeb2 -165 kb d1+2+3) mice for priming OVA-specific CD8 and CD4 T cells in response to various antigenic forms of ovalbumin. cDC1 were necessary and sufficient for priming CD8 and CD4 T cells in response to cell-associated OVA. cDC1 or cDC2, but not other APCs, were both sufficient for in vivo cross-presentation of OVA-antibody complexes, somewhat in contrast to earlier findings using OVA targeted to cDC surface receptors. Further, we excluded cross-dressing as a basis for presentation of OVA-antibody complexes. These results indicate that the differential process for loading peptides onto MHC-I or MHC-II is not simply intrinsic to different cDC subsets, but is dependent on the form of antigen, such as the route or mechanisms of antigen capture.

Poster – 35

ATDC (autologous tolerogenic dendritic cells) regulate CD8+ T cell population in kidney transplantation.

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In transplantation, kidney survival is impacted by allograft rejection, which is treated with nonspecific immunosuppressive drugs elevating the risk of infections, tumors, and toxicities. Tolerogenic myeloid cell-based therapy has been shown to be a promising strategy for treating patients undergoing transplantation due to specific antigen tolerance. Our team developed a procedure to generate autologous tolerogenic dendritic cells (ATDC), and the use of ATDC in cell therapy has been shown to improve transplant survival in animal models. As a result of these findings, a human GMP compliant ATDC procedure was developed. To evaluate the efficacy and safety of ATDC, a first-inman phase I/II clinical investigation was conducted. Regarding their in vitro characterization, ATDC have an enhanced metabolic profile, consuming considerable amounts of glucose and producing abundant levels of lactate. We also demonstrated that CD4+ T cells absorb the lactate produc ed by ATDC, lowering their glycolysis, activation, and proliferation levels. In our clinical trial, we found that ATDC reduced CD8+ T cell activation while increasing FoxP3 expression in the blood of kidney transplant recipients. In this project, we focus on discovering the mechanism of action of ATDC on the regulation of CD8+ T cells. In vitro, ATDC suppress CD8+ T cell proliferation, and this reduction is preserved when the ATDC supernatant is filtered (3Kda). This illustrates the involvement of secreted or depleted metabolites in the ATDC-regulated environment. However, in contrary to CD4+ T cells, lactate is not involved in CD8+ T cell suppression. One immunomodulator is known as indoleamine 2 3-dioxygenase (IDO), which is an enzyme that degrades and depletes tryptophan from the environment. Tryptophan depletion is known to inhibit the proliferation of lymphocytes. In our assays, the use of a specific IDO inhibitor proves clearly that tryptophan deficiency mediated by ATDC suppr esses the proliferation of CD8+ T cells. Furthermore, ATDC reduce CD8+ T cell activation and decrease their migratory capacity via a contact dependent mechanism. These findings clearly demonstrate the efficacy of ATDC as a therapeutic method to promote graft tolerance in kidney transplantation, as CD8+ T cells has a major role in allograft rejection in kidney survival.

Poster – 36

Back dans les bacs: rehabilitating the contribution of cDCs subsets in the maintenance of mucosal tolerance

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Peripherally induced regulatory T cells (pTreg) in the intestinal mucosa are critical in maintaining tolerance to the commensal flora. We and other have previously shown that pTreg differentiation is critically dependent on activation of latent TGFβ by αvβ8 integrin on CD11c-expressing antigen presenting cells (APC). Conventional dendritic cells (cDC) have long been thought to be the primary APCs of pTreg induction in the gut via αvβ8-mediated TGFβ activation. However, this prevailing concept was recently challenged by three groups. In their models, they excluded a role for cDC in the development of commensal-specific pTreg and shed light on new RORγt-expressing APC subsets in the development of commensal-specific pTreg, once again via αvβ8-mediated TGFβ activation. However, cDC remain the most abundant APCs in the gut, and while we do not challenge the importance of RORγt+APCs, our results put the DCs back into the picture. Here, thanks to mouse models that allow selective targeting of cDC subsets (and not RORγt+APC as confirmed by lineage tracing), we show that deletion of β8 integrin into either cDC1 or cDC2 is sufficient to decrease gut pTreg at homeostasis. αvβ8-expression by cDC1 or cDC2 is also required for optimal induction of dietary-specific pTreg. Finally, while cDC1- or cDC2- specific β8KO mice develop normally, mice lacking β8 integrin in both cDC1 and cDC2 develop late-onset ulcerative colitis. Thus, contrary to the recent exclusion of cDCs by aforementioned studies, our results instead show that αvβ8 expressing cDC1, -cDC2 and -RORγt+APCs play hand in hand in instructing tolerance at mucosal surfaces.

Poster – 37

No longer disguised: a novel flow cytometry panel to distinguish homeostatic from immunogenic mature dendritic cells

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As major antigen-presenting cells, dendritic cells (DCs) play a crucial role in determining the balance between tolerance and immunity. Depending on how the DC perceives an antigen, as dangerous or self, it will mature in an immunogenic or tolerogenic way, instructing the T cell to kill or tolerate antigen-bearing cells, respectively. Despite the clinical relevance, DC maturation state is seldomly monitored in the clinic due to the lack of appropriate markers. Furthermore, while signals driving immunogenic DC maturation have been well characterized, little is known about signals driving homeostatic DC maturation. Our lab recently established that the continuous uptake of apoptotic cells and cholesterol influx is a determining upstream trigger for homeostatic maturation of cDC1s. The process can be mimicked by engulfment of empty non-adjuvanted lipid nanoparticles (LNPs), while uptake of poly(I:C)-adjuvanted LNPs leads to immunogenic DC maturation. We now used this LNP-based approach to induce homeostatic versus immunogenic maturation to define markers that can distinguish both maturation states. To this end, we injected empty LNPs or pIC-coupled LNPs i.v. in mice, sorted splenic cDC1s after 2 or 8 hours and analyzed them by CITEsequencing. This generated a list of potential markers that were tested by flow cytometry in mouse and human. The “DC maturation panel” was validated by a Toxoplasma infection in mice, as well as by running tumor models to test its potential to monitor the outcome of anti-tumor immune responses. In summary, we generated a novel DC maturation flow cytometry panel that can be used to identify DC maturation states in mouse and human. This might aid in predicting the outcome of patient immune responses.

Poster – 38

Distinct migratory dendritic cell subsets cooperate to promote tissue-resident memory CD8+T cells specification in tumor-draining lymph nodes.

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Tumor infiltration by CD8+ T cells endowed with a tissue-resident phenotype (TRM) is generally associated to favorable outcomes in human tumors. Here, we delineate the requirements for migratory dendritic cells (DCs) during the activation of tissue-resident and effector cells within tumor-draining lymph nodes. We identify a discrete subset of activated CXCR6+CD103+ CD8+ T cells aligning with TRM in the lymph nodes. These cells are generated in tumor-draining lymph nodes in a process dependent on both XCR1+ migratory DC1s and IRF4-dependent migratory DC2s. By contrast, the wide majority of PD1- effector and PD1+TIM3+ exhausted cells solely rely on XCR1+ DC1s for their activation. Mechanistically, IRF4-dependent DC2s are less efficient at driving T cell proliferation but maintain CD103 expression during T cell activation, thereby enabling TRM specification. Altogether, these findings highlight the fine tuning of T cell fate and memory formation by the cooperation of multiple DCs subsets within draining lymph nodes.

Poster – 39

Decipher the role of dendritic cells in early immune surveillance during breast cancer development.

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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. In order to characterize the molecular and cellular mechanisms of early immune surveillance in triple negative breast cancer, we used a spontaneous mammary tumor model in mice (BLR-Cre, BRCA1f22- 24/f22-24, p53+/-) which recapitulates the different stages of development (Healthy, Pre-neoplasia and invasive carcinoma). Given the important role of dendritic cells (DC) in the initiation of antitumor immune response, we hypothesize they could play a central role in the anti-tumor immune surveillance. First, we shown that all DC subsets are present from the pre-neoplasia stage, but, the proportion of cDC1 and cDC2 decreases during tumor progression while plasmacytoid DC (pDC) remain stable. We also characterized the functional abilities of infiltrating DC ex vivo in response to TLR agonists. Furthermore, we performed the first in-depth analyses of DC types sorted from mammary tissues at the different stages by single cell RNA sequencing (scRNAseq). Preliminary analysis revealed DC heterogeneity (e.g., presence of mature DCs) and different activation states (e.g., DC in cell cycle) that will be compared between the different stages of development. By exploiting a spontaneous mouse mammary tumor model and powerful technologies to study early TNBC immune surveillance, our 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, in advanced tumors resistant to conventional immunotherapies.

Poster – 40

Mitochondrial metabolism regulates the immunogenic responsiveness of dendritic cells

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Dendritic cells (DCs) are antigen presenting cells that control T cell responses upon immunogenic activation. This is characterized by a metabolic reprogramming of DCs to elevate glycolysis. However, the relevance of mitochondrial metabolism and the electron transport chain (ETC) for the activation of conventional type 1 and 2 DC subsets (cDC1 and 2) is poorly defined. Here, we revealed that the immunogenic stimulation of cDC1s causes a rapid and sustained increase in their oxygen consumption, which is absent in cDC2s. To investigate the importance of this, we generated mice deficient for a component of the ETC complex III specifically in DCs. Indeed, the upregulation of costimulatory molecules, migratory capacity, ability to generate a T cell effector response and control cancer growth was notably impaired in complex III-deficient versus control splenic cDC1s upon activation, but only mildly affected in cDC2s. Mechanistically, the immunogenic functions of complex III-deficient splenic cDC1s were rescued by re-establishing electron flow through the ETC, but not ATP production, via transgenic expression of alternative oxidase in vivo. This suggests that a metabolic deregulation and not a bioenergetic crisis underlies the functional impairment of complex III-deficient cDC1s. In line, loss of complex III in splenic cDC1s, but not cDC2s, led to DNA hyp ermethylation in binding regions of the transcription factor PU.1, which is regulating DC activation. This caused impaired expression of early stimulus-induced genes in complex IIIdeficient cDC1s already in the steady state and hampered their timely immunogenic responsiveness. In conclusion, our findings manifest the importance and DC subset-specific requirement of a functional mitochondrial metabolism for the immunogenic activity of natural DCs.

Poster – 41

Immunotherapy with natural conventional type-1 dendritic cells excels at immune memory induction to eradicate cancer relapse

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The potential of dendritic cell (DC) vaccination against cancer is not fully achieved. While next generation vaccines using adoptive transfer of natural DCs are explored, little is known about the precise nature of the anti-cancer immune response triggered by different natural DC subsets and their relevance in preventing tumor recurrence. Here, we used mouse splenic conventional DC1s (cDC1s) or cDC2s pulsed with tumor cell lysates to generate DC vaccines. cDC1-based vaccination induced a stronger effector and memory CD4+ and CD8+ anti-tumor T cell response, leading to a better control of tumors treated therapeutically or prophylactically. In addition, using an experimental model of tumor relapse, we showed that adjuvant or neoadjuvant cDC1 vaccination improved natural anti-tumor immune memory, particularly by increasing the infiltration of CD4+ tissue resident memory T cells. This translated into complete rejection of tumor relapse. Our findings suggest that cDC1 vaccination excels at immune memory induction and prevention of cancer recurrence.

Poster – 42

Lactate sensing drive glioblastoma tumor regulation

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Glioblastoma is the most aggressive tumor of the central nervous system, with a median of survival less than 15 months after diagnosis. The standard of care, called “STUPP” protocol, consists of surgical resection when possible followed by a combination of radiotherapy and chemotherapy with Temozolomide, an alkylating agent. However, patients with glioblastoma who have followed the “STUPP” protocol are subject to a very high rate of tumor recurrence. This high rate of tumor recurrence can be explained by an immune environment rich in myeloid cells having immunosuppressive and/or pro-tumoral properties, such as macrophages promoting tissue repair, resolution of the inflammation and angiogenesis (macrophages having an anti-inflammatory and pro-tumor phenotype). We were interested in the acidic and hypoxic properties of brain tumors. More precisely, it has been shown that lactate, a metabolite derived from cell glycolysis to allow the production of ATP, used for the proliferation of tumor cells, is responsible for immuno-metabolic modifications of immune cells in other cancers. We were therefore interested in the effects of lactate on the phenotypes and functions of myeloid cells in the glioblastoma tumor microenvironment. To do this, we generated mouse models deficient for a lactate transporter in myeloid cells (Mct1Δmye) or for lactate signalling (Gpr81Δmye) or constitutively deleted for the lactate receptor Gpr132 (Gpr132-/-). In vivo studies indicate that Mct1Δmye deletion has a moderate impact on tumor development in males or females. Conversely, deletion of the Gpr132 receptor greatly impacts tumor development in both males and females, with Gpr132-/- mice developing much smaller tumors. Collectively, these results open two perspectives and fields of study on the impact of tumor resection and the effects of the lactate signalling pathway on the development of glioblastoma, in order to better understand the causes of tumor recurrence, with a view to improve treatments for patients suffering from brain tumors.