Technological Innovations in Immunology

Ecole doctorale thématique
en Immunologie

October 4th, 2022
Liège, Belgium

Scientific program
Abstract book

8h40 – 09h00: Welcome coffee and poster billsticking

09h00 – 09h05
Welcome address, *Thomas Marichal,* spokesperson of the EDT-Immunology

09h05 – 09h55
Opening Lecture
Chairs: TBD

Nicolas Gaudenzio, Infinity, Toulouse, France
MANTIS: an integrated digital system for 3-D deconstruction of human skin immune landscape

09h55 – 10h40
Short talk session 1
Chairs: TBD

Walther Brochier, UCLouvain
Defining the antigen specificity of $CD8^+$ T cells infiltrating bladder tumors in the context of BCG therapy

Elisa Brauns, ULB
Functional reprogramming of monocytes in acute and convalescent severe Covid-19 patients

Margot Meunier, ULiège
Lung neutrophil targeting has long-term consequences on cellular trafficking and endothelium homeostasis

10h40 – 11h10: Coffee break

11h10 – 12h00
Invited Lecture
Chairs: TBD

Kerstin Meyer, Wellcome Sanger Institute, UK
A spatial multi-omics atlas of the human lung reveals a novel IgA immune niche

12h00 – 12h10
Sponsor talk, *Vesna Melkebeek,* BD Biosciences
Discover BD's latest technologies to elevate your immunology research

12h10 – 14h00: Lunch & Poster session

14h00 – 14h50
Invited Lecture
Chairs: TBD

Maxime Dhainaut, Mount Sinai, NY, USA
Spatial CRISPR genomics – Perturb-map identifies regulators of the tumor microenvironment

14h50 – 15h35
Short talk session 2
Chairs: TBD

Domien Vanneste, ULiège
MafB-restricted local monocyte proliferation precedes lung interstitial macrophage differentiation

Guillem Sánchez Sánchez, ULB
Single-cell analysis of human fetal and pediatric γδ thymocytes reveals distinct functional thymic programming

Lucia Rodriguez Rodriguez, ULiège
The specific history of viral infections may pave the way for tumor development

15h35 – 15h45
Sponsor talk, *Manuel Cerban-Bautista,* Miltenyi
TBD

15h45 – 16h15: Coffee break

16h15 – 17h05
Flash talk session
Chairs: TBD

Pierre Maus, UCLouvain
Genetic analysis of rare families points to a pathogenic role for the cGAS/STING - IFN-I axis in Systemic sclerosis

Jelena Gabrilo, ULB
Identification of predictive biomarkers of response to anti-PD-1 treatment

Alexis Balthazar, ULiège
Respiratory gammaherpesvirus infection exacerbates experimental model of inflammatory bowel disease

Léna Puigdevall, UCLouvain
Role of the C-ter part of the IL-22Ra: Inflammation and mechanistic view

Clara Valentin, ULB
Maternal probiotics administration modulates the neonatal immune response against Influenza A infection

Meijiao Gong, ULiège
AlHV-1 infection causes oligoclonal expansion and activation of CD8+ T lymphocytes resulting in bovine malignant catarrhal fever via interaction with T cell signaling pathway

Arthur Poncelet, ULB
Deciphering the impact of EBV infection on functionnal reprogramming of monocytes using a relevant humanized mouse models

Joseph Jorssen, ULiège
High-dimension flow cytometry resolves human and murine eosinophilopoiesis and the unique dynamics of eosinophilia

17h05 – 17h55
Closing Lecture
Chairs: TBD

Yvan Saeys, VIB, UGhent, Belgium
Modeling immune system dynamics using single cell omics approaches

17h55 – 18h05
Awards & concluding remarks, *Stanislas Goriely, Laure Dumoutier & Frédéric Baron,* local representatives of the EDT-Immunology

Invited speakers

Maxime Dhainaut, Mount Sinai, NY, USA

Maxime received his PhD in immunology in 2015 from the Universite Libre de Bruxelles, under the supervision of Prof. Muriel Moser. During his graduate studies, he investigated the molecular mechanisms involved in regulatory T cells control of T cell priming, with a focus on Tregs/dendritic cells interaction (Dhainaut, Coquerelle et al., EMBO J, 2015). Maxime then joined the laboratory of Prof. Brian Brown at the Icahn Institute of Medicine at Mount Sinai, where he focused on the molecular mechanisms governing cancer cell escape from T cell immunoediting. Specifically, Maxime developed novel approaches for high-throughput CRISPR/Cas9 functional genomics with high dimensional phenotyping, using Protein Barcodes (Pro-Codes) (Wroblewska, Dhainaut et al., Cell, 2018). The main limitations of phenotyping screens, including Pro-Code/CRISPR genomics, is their incompatibility with spatial read out, which limits their application to phenotypes that can be measured in cell suspensions. Maxime recently developed Perturb-map, a platform for spatial CRISPR genomics, which allows to identify gene perturbations in tissue sections (Dhainaut, Rose et al, Cell, 2022). Using Pro-Code/CRISPR genomics and Perturb-map, Maxime identified genes regulating cancer cells sensitivity to T cell killing, expression of key inhibitory checkpoints, or orchestrating specific immune niche in the tumor microenvironment. Maxime is currently Associate Director in early discovery at Immunai.

Nicolas Gaudenzio, Infinity, Toulouse, France

Dr Nicolas Gaudenzio graduated in immunology from the University of Toulouse, France. Following a 4-year postdoctoral period in the laboratory of Stephen J. Galli, Department of Pathology, University of Stanford (CA USA), he became in 2019 Principal Investigator at the institute of immunology and infectious diseases of Toulouse (Infinity),Inserm France. In 2019, he was awarded a French ATIP-Avenir grant and a European Research Council (ERC) Starting grant and in 2020 he received the ACTERIA Early career Prize in Allergology and the Physiology-Medicine Prize from the French Academy of Sciences. Dr Gaudenzio heads a laboratory focused on the understanding how peripheral neurons and immune cells communicate to regulate inflammatory disorders via the use of transcriptomic and computational imaging approaches. https://www.infinity.inserm.fr/en/research-teams/team-3-n-gaudenzio/

Since 2019, Dr Gaudenzio is also the Chief Scientific Officer of the French-American biotech Genoskin that specializes in the generation of human data from bio-stabilized natural human skin.

Kerstin Meyer, Wellcome Sanger Institute, UK

Kerstin Meyer started her career in immunology, obtaining a PhD from the MRC Laboratory of Molecular Biology, University of Cambridge, where she studied the regulation of immunoglobulin gene expression. She continued to work in this field for a number of years before moving to the CRUK Cambridge Institute, where she investigated genetic risk for breast and lung cancer. She worked in close collaboration with clinicians and computational biologists, using transcriptional networks to understand the combined effect of inherited risk variants for cancer. She is fascinated by the way gene regulatory networks determine cell types and cell states. She is currently based at the Wellcome Sanger Institute, working towards generating a Human Cell Atlas and improving our understanding of how cell types cooperate in space to achieve organ function and how this can potentially be modified for therapy.

Over the last few years, Dr Meyer has led cell atlasing studies of the human lung over the lifetime, investigating lung development in the first two trimesters of pregnancy and charting the cell types in the healthy adult human lung and airways, which we have contrasted to those present in asthmatic individuals. She is particularly interested in lung immunity and has studied the immune responses to COVID-19 in the airways and blood of adults and children. In healthy adults, an unbiased cell atlasing approach has allowed her to describe a number of novel cell types and identified an IgA immune niche at the airway submucosal glands.

Yvan Saeys, VIB, UGhent, Belgium

Yvan Saeys is associate professor of Machine Learning and Systems Immunology at VIB and Ghent University. He is developing state-of-the-art data mining and machine learning methods for biological and medical applications, and is an expert in computational models to analyse high-throughput single-cell data. The methods he develops have been shown to outperform competing techniques, including computational techniques for regulatory network inference (best performing team at the DREAM5 challenge) and biomarker discovery from high-throughput, single cell data (best performing team at the FlowCAP-IV challenge). Yvan Saeys has published >200 papers in top ranking journals and conferences, ranging from methodological development in machine learning and bioinformatics to applications in cancer, immunology and medicine (Nature Immunology, Nature Methods, PNAS, Bioinformatics). The tools he develops have received several awards and are being used by international consortia.

Abstract book

#1. Approach using « second generation » immune checkpoint inhibitors for the treatment of triple-negative breast cancer.

Ancion M.[1], Bruyère D.[1], Hendrick E.[1], Pilard C.[1], Roncarati P.[1], Reynders C.[1], Lerho T.[1], Luyckx M.[1], Renard M.[1], Peulen O.[2], Delvenne P.[1], Herfs M.[1], Hubert P.[1]

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Immunotherapy is revolutionizing cancer treatment. However, only a subset of patients benefit from it, the majority of them showing limited or no response. Triple-negative breast cancer (TNBC) represents 10-20% of invasive breast cancers and has no specific treatment. The blockade of novel "second generation" immune checkpoints could be promising to enhance the number of responders. This project aims at highlighting new immune checkpoints and to study the impact of their inhibition on TNBC progression. To this end, we selected potential immune checkpoints that showed high mRNA expression in TNBC using bioinformatic analyses. Next, we chose the targets displaying a higher protein expression in TNBC compared to the 3 other categories of breast cancer (LumA, LumB and HER2+) by using immunohistochemistry. The proteins VISTA, sirp-α, CD47 and PVR were selected. Several murine syngeneic tumor models were used. Checkpoint expression was thoroughly investigated in 12 specific immune populations. Monoclonal antibodies and inhibitors against said immune checkpoints were selected. Using two syngeneic mouse models, the effect of the monoclonal antibodies on tumor growth as well as on composition of the immune tumor microenvironment was assessed. Tumor growth was significantly slowed down in Balb/C mice bearing 4T1 tumors treated with anti-VISTA and anti-TIGIT (PVR ligand) antibodies. Interestingly, anti-VISTA response seems to be mediated through $CD4^+$ and $CD8^+$ recruitment. In C57Bl/6 mice bearing E0771 tumors, treatment with anti-VISTA, anti-sirp-α, anti-CD47 and anti-TIGIT antibodies all significantly slowed down tumor growth. Further investigation will be carried out in order to highlight the immune cell populations and mechanisms at play.

#2. Influence of gammaherpesvirus infections on the antibody repertoire of their host.

Baiwir J.[1], Xiao X. [1], Lété C. [1], Machiels B.[1], Gillet L.[1]

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Gammaherpesviruses (GHVs) are ubiquitous viruses that have co-evolved with their hosts. Although these infections remain asymptomatic in most of the individuals, they can cause cancers, mainly lymphoproliferative disorders, in immunocompromised people. After primary infection, most of GHVs undergo latent expansion in germinal center (GC) B cells and persists in memory cells. In this project, using next generation sequencing and Murid Herpesvirus 4 (MuHV-4), a mouse GHV, we characterized the effect of a GHV infection on the antibody repertoire of its host. Firstly, we showed that B cells of MuHV-4 infected mice display distinct VDJ recombination frequencies and isotypes switching routes compared to the ones of non-infected mice. Secondly, using a YFP expressing MuHV-4 strain, we compared the repertoire of MuHV-4 infected and non-infected B cells. We observed that infected cells could display a distinct repertoire than the one of their non-infected counterparts and especially that these infected cells exhibit a different pattern of clonal expansion. These results suggest therefore that MuHV-4 infection is not random and establishes preferentially in some B cells and mainly affects the future of infected cells. In the future, identifying the determinants of these infectable B cell subsets and what triggers these differences of clonal evolution could help us to better understand GHVs lifecycle and the lymphoproliferative disorders that they induce. More generally, it could help us to better understand how our environment and especially some infections agents shape our immune responses.

#3. Respiratory gammaherpesvirus infection exacerbates experimental model of inflammatory bowel disease.

Balthazar A.[1], Sandor R. [1], Javaux J. [1], Gillet L.[1], Machiels B.[1]

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Inflammatory bowel diseases (IBD) is a widespread disease worldwide that affects the quality of life of all age groups. Although the precise etiology is still poorly defined, multiple factors, such as genetic background, environmental triggers, and mucosal immune dysregulation, have been associated to the pathogenesis. Epidemiological studies in humans have proposed a key influence of gammaherpesvirus (γHV) infections given that Epstein Barr virus has been associated with the development and exacerbation of IBD. However, the presence of confounding factors makes it difficult to distinguish between a causal role from an innocent side effect. In this study, we used dextran sulfate sodium (DSS)-induced colitis and Murid herpesvirus 4 (MuHV-4) to investigate in mice whether and how γHV infection could shape deleterious immune responses responsible for IBD exacerbation. By monitoring weight loss, clinical scoring and pathological lesions, we demonstrated a significant exacerbation of experimental colitis in MuHV-4-infected individuals. Interestingly, immunophenotyping of leukocytes isolated from the *lamina propria* revealed a massive increase in T cell infiltration in the colon of MuHV-4 pre-infected mice. These T cells show major phenotypic changes such as overexpression of activation markers and exacerbation of cytotoxic properties. These differences correlate with an enhanced Th-1 response in draining lymph nodes, consistent with a significant accumulation of CXCL9-producing monocytes that could attract T cells but also dictate their cytotoxic polarization. Overall, these initial data highlight the ability of certain γHV to trigger deleterious intestinal immune responses that could explain some inter-individual differences in IBD susceptibility.

#4. Highlights on Usutu virus infection in a susceptible bird species: the domestic canary (*Serinus canaria)*.

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Usutu virus (USUV) is a mosquito-borne Flavivirus pathogenic to avian species. It is often associated with massive die-off events, some of which occurred during the winter, a vector-free period. The main hypothesis to explain how USUV "overwinters" is a bird-to-bird transmission, as shown for the closely-related West Nile virus (WNV). To assess this hypothesis, we first experimentally challenged canaries with intranasal inoculation of USUV, which led to a systemic dissemination of the virus comparable to that observed after parenteral inoculation, provided the inoculated dose was sufficient (> 10^2 $TCID_{50}$). Next, we co-housed infected birds (after intranasal or intradermal inoculation) with naive sentinels, to determine if an horizontal transmission could be reproduced experimentally. However, since no evidence of seroconversion or viral RNA was noticed in the sentinel group, we failed to prove such a transmission. Besides, although we highlighted the oronasal excretion of infectious viral particles in infected birds, the shed titres and/or the direct contacts between birds seemed insufficient to allow a direct transmission, in our experimental conditions. To complete our study, we infected *ex vivo* tracheal explants of canaries to determine the viral tropism at the inoculation site, but we observed only very limited signs of viral replication or amplification. Contrary to its human counterpart, the avian tracheal epithelium seem thus to not be permissive to USUV infection. Further research on the cell tropism is needed to identify the cell type(s) involved in the initial replication and dissemination of USUV after intranasal inoculation and to understand how the bird-to-bird transmission might occur in wildlife.

#5. Mitochondria as a potent driver of T cell dysfunction mediated by Tryptophan-degrading enzymes.

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Expression of tryptophan-catabolizing enzymes IDO and TDO was found in various tumors and associated with local immune suppression, notably by inhibiting anti-tumor T cell functions. However, the exact mechanisms making T cells sensitive to these enzymes remain unclear. Using an *in vitro* CRISPR knockout screening approach, we identify mitochondria as a potent driver of T cell dysfunction mediated by tryptophan-degrading enzymes.
Primary $CD8^+$ T cells were isolated from Cas9 transgenic mice expressing a P1A antigen-specific TCR, stimulated with P1A-expressing tumor cells, and transduced with a genome-wide CRISPR knockout library. Seven days after the first stimulation, $CD8^+$ T cells were re-stimulated in a control ($Tryptophan^{high}$, $kynurenine^{low}$) or selective ($Tryptophan^{low}$, $kynurenine^{high}$) medium, mimicking the function of trp-degrading enzymes *in vitro*. After four days of selection, gDNA was extracted from remaining living cells and sgRNA representation was assessed by sequencing.
Among genes whose deletion improved T cell survival when exposed to trp deprivation, we identified genes involved in mitochondrial functions, including OXPHOS (e.g. *NDUFA1*) and translation (e.g. *MRPL20*), indicating the involvement of mitochondria in T cell dysfunction mediated by trp deprivation. Individual deletion of these genes in T cells increased their survival in the selective medium, but reduced cell growth in the control medium. Mitochondrial potential was increased in T cells restimulated in absence of trp, but mass was decreased after four days of culture. Respiration and glycolysis, as well as NAD/NADH ratio were also altered in these cells. Altogether, our results suggest a link between mitochondrial alterations and T cell dysfunction mediated by tryptophan-catabolizing enzymes.

#6. Identification of a TAP-independent antigen.

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Among the mechanisms of tumor escape, down-regulation of the TAP transporter has been observed in a wide range of tumors and leads to a dramatic decrease of surface MHC class I expression. Nevertheless, some antigenic peptides called TEIPP (T cell epitope associated with impaired peptide processing) were shown to be expressed in TAP-deficient tumors. These peptides could therefore represent antigenic targets of interest in the frame of cancer immunotherapy.
Here we describe a CTL, which was isolated from a melanoma patient and recognizes an antigenic peptide that is presented in the absence of the TAP transporter. Treatment of TAP-negative cells with a signal peptide peptidase (SPP) inhibitor, ZLL-2-Ketone, resulted in a complete loss of recognition of these cells by the CTL. This suggests that the TAP-independent peptide recognized by CTL6 is derived from a protein signal sequence. Approaches based on immunopeptidomic or the screening a lentiviral cDNA library, constructed using mRNA from TAP negative cell lines will be used to try to identify the peptide recognized by the CTL.

#7. Functional reprogramming of monocytes in acute and convalescent severe COVID-19 patients.

Brauns E.[1], Azouz A.[1], Grimaldi D.[2], Xiao H.[3], Thomas S.[1], Nguyen M.[1], Olislagers V.[1], Vu Duc I.[1], Orte Cano C.[4], Del Marmol V.[4], Pannus P.[5], Libert F.[6], Saussez S .[7], Dauby N.[1,8], Das J.[3], Marchant A.[1], Goriely S.[1]

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Severe COVID-19 disease is associated with dysregulation of the myeloid compartment
during acute infection, with the emergence of HLA-DR^{lo} monocytes. This population had already been described in other diseases like sepsis and in this circumstance impact the outcome of the acute event but eventually also the long-term mortality. Little is known about the eventual persistence of this immune alteration in COVID-19. Herein, we evaluated Toll-like receptor-induced cytokine responses in a cohort of mild to critical patients during acute or convalescent phases (n=97). In the acute phase, we observed impaired cytokine production by monocytes in the most severe patients. This capacity was globally restored in convalescent patients. Yet, we observed increased responsiveness to TLR1/2 ligation in patients that recovered from severe disease, indicating that these cells display distinct functional properties at the different stages of the disease. We identified a specific transcriptomic and epigenomic state in monocytes from acute severe patients that can account for their functional refractoriness. This profile displays similarities with monocytes from patients that recovered from sepsis. The molecular profile of monocytes from recovering patients was distinct and characterized by increased chromatin accessibility at AP1 and MAF loci. These results demonstrate that severe COVID-19 infection has a profound impact on the differentiation status and function of circulating monocytes both during the acute and the convalescent phases in a completely distinct manner. In order to better understand the key events underlying the cytokine-driven emergence of HLA-DR^{lo} monocytes in the context of severe inflammation, we are now developing dedicated humanized mice models.

#8. Defining the antigenic specificity of CD8$^+$ T cells infiltrating bladder tumors in the context of BCG therapy.

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Despite having been used for decades in routine clinical practice, the mechanisms underlying the antitumor effect of BCG (Bacillus Calmette-Guérin) in the context of non-muscle invasive bladder cancer (NMIBC) is not yet fully understood. We investigate the role of T cells in the context of BCG therapy, with a focus on their specificity.
We collected tumor, blood and urine samples from 4 bladder cancer patients, before, during and after induction BCG treatment. We extracted the T cells from these samples, and performed single-cell RNA sequencing. Then, we analyzed the frequency and the phenotype of extracted T cell clonotypes in each sample and we selected clonotypes of interest.
Next, we expressed the TCRs (T cell receptors) of the selected clonotypes in a reporter cell line and screened them for recognition of mutant peptides predicted from RNA-seq and WES data from the tumor of the patients. In one patient, we detected tumor-infiltrating lymphocytes (TILs) that recognize tumor-specific mutations. In this patient, these T cells do not seem to play a major role in the antitumor mechanism of BCG. To further define the specificity of the T cells that did not show reactivity against the mutant peptides, we are performing cDNA library screenings from tumoral RNA from the patients. For this purpose, we developed a technique that allows for a fast and sensitive detection of cDNA fragments encoding a recognized antigen. Using this technique, we found a cDNA fragment that encodes an antigen recognized by another TCR from the same patient in which we found tumor-specific TCRs using the mutant peptides screenings. We are currently characterizing this antigen, and proceeding with the cDNA screenings for the other patients.

#9. STAT3 activation by the type I IL-20 receptor: two ways are better than one.

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The IL-20 subfamily of cytokines is composed of several members, including the interleukin-19 (IL-19), interleukin-20 (IL-20) and interleukin-24 (IL-24). These cytokines are produced by immune cells and mostly act on non-immune cells, as keratinocytes. They participate in the maintenance of epidermal barrier integrity by promoting antimicrobial peptide production, keratinocyte proliferation and differentiation, and also chemokine expression. However, an excessive and uncontrolled production of these cytokines can cause inflammatory diseases, such as psoriasis.
To induce their biological effects, IL-19, IL-20 and IL-24 bind to the type I IL-20 receptor (IL-20R), composed of the IL-20Rα and IL-20Rβ chains. IL-20 and IL-24 also bind to the type II IL-20R, consisting of the IL-22Rα and IL-20Rβ chains. Once bound to their receptor, these three cytokines activate the JAK-STAT signaling pathway.
By studying the type I IL-20R signaling, we recently discovered that, besides the classical STAT activation pathway, STAT3 can also be activated via a non-canonical pathway which is independent of receptor tyrosine residues. Indeed, cells expressing a mutant tyrosine-less alpha chain in their type I IL-20 receptor still have an activation of STAT3 but not of STAT1 or STAT5, after being stimulated by IL-24. This non-canonical STAT3 activation pathway is dependent on the IL-20R-Cter region and is similar to the one discovered in the IL-22R, showing that several members of the IL-20 subfamily of cytokines share a tyrosine-independent STAT3 activation. As STAT3 plays a considerable role in the development of psoriasis, we believe that preventing this non-canonical STAT3 activation might be a targeted therapy. In fact, we would be blocking the deleterious effects of IL-19, IL-20 and IL-24 while leaving the good ones intact. In the future, we will define the role of the IL-20R-C-ter region on reconstructed epidermis models.

#10. Integrative Approach in the Context of BNT162b2 mRNA COVID-19 Vaccine in Allo-HCT Patients: System Vaccinology.

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Systems vaccinology contributed to elucidate molecular responses to vaccines in healthy subjects. The diffusion and periodic outbreaks of SARS-CoV-2 virus further compelled the early protection of allo-HCT patients with efficient immunization programs.

In this project, we aim to identify predictors of immune response following BNT162b2 mRNA vaccine administration through a systems biology approach integrating traditional and spectral flow cytometry to multiplex ELISA and bulk RNA sequencing data in allo-HCT patients. We observed that anti-RBD IgG and neutralizing Ab against wild-type SARS-CoV-2 were detected in 86% and 49% of allo-HCT recipients after two doses, versus 100% and 88% in healthy adults (n= 40), respectively. Clinical factors associated with high Ab titers included absence of cGVHD and rituximab one year prior to vaccination. Third dose, administered a median of 153 days after the first dose, significantly increased Ab response. Consequently, 87%, 82% and 61% of allo-HCT patients had detectable neutralizing Ab against wild-type, delta and omicron variants, respectively. cGVHD remained associated with a lower response. Multiplex ELISAs on samples at day 0 and +1 after the third dose revealed that several cytokines significantly increased following vaccination. This demonstrates the activation of both innate and adaptive immune system following booster dose. Spectral flow cytometry will also be used to study T cells-specific (i.e. anti-Spike) responses to the vaccine at days 0, +21 and +49 after the first dose, at days 0 and +28, and months 6 and 12 from booster (unpublished). Since chronic GVHD and immune suppressive therapy with rituximab has a major impact on B cells response, we will further stratify the cohorts to taking such variables into account.

#11. scRNA-sequencing of Mouse and Human Tumors reveals new cDC2 subsets.

Caro A.A.[1,2,3], Kancheva D.[1,2], Hadadi E.[1,2], Murgaski A.[1,2], Elkrim Y.[1,2], Brughmans J.[1,2], Debraekeleer A.[1,2], Laoui A.[1,2]

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While cDC1s represent a rather homogenous population, the functional and phenotypic heterogeneity of cDC2s remains enigmatic. Although several cDC2 subsets have been identified in mice and humans subsets in steady-state and inflammation, their concurrent and comprehensive understanding in different murine and human tumors is lacking. Indeed it is key to also understand these cDC2 subsets in cancer, especially for the development of immunotherapies.
To appreciate the cDC2 heterogeneity across mouse and human tumors at the transcriptomic level, we generated murine and human cancer scRNA-seq meta-datasets. The murine meta-data was generated from 5 murine tumor models, while the human meta-data was made from 8 different cancers to assess the translatability of our observations in murine cancers and appreciate species-specific variations.
cDC2s exhibited a profound heterogeneity in cancer, wherein some known subsets including Inf-cDC2, CD301b$^+$ cDC2, and DC3 could be identified, while some others were novel, including the cDC2-neutrophil cluster, and CD7$^+$ cDC2. We validated some of the new cDC2 subsets at the protein level using flow cytometry and assessed their intratumoral localization via spatial transcriptomics. Importantly, cDC2 heterogeneity in human cancers revealed a certain level of conservation with their murine counterparts.
Overall, using cutting-edge techniques we show that cDC2s in different murine and human cancers exhibit additional heterogeneity to what is known in literature. We provide a comprehensive understanding of these novel cDC2 subpopulations at the transcript and protein levels, which could serve as the foundation for employing these cells in the development of anti-cancer immunotherapies including DC vaccines.

#12. Diesel particles exposure favors lung cancer progression through induction of an immunosuppressive microenvironment.

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Air pollution, and more specifically fine particulate matter, is a major health problem. These particles are characterized by a small diameter and a deep penetration into the respiratory track. Indeed, several epidemiological studies have reported a significant association between exposure to fine particles and the development of both lung cancer and chronic respiratory/cardiovascular diseases. However, the impact of fine particles on the pathogenesis of lung cancer progression and the underlying mechanisms remain unclear.
The aim of this study was to determine whether exposure to fine particles may create a lung microenvironment suitable for lung cancer progression.
To this purpose, C57BL/6 mice were exposed to diesel exhaust particles (DEP) by intratracheal instillations. After 2 instillations of DEP (200µg/mL), lungs were collected and the immune microenvironment was analyzed by flow cytometry using a set of specific antibodies. Among neutrophils, polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) are of particular interest for their role in cancer progression.
To evaluate whether the inflammatory microenvironment conditioned by DEP had an impact on tumor progression, mice were injected orthotopically with Lewis Lung Carcinoma (LLC) cells.
DEP-instilled mice display an increased lung tumor progression as compared to sham-treated mice. Tumor microenvironment neutrophil counts and FACS analysis have revealed increase neutrophil counts. Our results indicate that $CD14^{int}$ and $CD14^{high}$ PMNs are increased in the lung of DEP instilled mice.
Exposure to DEP contributes to the creation of a supportive lung microenvironment for tumor progression in which recruited neutrophils are probably involved. We will perform systematic T-cell proliferation assays to characterize PMN-MDSCs immunosuppression capacity.

#13. Differential innate inflammatory response and macrophage polarization in male and female mice with *Streptococcus agalactiae*-induced pneumonia and potential role of microRNA-223-3p.

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While number of studies have shown that biological sex is a risk factor in the incidence and severity of infection-induced inflammatory diseases, the underlying mechanisms are still poorly understood. In this study, we compared the innate inflammatory response in male and female mice with group B streptococcal (GBS)-induced pneumoniae. Although male and female mice displayed similar bacterial burdens, males exhibited more innate inflammatory cytokines and chemokines and a higher proportion of infiltrating monocytes/macrophages. The analysis of the distribution of macrophage subtypes M1 (pro-inflammatory) versus M2 (anti-inflammatory) yielded a higher M1/M2 ratio in infected males compared with females. Given the importance of the chromosome X-linked microRNA-223-3p (miR-223-3p) in modulating the inflammatory process and macrophage polarization, we investigated its potential contribution in sex bias of GBS-induced innate inflammatory response. Knock-down of miR-223-3p with specific antagomiR resulted in increased inflammatory response and higher M1/M2 ratio following GBS infection. Notably, compared to male mice, we detected higher amount of miR-223-3p in macrophages from females that correlated negatively with M1 phenotype. These results suggest that differential expression of miR-233-3p may impact macrophage polarization, thereby contributing to finetune sex difference in inflammatory response.

#14. Modifications of pulmonary macrophages profile after exposure to heated tobacco products.

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Since 2015 a new method of tobacco consumption, consisting of heating tobacco leaves with the help of an electric device, has been placed on the market. According to the industries, heating instead of burning tobacco reduces the amount of carbon monoxide and carcinogenic components usually found in cigarette smoke, making this tobacco consumption much less harmful than conventional cigarettes. However, few independent studies have investigated the impact of heated tobacco consumption on the lung. In the present work, we are studying the potential effects of heated tobacco smoke on pulmonary homeostasis and immune response using in vivo expositions models.
Mice were exposed to heated tobacco smoke daily for three weeks and the bronchoalveolar lavages (BAL) were collected for analysis of the immune cell populations. Our preliminary data showed an increase in the total number of alveolar macrophages in the BAL of heated tobacco exposed mice when compared to control mice. After further analysis, we noticed that these macrophages are loaded with lipid droplets, and an increase in the amount of total lipids and peroxidized lipids has been demonstrated. The source of these lipids remains to be determined, whether it is a de novo synthesis or a change in the composition of the surfactant after exposure to heated tobacco that could disrupt the lipid capture. Interestingly, the emergence of lipid-laden macrophages in the lung has often been linked to many respiratory diseases (fibrosis, asthma and COPD, …) as well as a larger susceptibility to bacterial and viral infections. In addition, an ex vivo model showed that these macrophages had a reduced capacity to phagocyte bacterial particles compared to our controls. Other analyses revealed that these lipid-laden macrophages express more anti-inflammatory cytokines like interleukin-4 and interleukin-13, suggesting that these immune cells could be less responsive to infection.
These preliminary data suggest that the daily consumption of heated tobacco is not as harmless as claimed by the tobacco industry.

#15. Characterization of different exhausted CD8 T cell populations associated to tumor progression.

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The failure of tumor-infiltrating T lymphocytes to eradicate cancer cells is mainly due to dysfunction (or exhaustion) of T cells becoming progressively hyporesponsive to the tumor, which poses constraints on anti-tumor T cell-based therapies.
Our lab has recently developed a versatile model of autochthonous hepatocarcinoma based on hydrodynamic transfection of mice with a combination of oncogene-expressing plasmids, associated with ovalbumin (OVA) as a surrogate for a tumor-associated neoantigen. In the absence of oncogenes, OVA is expressed by non-cancerous hepatocytes.
Transfer of OVA-specific OT-I lymphocytes in these groups of mice allowed us to follow antigen-specific T cell fate in response to the same antigen in the context of healthy, precancerous, or advanced hepatocarcinoma liver environment. Our preliminary results reveal that hepatocarcinoma drives a specific exhaustion program in liver infiltrating T cells, with the development of a higher proportion of terminally exhausted T cells, compared to a chronic non-tumor induced liver stimulation.
These observations suggest that, in addition to chronic antigenic stimulation, the hepatocarcinoma environment plays a specific role in driving terminal T cell dysfunction. Further experiments will (i) define the metabolic, transcriptional, and epigenetic signatures of CD8+ T cell exposed to tumor-associated or "healthy" liver chronic antigenic stimulation; (ii) identify the roles of metabolic regulators and selected gene candidates in the exhaustion profile driven by healthy and cancerous hepatocytes.

#16. A single oral immunization with a replication-competent adenovirus-vectored vaccine protects mice from influenza respiratory infection.

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Abstract

The development of effective and flexible vaccine platforms is a major public health challenge as recently highlighted by the COVID-19 pandemic. Adenoviruses (AdVs) are easy to produce and have a good safety and efficacy profile when administered orally as demonstrated by the long-term use of oral AdV 4 and 7 vaccines in the US military. These viruses therefore appear to be the ideal backbone for the development of oral replicative vector vaccines. However, research on these vaccines is limited by the ineffective replication of human AdVs in laboratory animals. The use of mouse AdV type 1 (MAV-1) in its natural host allows infection to be studied under replicative conditions. Here, we orally vaccinated mice with MAV-1 vectors expressing the full length or the "headless" hemagglutinin (HA) of influenza to assess the protection conferred against an intranasal challenge of influenza. We showed that while the headless HA vector did not generate any significant humoral or cellular immune response to influenza, a single oral immunisation with the full-length HA vaccine generated influenza-specific and neutralizing antibodies and completely protected the mice against clinical signs and viral replication.

#17. Impact of anticodon tRNA modification on antitumor immune response in melanoma.

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The stimulation of the immune system in order to direct an anti-tumoral response is now holding significant promises in the treatment of cancer patients. Specifically, the development of immune checkpoint inhibitors and immunotherapy is changing the treatment perspectives of cancer patients. Melanoma is the most aggressive form of skin cancer whose incidence and mortality constantly increased over the last 40 years. Recent discoveries changed the therapeutic intervention landscape of melanoma patients. In our lab, we discovered the key role of anticodon tRNA modifications and specific mRNA translation reprogramming in melanoma resistance to targeted therapy (*Rapino et al, Nature 2018*). In this study, we aim to uncover the importance of anticodon tRNA modification in the regulation of the melanoma immune response. We assessed the propensity of >50 tRNA modifying enzymes to regulate proteome homeostasis and to promote anti-tumoral immune regulation. *In vivo* validation experiments highlighted the importance of specific enzymes in the trigger of an anti-tumor immune response. We discovered that the loss of some anticodon tRNA modification enzymes in melanoma tumors reduced tumor growth in mice in a T-cell dependent manner. Systematic ribosome profiling experiments are currently ongoing to investigate how the loss of anticodon tRNA modification enzymes impacts on specific mRNA translation. Together, our work uncovered the importance of anticodon tRNA modification in the regulation of the melanoma immune response and highlight the importance of specific tRNA modification in cancer immunology.

#18. Regulation of type 2 helper T cell by the CD27/CD70 pathway in the context of the adipose tissue and obesity.

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During obesity, the immune landscape of adipose tissue (AT) is profoundly modified, leading to the development of a systemic low-grade chronic inflammation responsible for obesity-associated disorders such as type 2 diabetes, cardiovascular disease, liver disease and cancer. Increasing evidence suggests that the maintenance of a type 2 anti-inflammatory environment is key for AT homeostasis and resistance to obesity. In particular, ILC2s are the main producers of IL-5 and IL-13 and have been shown to promote AT homeostasis by several mechanisms involving eosinophils and alternatively activated macrophages as well as beige adipocyte precursors. By contrast, the role of Th2 cells, a minor population of AT, is less clear. In this work, we sought to better characterize AT resident Th2 cells and their role in tissue homeostasis and resistance to obesity. We identified CD27, a costimulatory receptor known to promote the activation and survival of Th1 type cells and the accumulation of memory T cells, as a negative regulator of Th2 development in the adipose tissue.
Indeed, CD27KO mice displayed higher number of Th2 cells in the adipose tissue at steady state and gained less weight when fed a high fat diet. More importantly, CD27 KO mice showed improved glucose resistance, as well as reduced liver steatosis and blood triglyceride levels compared to WT. This was linked to greater numbers of functional Th2 cells, eosinophils and alternatively activated macrophages. Some evidence points to PD-1 as a mediator of CD27 regulation. Indeed, CD27 promoted PD-1 expression in AT Th2 cells and PD-1 blockade or deficiency led to their accumulation. Overall, our results show a novel role for the costimulatory receptor CD27 in the regulation of AT homeostasis and type 2 adaptive responses.

#19. The importance of lactate in T cell-mediated anti-tumour response.

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Cells need to supply themselves with energy using different biochemical pathways. They can produce ATP by performing oxidative phosphorylation and/or glycolysis. During T lymphocyte activation, glycolysis is initiated and lactate must be evacuated from the cytoplasm. Monocarboxylate transporter 1 (MCT1) is primarily responsible for cellular lactate removal and is essential for T cell proliferation. To study its final function after T cell activation, MCT1 was specifically deleted in T cells (SIc16a1.CD4Cre, designated as knockout, KO). Metabolism of T cell in wild-type (WT) and KO mice was investigated thanks to a flow cytometry assay that uses Puromycin to quantify the rate of mRNA translation on a cell-by-cell basis. Puromycin is an inhibitor of ribosome-catalysed protein synthesis. Thus, puromycin is incorporated into the C-terminal side of the nascent chain and can be detected by using flow cytometry and antibody specific to puromycin. By incorporing inhibitors of different metabolic pathways, the preferential metabolic pathways used by T cells to support protein synthesis can be demonstrated. Three inhibitors are used: 2-DG, an inhibitor of glycolysis, oligomycin, an inhibitor of oxidative phosphorylation and DON, an inhibitor of glutaminolysis. Metabolism and lactate are important elements in the field of anti-tumor immunity. Using our puromycin-based assay, we plan to characterize metabolically the different subsets of tumor-infiltrating MCT1-deficient T cells in a mouse model of hepatocellular carcinoma (HCC).

#20. Identification of predictive biomarkers of response to anti-PD-1 treatment.

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Despite their well-established clinical efficacy, durable clinical response to anti-PD-1 therapy has been observed in only a fraction of cancer patients. Therefore, the aim of our project is to understand regulatory mechanisms underlying the resistance/response to anti-PD-1 treatment and to identify predictive biomarkers which could help us select the patients that are more likely to respond to PD-1 blockade.
In order to do so, we have taken advantage of a mouse model of bilateral tumor implantation displaying a dual response. DBA/2 mice implanted with the P815 mastocytoma respond to anti-PD1 treatment in a bimodal fashion, with a fraction of mice displaying a complete response, while a similar proportion of the mice fail to respond to this treatment. The development of a bilateral tumor implantation enabled us to surgically remove one tumor sample before the treatment, purify tumor-infiltrating immune cells and characterise them by flow cytometry and RNA-Seq analysis. Following anti-PD-1 treatment, mice were assigned to a progressor vs regressor group based on the growth of the contralateral tumor mass.
Our data suggest that MHC class II expressing myeloid cell subsets showing increased expression of PD-L1 seem to correlate with better response to anti-PD-1 treatment. Single-cell RNA-seq reveals pro-inflammatory character of tumor infiltrating myeloid cells in regressors (INF signature) but also associates the enrichment of immune regulatory TAM subset with better response. Response to anti-PD-1 treatment seems to be correlated as well with the increased frequency of tumor-reactive, $P1A^+$ $CD8^+$ TILs. However, identification of myeloid cell clusters revealed by the scRNA-seq and understanding their functional heterogeneity and developmental trajectory is still ongoing.

#21. Targeting the CCL5-CCR5 axis to tackle chemoresistant malignant pleural mesothelioma.

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Malignant pleural mesothelioma (MPM) is an aggressive cancer with limited treatment options. Despite advances in immunotherapy, the standard treatment consists of chemotherapy combining pemetrexed and cisplatin. Unfortunately, in a majority of cases, tumor chemoresistance limits average patients' survival to one year. CCL5 is a chemokine overexpressed in various types of cancer and interaction with its main receptor, CCR5, increases tumor development. Pharmacological inhibitors are currently studied in clinical trials. Evidence for a potential role of CCL5 in tumor resistance to chemotherapy is still scarce.
First, we generated cisplatin-resistant human and mouse mesothelioma cell lines. In order to better understand the potential mechanisms leading to cisplatin resistance and to determine if CCL5 and CCR5 are implicated, we performed several cellular and biochemical analysis both *in vitro* and *in vivo*.
In vitro, measurement of CCL5 expression in conditioned media of murine mesothelioma cells (AB12) and human cells (MSTO-211H) showed that cisplatin-resistant cells secreted higher amounts of CCL5 as compared to parental cells.
BALB/C mice and Nude mice were subcutaneously injected in both flanks with cisplatin-resistant and parental AB12 and MSTO-211H cells. Cisplatin-resistant tumors showed increased growth rate and higher CCL5 and CCR5 expression when compared to their parental counterparts. Flow cytometry analysis of dissociated cells from these tumors showed an increased recruitment of F4/80 macrophages with a M2 phenotype (CD206 positive cells) in resistant tumors compared to parental tumors. M2 macrophages present in resistant tumors express CCR5. Treatment with Maraviroc, a CCR5 inhibitor, decreased the growth rate of tumors compared to the control group.
In conclusion, our results suggest that the CCL5-CCR5 axis might contribute to cisplatin resistance in MPM. These results also underline the potential involvement of changes in tumor microenvironment in resistance to chemotherapy via the recruitment of CCR5+ M2 macrophages. Furthermore, *in vivo* treatment with Maraviroc significantly decreased the growth of resistant tumors which reinforces our initial hypothesis.

#22. Subcutaneous Inoculation of Usutu Virus Induces a Lethal Neuroinvasive Disease in Wild-Type 129/Sv Pups.

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Usutu virus (USUV) is an emergent mosquito-borne flavivirus. Originating from Africa, it spread across Europe over the last decades. USUV is now becoming endemic and co-circulates with its close parent the West Nile virus, causing seasonal mass mortalities in diverse bird species as well as occasional neurological diseases in healthy and immunocompromised mammals, including humans. The risk factors responsible for these sporadic events remain unknown and thus, the growing number of cases diagnosed in EU in the past few years highlights the crucial need for a relevant and reproducible mammalian model of USUV infection. Adult immunocompetent mice proved to be quite resistant to USUV infection, with scarce individuals suffering from a neuroinvasive disease. Although newborn mice seem highly prone to this condition, no study specifically addressed this question so far. We assessed the age-dependent susceptibility of immunocompetent 129/Sv mice to USUV by comparing 6 groups (5, 7, 9, 11, 13 or 15 days-old) of 8 female pups. Each pup was inoculated subcutaneously with 10^6 TCID50 of USUV (strain USU-BE-Seraing/2017, lineage Europe 3). While all the pups infected at 5 days-old died before 10dpi, lethality dropped to 75%, 50%, 37.5%, 12.5% and 0% for the 7, 9, 11, 13 and 15 day-old group, respectively. All symptomatic individuals shared clinical and molecular evidence of neurotropic infection whatever the age group. Interestingly, survivors did not show any symptom and presented a marked decrease in viral titers and dissemination, suggesting that the outcome of the infection is determined at a very early stage after inoculation. Future studies are needed to identify the exact host-pathogen interactions involved in the development of susceptible or resistant phenotypes in mice.

#23. AlHV-1 infection causes oligoclonal expansion and activation of CD8$^+$ T lymphocytes resulting in bovine malignant catarrhal fever via interaction with T cell signaling pathway.

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Alcelaphine herpesvirus 1 (AlHV-1) is a member of the *Gammaherpesvirinae* subfamily and establishes asymptomatic latent infection in its natural host species, the wildebeest. Cross-species transmission to various ruminant species including cattle can occur, resulting in the induction of malignant catarrhal fever (MCF), a deadly peripheral T cell lymphoproliferative disease. Here, we first confirmed in the bovine species that AlHV-1 latency-associated gene expression is essential for persistent infection of CD8$^+$ T cells and MCF development. Next, we performed an in-depth characterization of peripheral CD8$^+$ T cells during bovine MCF. T cell receptor sequencing of both CDR3α and β revealed oligoclonal expansion of CD8$^+$ T cells, and we observed severe transcriptomic and epigenetic changes in CD8$^+$ T cells using RNA-seq and ATAC-seq analyses. MCF was associated with significant enrichment of gene expression involved in proinflammatory cytokine signaling, cell cycle, TCR signaling, chromatin remodeling but reduced expression of genes involved in adhesion. We observed upregulation of *MKI67*, as well as effector molecules like *GZMA*, *GZMK* and *GNLY*. Whereas *TCF7*, *CCR7* and *CD226* were downregulated, exhaustion genes like *PDCD1*, *EOMES* and *TOX2* were upregulated in MCF, confirmed by analysis of open chromatin. Such unique MCF-related transcriptomic program was confirmed in clusters containing infected CD8$^+$ T cells by single-cell RNA-seq analysis. Analysis of the viral

genome transcription identified viral genomic regions being expressed in infected bovine $CD8^+$ T cells, such as the region predicted to encode the gene A10. A10 encodes a transmembrane protein containing an immunoreceptor tyrosine-based activation motif (ITAM) and a SRC homology 3 domain (SH3), suggesting interaction with intracellular T cell signaling. We demonstrated that A10 is phosphorylated in T cells *in vitro* and affects T cell signaling. Impaired expression of A10 did not affect AlHV-1 replication *in vitro* but rendered AlHV-1 unable to induce MCF in the rabbit model. Furthermore, AlHV-1 expressing mutated forms of A10 devoid of ITAM and/or SH3 domains induced MCF with a significant delay compared to a wildtype virus. Overall, we provide a thorough description of $CD8^+$ T cell responses during MCF to uncover a novel mechanism explaining how AlHV-1 dysregulates T cell signaling leading to MCF.

#24. Kupffer cells mediate early anti-tumour responses in liver metastasis.

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Introduction: Metastasis is the major cause of death in patients with solid tumours. Thus, a better understanding of the metastatic niche, including immune cells, is a key step to identify novel therapeutic targets to reduce metastatic growth. The liver is one of the most common metastatic sites for many solid tumours (e.g. colon cancer). The liver-resident Kupffer cells (KCs), due to their exposed position inside the liver sinusoids and their scavenging behaviour, are hypothesised to be involved in the regulation of liver metastasis. However, the existing data are controversial and the precise role of KCs in metastatic processes is unclear.
Objective: We aim to shed light on the role of KC and KC-derived molecules during early stages of metastatic development using KC-specific transgenic mouse models.
Results: Bulk mRNA-seq of isolated KCs revealed dynamic changes in chemokine/cytokine expression upon tumour cell inoculation. Transcriptional changes suggest that KCs first drive the early influx of neutrophils and monocytes, then later they mediate the recruitment and activation of lymphocytes including NK cells. Specific depletion of the KCs a priori to tumour cell inoculation resulted in significantly increased metastatic outgrowth that we could further enhance with additional NK cell depletion. Intriguingly, when KCs were depleted at later stages we did not observe alteration in metastatic burden. In accordance with these data, we found that KCs are the most potent phagocytic cells in the liver to eliminate the arriving tumour cells. Altogether, we can conclude that KCs harbour several anti-metastatic properties but their protective role is restricted to the early phases of metastasis.

#25. The microenvironment of mesothelioma tumors impairs the immune-editing activity of primary human macrophages.

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Malignant pleural mesothelioma (MPM) is an aggressive and a fatal cancer that affects the pleural, pericardial and peritoneal mesothelium. In MPM, macrophages are particularly abundant and represent approximately 27% ± 9% of the tumor. The degree of macrophage infiltration negatively correlates with overall survival of MPM patients. Besides phagocytosis, cytokine expression and antigen presentation, macrophages are also able to be directly cytotoxic to MPM tumors (Hamaidia et al, *JCI Insight 4:e128474*). In fact, macrophages display a diverse continuum of phenotypes between classical (M1) and alternatively-activated (M2) patterns. Macrophages in MPM tumors do not correspond to these extreme phenotypes but, instead, are shaped by local inflammatory mediators released in the pleura.
The goal of the study is to investigate the cytotoxic activity of macrophage-associated MPM tumors. Monocyte-derived macrophages were polarized either in M1 with IFN-γ and LPS or in M2 with IL-4, or pulsed with pleural effusion isolated from MPM patients (i.e. pleural effusion macrophages). Macrophages were co-cultivated with CFSE-stained M14K mesothelioma cells. The direct killing of mesothelioma cells by macrophages was recorded by time-lapse microscopy and quantified by flow cytometry after staining with annexin-V. The ability of macrophages to control tumor growth was assessed in NSG mice.
Our data shows that M1-activated macrophages were more cytotoxic for M14K cells than M2. The killing activity of M1 macrophages was dependent on NADPH oxidase and peroxynitrites. Mesothelioma cells and M2 macrophages interacted through an inhibitory synapse characterized by engagement of the PD-1 receptor. Consistently, the immune-editing activity of M2 macrophages was partially restored in presence of neutralizing anti-PD1 antibody. Primary human macrophages cultured in presence of pleural effusions of MPM patients were less cytotoxic than M1 and were unable to inhibit tumor growth in mice. Macrophages differentiated in pleural effusions were either completely inactive or, at most, equivalent to M2. This heterogeneity of pleural fluids was then characterized by Luminex. Subsequent bioinformatic analysis identified a series of key mediators associated with the cytotoxic phenotype. The best score was obtained with

Resistin also known as found inflammatory zone-3 (FIZZ3). Finally, treatment of pleural effusion macrophages with human recombinant resistin ameliorated their killing activity.
Pleural effusions from MPM patients impair the cytotoxicity of primary human macrophages directed towards mesothelioma cells. Resistin (FIZZ3) is the main factor that mediates this immunoediting activity, opening new prospects for therapeutic intervention.

#26. Identification of co-activator(s) of TGF-β1 at the surface of activated Tregs.

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Regulatory T cells (Tregs) are a subset of CD4+ T lymphocytes specialized in the inhibition of immune responses. They are indispensable to prevent auto-immunity but play detrimental roles in cancer patients. Tregs suppress other immune cells by producing active TGF-β1 in a GARP- and integrin αVβ8-dependent manner. These surface proteins are required, but not sufficient, for TGF-β1 activation by Tregs. Indeed, T cells expressing both GARP and αVβ8 still need to be stimulated through their TCR to activate latent TGF-β1. In this project we aim to identify the missing partner(s) expressed at the surface of Tregs that participate, with GARP and αVβ8, in the activation of TGF-β1. We first screened a cDNA library of activated Tregs in TGF-β1 reporter HEK cells overexpressing GARP and latent TGF-β1. We identified one candidate coding for GALECTIN-9 (GAL9), a β-galactoside-binding protein that can be secreted in the extra-cellular milieu. Preliminary co-IP results suggest that GAL9 interacts with GARP and TGF-β1 and that interactions between GARP and the αV integrin chain are reduced in the absence of GAL9. GAL9-KO T cells expressing GARP and αVβ8 showed a lower (or no) capacity to activate TGF-β1 upon TCR stimulation compared to WT cells. Interestingly, loss of TGF-β1 activation in GAL9-KO T cells was associated with loss of surface αVβ8. However, re-expression of GAL9 by lentiviral infection did not restore surface αVβ8 nor the ability to activate TGF-β1. These data lead us to hypothesize that GAL9 could promote activation of latent TGF-β1 on the surface of Tregs by stabilizing the GARP:latent TGF-β1:αVβ8 complexes, but that it is not essential. We are thus re-screening the Treg cDNA library to search for another essential partner contributing to TGF-β1 activation.

#27. Neutrophil Extracellular Traps Are Found in Bronchoalveolar Lavage Fluids of Horses With Severe Asthma and Correlate With Asthma Severity.

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Asthma encompasses a spectrum of heterogenous immune-mediated respiratory disorders sharing a similar clinical pattern characterized by cough, wheeze and exercise intolerance. In horse, equine asthma can be subdivided into severe or moderate asthma according to clinical symptoms and the extent of airway neutrophil inflammation. Yet, the physiopathology of different phenotypes of equine asthma remains poorly understood and there is a need to elucidate the underlying mechanisms tailoring those phenotypes in order to improve clinical management and elaborate novel therapeutic strategies. In this study, we sought to quantify the presence of neutrophil extracellular traps (NETs) in bronchoalveolar lavage fluids (BALF) of moderate or severe asthmatic horses and healthy controls, and assessed whether NETs correlated with disease severity. To this end, we evaluated the amounts of NETs by measuring cell-free DNA and MPO-DNA complexes in BALF supernatants or by quantifying NETs release by BALF cells by confocal microscopy. We were able to unequivocally identify elevated NETs levels in BALF severe asthmatic horses. Moreover, we provide evidence that BALF NETs release was a specific feature seen in severe equine asthma, as opposed to moderate asthma, and correlated with disease severity. Finally, we showed that NETs could act as a predictive factor for severe equine asthma. Our study thus uniquely identifies NETs in BALF of severe asthmatic horses using three distinct methods and supports the idea that moderate and severe equine asthma do not rely on strictly similar pathophysiological mechanisms. Our data also suggest that NETs represent a relevant biomarker, a putative driver and a potential therapeutic target in severe asthma disease.

#28. High-dimension flow cytometry resolves human and murine eosinophilopoiesis and the unique dynamics of eosinophilia.

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Eosinophils are a specific type of granulocytes. They develop from hematopoietic progenitors in the bone marrow before populating an array of organs where they are thought to exert various homeostatic and immune functions. Eosinophils are yet mostly known for their involvement in a variety of immune related pathological disorders called Eosinophil Associated Diseases (EADs). EADs are essentially characterised by abnormal increases in blood and tissue eosinophils in a process known as eosinophilia. The precise ontogeny of eosinophils and the dynamics of their amplification in EADs yet remain very little-studied. Therefore, we first combined single-cell RNA sequencing with high dimensional flow cytometry to characterize the full developmental pathway of murine and human eosinophils. This approach showed that eosinophils arise from a small pool of progenitors shared with basophils. Maturing eosinophil progenitors subsequently transit through 4 morphologically, immunophenotypically and transcriptionally distinct stages to generate mature eosinophils. We next assessed the changes to eosinophilopoiesis that underlie eosinophilia in mice exposed to parasitic helminths, allergens, or recombinant alarmins. We thereby observed that eosinophil expansion relies on transit amplification of committed maturing eosinophil progenitors through a combination of delayed maturation and prolonged proliferative capacity. Altogether this work provides a useful resource that characterizes the transcriptome and surface proteome of eosinophil development with unprecedented resolution. Moreover, it reconciles human and murine eosinophilopoiesis and reveals a particular process of transit amplification that underlies eosinophilia.

#29. Understanding the mechanisms underlying gammaherpesvirus- triggered modulation of immune and pro-fibrotic responses using a multispectral imaging model.

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The lung is continuously exposed to different environmental insults like microbial products, smoke, pollutants and pathogens that can damage the tissue. Therefore, a large reparative capacity is required. When inadequately controlled, this repair process can lead to pathology such as seen in pulmonary fibrosis.
Many studies have shown that idiopathic pulmonary fibrosis is associated with environmental risk factors. Epstein bar virus, a γ-herpesvirus or its mouse homologue, murid herpesvirus 4 (MuHV-4), is highly prevalent and is responsible for persistent latent infection. While these infections are associated with lymphoproliferative disorders in immunocompromised individuals, there is also evidence that γ-herpesviruses correlate with the aggravation of various immunopathologies such as lung fibrosis.
While lung immune cells may play a beneficial or detrimental role in regulating tissue repair responses, it is not clear how this occurs in a spatio-temporal manner. A better understanding of the mechanisms by which environmental factors shape the lung immune system to promote or prevent lung regeneration is a pressing unmet need that may open new avenues to improve lung regeneration. Thus, to study the mechanisms of MuHV-4-induced exacerbated pulmonary fibrosis and the interactions between immune cells and their microenvironment, an innovative multiplex immunofluorescence technique will be employed. This technique uses a multispectral Nuance camera that can visualize up to 15 markers in a single tissue section, which can then be overlapped with classical histopathological analyses.
As a first step, respiratory infection by MuHV-4 followed by bleomycin intratracheal administration one month later is characterized. Lung cryosections are employed to quantify collagen deposition, using Sirius Red stainings, as well as for multispectral imaging.

#30. The role of follicular helper T cells in the immune response to breast cancer.

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We previously demonstrated that tumor-infiltrating lymphocytes (TIL) in human breast cancer sometimes form organized tertiary lymphoid structures (TLS) characterized by CXCL13-producing T follicular helper (Tfh) cells. The present study found that CD4+ Tfh TIL, CD8+ TIL, and TIL-B, colocalizing in TLS, all express the CXCL13 receptor CXCR5. An ex vivo functional assay determined that only activated, functional Th1-oriented Tfh TIL (PD-1hiICOSint phenotype) provide help for immunoglobulin and IFN-γ production. A functional Tfh TIL presence signals an active TLS, characterized by humoral (immunoglobulins, Ki-67+ TIL-B in active germinal centers) and cytotoxic (GZMB+ CD8+ and GZMB+ CD68+ TIL plus Th1 gene expression) immune responses. Analysis of active versus inactive TLS in untreated patients revealed that the former are associated with positive clinical outcomes. TLS also contain functional T follicular regulatory (Tfr) TIL, which are characterized by a CD25+ CXCR5+ GARP+ FOXP3+ phenotype and a demethylated FOXP3 gene. Functional Tfr inhibited functional Tfh activities via a glycoprotein A repetitions predominant (GARP)-associated TGF-β–dependent mechanism. The activity of tumor-associated TLS was dictated by the relative balance between functional Tfh TIL and functional Tfr TIL. These data provide mechanistic insight into TLS processes orchestrated by functional Th1-oriented Tfh TIL, including TIL-B and CD8+ TIL activation and immunological memory generation. Tfh TIL, regulated by functional Tfr TIL, are an expected key target of PD-1/PD-L1 blockade.

#31. Vaccination with tumour-derived conventional dendritic cells delays lung metastasis.

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Optimising therapies aiming at promoting systemic antitumour immune responses that prevent metastasis holds considerable clinical potential. In a prophylactic setting, we assessed whether using conventional dendritic cells (cDCs) isolated from donor tumours could induce an antitumour memory capable of slowing tumour growth upon challenge. In models of murine lung carcinoma and melanoma, we found that vaccinating with cDCs isolated from tumours of anti-CD40 pre-treated donors conferred better protection after tumour challenge. The prophylactic effect further synergised with anti-PD-1 therapy that resulted in complete tumour regression in 60% of melanoma tumour-bearing mice.

To assess whether tumour-derived cDCs could be used as a therapy to specifically prevent metastasis, we generated a Lewis lung carcinoma-cell line that spontaneously metastasises to the lung upon complete surgical resection of the primary tumour. We treated tumour-bearing mice with Flt3L and anti-CD40 to augment and activate cDC populations within tumours prior to surgery. Strikingly, mice vaccinated with cDCs from their own tumours showed significantly extended metastasis-free survival after surgery. The effect on survival was dependent on the anti-CD40 pre-treatment and mainly induced by cDC2s, as exclusion of either component from the regimen abrogated any survival benefit.

We believe our data represents the first preclinical evidence that *in vivo* modulation of tumour-derived cDCs can improve DC vaccination strategies and specifically prevent metastasis. In the longer term, this could lead to the development of a personalised vaccination strategy using a patients' own tumour-derived DCs to prevent metastatic relapse.

#32. A systems immunology approach reveals distinct roles of non-genetics and genetics factors in shaping variation of immune responses in cattle.

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Immune system has primarily evolved to protect the host against infections. Given the breadth of the effects of immune responses on pathologies, it is necessary to understand the variability of immune responses in a given population and how this variability relates to susceptibility to infections and immune-mediated diseases. Recent studies have identified several host, environment, and genetic factors that influence the landscape of the immune system in human. However, such approaches have not yet been implemented in animal populations. In this study, the immune response of the Belgian White and Blue (BWB) breed was studied in a comprehensive manner by integrating host, environmental, and genetic factors that lead to immune variation in cattle. A systemic immunophenotyping approach was established to quantify more than 200 immune parameters at steady state and after ex vivo restimulation of blood leukocytes. It was found that genetics and season rather than sampling point and age were important drivers of immune variation in this BWB population. Particularly, the genetic variants associated with IL-8, IL-10, and CD8+ T cells were identified using the univariate genome-wide association study (GWAS) method. Following *ex-vivo* stimulations, we identified genetic variants associated with IL-1β IL-6 and IFN-γ production. Furthermore, using PCA GWAS and multivariate GWAS methods, we identified genetic variants associated with WC1.1+ expression among gamma delta T cells and with IL-1β production induced by stimulation with bacterial products. Finally, we also demonstrated that cytokine production in response to stimuli can be predicted on the basis of an individual's cell composition and genetics factors. Overall, this approach may provide new information on the mechanisms underlying disease pathogenesis in cattle and will help us to better understand the genetic determinism of these mechanisms, allowing the application of genomic selection methods for these traits to improve these cattle breeds.

#33. Functional Testing of candidate genes for Familial Hodgkin Lymphoma.

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Hodgkin lymphoma (HL) is a hematological B cell malignancy characterized by the presence of rare, morphologically distinctive tumor cells surrounded by reactive immune cells. We assessed for rare, predicted-pathogenic, disease-cosegregating variants that potentially predispose to familial HL (fHL), in whole exome sequencing (WES) data from a series of 22 affected members from 11 families. We prioritized two genes for functional testing: *CCNF* and *HK3*. CCNF (Cyclin F), which is highly expressed in B cells, is the target-binding component of the cell cycle regulated $SCF^{CyclinF}$ E3 ubiquitin ligase. The *CCNF* variants identified (p.R375W and p.Y341C, in one HL family each) decrease its binding to ubiquitylation targets. This could contribute to defects in DNA replication and repair, and cell cycle progression and checkpoints. EBV-B cell lines with *CCNF* knockout are currently being characterized for these features. HK3 (hexokinase 3) which is expressed in the surrounding tumor niche, mainly by neutrophils and macrophages, catalyzes the first committed step of glycolysis. Enzymatic tests showed that the *HK3* variants identified in HL families (p.L163I and p.G605D, in one family each) hamper or abolish enzyme function. A double-variant (p.Q578R-p.Q600H) identified on the second allele of one of these families (with the p.G605D variant that abolishes HK3 catalytic activity) will also be tested. The occurrence of variants in both *CCNF* (p.R375W) and *HK3* (p.G605D on one allele and p.Q578R-p.Q600H on the other) in the same HL family may suggest complementary contributions of these genes to HL: *CCNF* alterations promoting B cell tumorigenesis and *HK3* defects conferring a more permissive microenvironment for tumor cell survival.

#34. Genetic analysis of rare families points to a pathogenic role for the cGAS/STING-IFN-I axis in Systemic sclerosis.

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Systemic sclerosis (SSc) is a rare, chronic condition characterized by vascular, immunological and connective tissue abnormalities. No monogenic or oligogenic causes have been demonstrated, likely due to the infrequency of familial clustering in this disease. We are fortunate to have access to DNA samples from five families with two affected first-degree relatives each, in which we hypothesize that SSc may be transmitted as a di/oligogenic trait.
Whole exome sequencing (WES) was performed on all available blood-DNA. We filtered for variants that are (a) shared by both affected individuals within each family, (b) absent-to-rare in the general population, and (c) predicted to affect protein function by multiple *in silico* tools; this yielded 23-45 genes per family. We assessed for pathways represented in gene-lists from families, and found that participants in the cGAS/STING - type I-IFN axis are identified in all. This may point to a unifying disease mechanism. cGAS/STING is a sentinel system responsible for the production of anti-viral type I interferons and pro-inflammatory cytokines in the presence of cytoplasmic double stranded DNA (dsDNA). This sensing system has been implicated in silica-induced inflammation: a risk factor in SSc. A type I IFN-response signature is identified in SSc patients and correlates with disease severity. Mutations in two of our candidates cause Mendelian diseases with phenotypic overlaps with SSc, and all but one cause disease-relevant phenotypes when perturbed in mice. The identification of potential "driver" gene variants in this pathway across all families points to an important pathogenic role in SSc.

#35. Exploring neutrophil functional heterogeneity in non-small cell lung cancer for new target discovery.

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Neutrophils are initial responders to inflammation and infection and much of the bone marrow is dedicated to their production. One of the hallmarks of cancer is the emergence of an inflammatory tumour microenvironment (TME), which often displays high infiltration of neutrophils. Often these cells are skewed toward a pro-tumour phenotype in the TME as they enhance myeloid cell recruitment, angiogenesis, and T-cell suppression. Interestingly, pre-clinical models of lung squamous cell carcinoma (LUSC), a subtype of non-small lung cancer, show high prevalence of tumour-associated neutrophils (TANs). Previously, we have identified a state of long-lived TANs in a model of lung adenocarcinoma, which show reduced turnover and abnormal metabolism compared to normally short-lived neutrophils, thus enhancing their pro-tumour capacity. Using a recently generated model of LUSC driven by overexpression of SOX2 and inactivation of NKX2-1 and LKB1 (SNL model), we hope to identify the roles of TANs within the LUSC TME and, particularly, to elucidate whether targeting long-lived neutrophils in LUSC will curb tumour growth. More broadly, we hope our functional characterization of TANs in LUSC will provide new targeting opportunities for combination with existing immunotherapies.

#36. Lung neutrophil targeting has long-term consequences on cellular trafficking and endothelium homeostasis.

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The lung exerts vital functions that are sustained by both structural and immune components. The lung microenvironment has indeed been shown to shape the functional identity of immune cells to ensure appropriate responses that maintain its immunophysiological functions under homeostasis and pathogenic conditions. In line with this, one intriguing feature of the lung is the presence, at steady-state, of a substantial pool of neutrophils located in the microvasculature, called marginated neutrophils (MarNeu). Lung MarNeu display a unique transcriptomic profile as compared to other neutrophils across the whole body. Interestingly, the gene signature of MarNeu is mainly associated with vascular growth and repair. Here, we show that targeting MarNeu during a few days has a substantial long-term impact on cellular trafficking and endothelial cell (EC) homeostasis. Indeed, two weeks after MarNeu depletion, the endothelium permeability was decreased, leading to an impairment of leukocyte transendothelial migration upon exposure to various inflammatory stimuli (allergen or lipopolysaccharide [LPS] exposures, infection with influenza virus). Moreover, while the adult lung was thought to be a relatively quiescent organ, recent studies have demonstrated an unexpected tissue-specific heterogeneity of ECs and the presence of lung-specific EC subsets that exert particular functions, like $CD34^+$ ECs implicated in angiogenesis after tissue damage. Here, we show that targeting MarNeu is associated with a decrease in the number of $CD34^+$ ECs and their ability to proliferate after a tissue damage. We are currently further investigating the MarNeu-EC axis and the long-term control of EC stemness, identity and functions by MarNeu.

#37. Sputum IL-5 predicts the response to anti-IL-5/IL-5R therapy.

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Introduction: Severe eosinophilic asthma represents a high burden in term of healthcare costs. Biotherapies targeting interleukin (IL-5) or its receptor (IL-5R) have been developed and allow a tangible improvement of the asthma disease condition. However, all patients do not respond in the same manner to these treatments. Even if high blood eosinophil counts appear to be associated with a reduction in exacerbations with treatment targeting IL5, we lack of biomarkers for the prediction of a super-response to these very expensive treatments. The goal of this study was to highlight biomarkers of "super response" in the sputum of severe eosinophilic asthmatics.
Methods: Eighty subjects were recruited in our asthma clinic. Super-responders to biotherapy were defined as patients combining no chronic treatment with oral corticosteroids, no exacerbation, ACQ lower than 1.5 and/or ACT greater than 19. Eosinophil peroxidase (EPX), IgE, IL-3, IL-4, IL-5, IL-13, IL-25, IL-33, GM-CSF, TSLP and eotaxin-1 levels were measured in the sputum of these patients before anti IL-5/anti IL-5R treatment.
Results: Fifty-three patients treated with anti-IL-5 and 27 with anti-IL5R were assessed. Among them, 20 were classified as super-responders. We observed that these super responders were characterized by an increase in sputum macrophages and lymphocytes counts compared to other patients and a trend for an increase in sputum eosinophils. In addition, the level of sputum IL-5 was higher in the super responder group compared to the other with a trend for EPX.
Conclusions: Sputum IL-5 appeared to be a marker of super response to anti IL-5/IL-5R in a cohort of severe eosinophilic asthmatic. These preliminary results need to be validated in a larger cohort.

#38. The INPP5K/PtdIns(4,5)P2 axis controls the dynamic structure and signaling of wild type and mutated, leukemia-associated IL7 receptors.

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Signaling downstream of the IL7 receptor plays important physiological and pathological roles, including differentiation of lymphoid cells and proliferation of acute lymphoblastic leukemia cells. Gain of function mutations in the IL7Rα chain, the specific component of the receptor for IL7, result in constitutive, IL7-independent signaling and trigger acute lymphoblastic leukemia. Here, we show that loss of the phosphoinositide 5-phosphatase INPP5K is associated with increased levels of the INPP5K substrate PtdIns(4,5)P2 and causes altered dynamic structure of the IL7 receptor. We discovered that the IL7Rα chain contains a very conserved positively-charged polybasic amino acid sequence in its cytoplasmic juxtamembrane region; this region establishes stronger ionic interactions with negatively-charged PtdIns(4,5)P2 in the absence of INPP5K, freezing its structure. This dynamic structural alteration causes defects in IL7 receptor signaling, culminating in decreased expression of EBF1 and PAX5 transcription factor, in microdomain formation, cytoskeletal reorganization and bone marrow B cell differentiation. Similar alterations following reduced INPP5K expression also impacted mutated, constitutively activated IL7Rα chains that trigger leukemia development, leading to reduced cell proliferation. Altogether, our results indicate that the lipid 5-phosphatase INPP5K hydrolyses plasma membrane PtdIns(4,5)P2, allowing the requisite conformational changes of the IL7Rα chain for optimal signaling, and that targeting INPP5K may be of interest in the therapy of acute lymphoblastic leukemia and other IL7R-dependent diseases.

#39. Analyzing the role of maternal IgGs in the *L.rhamnosus*-induced maturation of cDC1s compartment in early-life.

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Conventional dendritic cells (cDCs) are specialized in antigen presentation and T-cell polarization, making them target for therapeutical and vaccination strategies. In early life, type 1 conventional dendritic cells (cDC1s) are the main subset of cDCs in the spleen. Neonatal cDC1s are distinguished from their adult counterparts by their unique ability to secrete IL-10 and not IL-12p70. We have recently shown that commensal microbiota influences the maturation of splenic cDC1s compartment in newborns, as demonstrated by the administration of antibiotics (*Köhler et al, Gut, 2020)* or probiotics (*L.rhamnosus)* (*unpublished data*) to their mother. Interestingly, this maturation is myeloid TNF- and maternal IgG-dependent.
Moreover, in the context of maternal *L.rhamnosus* supplementation, we have also demonstrated an increased level of transferred maternal IgGs to the pups. Using capillary electrophoresis to analyze the Fc fragment glycosylation profile of those IgGs, we show that the N-glycome of bulk IgGs does not seem to be significantly impacted. In parallel, we have assessed the expression of the Fc receptors of IgGs (FcγRs) on the potential target cells in the spleen of newborns. We have demonstrated that *L.rhamnosus* increases the ratio of activating/inhibitory FcγRs on innate myeloid cells.
Taken together, maternal probiotic supplementation seems to act quantitatively on maternal transfer of IgGs and modulate the activation threshold set by FcγRs expression on myeloid cells in the neonatal spleen. However, the precise mechanisms by which probiotic supplementation can influence maternal IgGs transfer and how those IgGs can act on neonatal splenic cells remain unclear.

#40. Deciphering the role of lactate in the functional reprogramming of tumor-associated macrophages (TAMs).

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In several cancers, high tumor-associated macrophages (TAMs) densities are correlated with poor clinical outcomes. As macrophages infiltrate the tumor, they are exposed to several signals from the tumor micro-environment (TME), leading to their reprogramming into a M2-like immunosuppressive phenotype. Among these signals, lactate was shown to polarize macrophages towards a M2-like state, critical for tumor growth. Lactate is a metabolic end-product of tumor cells which can be internalized by macrophages through monocarboxylate transporters and can in turn induce M2-like genes through different signalling pathways activation and epigenetic modifications. Besides, extracellular lactate also acts as a signalling molecule in the TME and interacts with some G-protein coupled receptors. However, the mechanisms enabling lactate-mediated immunosuppression are not elucidated yet. The goal of this project is to study the role of lactate in the functional reprogramming of human TAMs, and the mechanisms underlying their acquisition of an immunosuppressive phenotype. First, an *in vitro* cell line model will allow us to decipher the molecular mechanisms and select the best molecular targets. Then, to study the interactions between macrophages and tumor cells, we will use a model of human tumor spheroid, developed by P. van Der Bruggen (UCL). These *in vitro* platforms will allow us to select efficient targets before the humanized mouse model (HM). To assess the impact of specific targets on anti-tumor responses, we will use a model of HM reconstituted from modified CD34+ cells, implemented with tumor cells and tumor-specific T cells. Altogether, these complementary platforms will help us to identify new mechanisms involved in the immunosuppressive polarization of TAMs.

#41. Tgfβ-Tgfβ receptor signaling is essential for lung interstitial macrophage differentiation and identity.

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In the lung, interstitial macrophages (IM) spontaneously produce the immunosuppressive cytokine interleukin (IL)-10, thereby maintaining lung homeostasis and preventing the development of allergic inflammation to aeroallergens. Recently, we discovered two distinct IM subsets and found that MafB was an important transcription factor that restricted local monocyte proliferation and mediated IM differentiation and identity of both subsets. While macrophage colony-stimulating factor (M-CSF) acts as a local signal contributing to this process, it remains to be determined whether additional factors from the lung microenvironment are imprinting the identity of IM. We performed single cell RNA-sequencing of whole lung cells in steady-state and performed NicheNet analysis, allowing us to identify the *Tgfb-Tgfbr* axis as a promising ligand-receptor interaction mediating IM identity. Both IM subsets expressed high protein levels of Tgfβ-RII, and BMDM stimulation with Tgfb triggered expression of IM-associated genes. Then, we generated myeloid-restricted *Tgfbr2*-deficient mice (i.e., $LysM^{Cre}Tgfbr2^{fl/fl}$ mice) and found that numbers of IM were lower in $LysM^{Cre}Tgfbr2^{fl/fl}$ mice as compared to littermate controls. Of note, IM differentiation from monocytes seemed to be impaired and blocked in a monocyte-to-IM transition state in those mice. We also found that MafB directly or indirectly regulated IM-specific Tgfβ receptor expression in myeloid-restricted Mafb-deficient mice. This work adds to our understanding of IM biology by showing how the lung-specific microenvironment shapes IM identity, thus providing foundations for future IM-targeted therapeutic interventions in the context of lung chronic inflammatory disorders.

#42. Towards identifying novel eQTLs driving inherited predisposition to IBD in 22 distinct circulating immune cell populations.

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Inflammatory bowel disease (IBD) is characterized by a chronic idiopathic inflammation of the gastrointestinal tract and consist of two main forms: ulcerative colitis and Crohn's disease. The importance of genetic susceptibility has been well established through genome-wide association studies (GWAS), which have identified over 200 risk loci for IBD. However, the « true causative » genes in these loci have been identified for only few on the basis of independently associated coding variants. Fine-mapping studies suggested that most risk variants cause "cis"- expression quantitative trait loci (eQTLs) in disease relevant cell types, but recent post-GWAS studies, including from our laboratory, could not find matching cis-eQTLs for the majority of risk loci (137/200). This indicates that the relevant cell types were either not present among the analyzed cell populations or under-represented. In this study, we set up a protocol aiming to identify the remaining cis-eQTLs on as many cell types as possible from peripheral blood using highly pure FACS and magnetic cell sorting of at least 22 immune cell populations followed by bulk RNA-seq. After comparing several commercially available low to very low RNA input kits for library preparation, we selected the Takara SMART-Seq-HT kit for this study since it allowed the highest gene coverage even when RNA input was as low as 10pg.
In total, 300 healthy individuals and 100 Crohn's disease patients blood samples were collected. About 10,000 libraries are in the process of being RNA sequenced. Simultaneously, the cohort was SNP genotyped using the Infinium OmniExpress-24v1 chip from 1 mL of blood and imputed. eQTL analyses are ongoing and are expected to yield a unique collection of cell type-specific eQTLs including some that colocalize with IBD risk loci and contribute to inherited predisposition to IBD.

#43. IL-4 receptor signaling regulates lung macrophages during helminth coinfection resulting in enhanced gammaherpesvirus permissiveness.

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Helminth infection conditions lung macrophages in the long term, but little is known about how helminths affect the lung macrophage responses to respiratory viral coinfection. While comparing BALB/c and C57BL/6 mice, we found that helminth infection in C57BL/6 mice resulted in enhanced permissiveness to a subsequent infection with murid gammaherpesvirus 4 (MuHV-4), and that viral early tropism was mainly restricted to lung

macrophages. Helminth infection resulted in enhanced type 2 airway inflammation and M(IL-4) polarization of interstitial macrophages (IntMs) in C57BL/6 mice, associated with an IL-4Rα-dependent disappearance reaction of alveolar macrophages (AlvMs) and enriched monocyte-derived IntMs proportions. Competent IL-4Rα responsiveness or intra-tracheal instillation of recombinant IL-4 or IL-13 significantly resulted in reduced numbers of AlvMs and enriched IntMs as well as increased permissiveness to MuHV-4 infection, which was restricted to AlvMs. Thus, direct IL-4Rα signaling during helminth infection affects macrophage permissiveness to gammaherpesvirus infection.

#44. Enteric helminth infection dampens the induction of antigen-specific CD8 T lymphocytes after vaccination with a recombinant Vesicular Stomatitis virus vector.

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Current vaccines developed against viral diseases have mostly been designed to induce a specific humoral response. However, antibodies are not always sufficient due to their evasion strategies. Eliciting appropriate CD4 and CD8 T cell responses and effective memory after vaccination is essential for strong and long-lasting protection. Vaccination in tropical regions often achieves insufficient protection. Gastrointestinal helminths are widespread there and have developed immunoregulatory mechanisms to persistently infect their hosts. Such modulation of the immune response has been proposed to explain a low efficacy of the response to vaccination. We used the parasite *Heligmosomoides polygyrus* (Hp) and recombinant Vesicular Stomatitis virus (rVSV) vectors as model to investigate how persistent helminth infection affects the establishment of effective memory T responses to vaccination. Mice were infected with Hp before intramuscular vaccination with rVSV-GP-OVA expressing the glycoprotein GP1 of LCMV and the ovalbumin. Monitoring of the CD4 and CD8 T cell responses in the spleen, peripheral lymph nodes and the bone marrow revealed a significant reduction of the total CD8 T cell response, including antigen-specific CD8 T cells. While Hp induced an increase of $Foxp3^+$ Tregs and an expansion of virtual memory T cells, effector T cells expressed less KLRG1 or PD-1, suggesting impaired function. Together, these initial results suggest that enteric helminth infection impairs the induction of effective effector CD8 T cell responses to rVSV vector vaccines, confirming that parasitic infection plays an important role in modulating vaccine responses. In order to focus on the memory response and more particularly on antigen-specific CD8 T cells, challenge experiments are in progress.

#45. Deciphering the impact of EBV infection on functionnal reprogramming of monocytes using a relevant humanized mouse models.

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The immune system is constantly shaped by environmental stimuli, mainly from commensal or pathogenic microorganisms. In particular, monocytes integrate multiple signals from the microenvironment that can have a lasting impact on their differentiation and function. In this context, persistent viruses appear to have a major influence on the composition and function of the immune system. Epstein-Barr virus (EBV), a widespread human gammaherpesvirus (ɣHV) is usually acquired silently early in life and the carried as a lifelong asymptomatic infection. However, increasing epidemiological evidence links EBV infection to major immunomodulatory effects influencing the development of diseases such as allergic asthma or multiple sclerosis. The high prevalence of EBV and presence of multiple confounding factors make it difficult to establish a causal link in humans. In order to investigate whether latent EBV infection may represent a major determinant of monocyte development and functional programming, we take advantage of the MISTRG humanised mice model. This knock-in mouse strain expresses human M-CSF, IL-3/GM-CSF, SIRPα and thrombopoietin thereby allowing efficient reconstitution of the human myeloid compartment upon transplantation of hematopoietic stem cells. We validated the humanization procedure by intrahepatic injection of foetal liver CD34+ cells in neonatal mice. Using flow cytometry, we verified the substantial reconstitution of human monocytes in adult mice and assessed their functional properties upon in vivo or ex vivo stimulation. Preliminary experiments also demonstrate the feasibility of EBV infection in these mice. These initial findings reinforce the relevance of this model to decipher the impact of EBV on the functional programing of human monocytes.

#46. Role of the C-ter part of the IL-22Ra : Inflammation and mechanistic view.

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Interleukin-22 (IL-22) is a cytokine that acts on various tissues including the skin, the gut, the pancreas and the liver. In these organs, IL-22 is involved in tissue regeneration, homeostasis and defense against pathogens by inducing cell proliferation, recruitment of neutrophils and production of anti-microbial. Although these functions are beneficial in various contexts such as wound healing, colitis and pancreatitis, prolonged and exacerbated production of IL-22 induces tissue inflammation and immune disorders such as psoriasis and cancer. Therefore, targeting IL-22 activity to treat psoriasis or cancer bears the risk of deleterious effect at mucosal sites. We think that it might be useful to develop methods to partially block IL-22 functions.
IL-22 signaling involves the activation of STAT proteins such as STAT-1,-3,-5 with a massive activation of STAT3. However, unlike most cytokines, STAT3 is pre-associated with the IL-22R and its activation is induced in a receptor tyrosine-independent manner. This alternative STAT3 activation relies on the coiled-coil domain of STAT3 and the C-terminal part (Cter) of the IL-22R, which lacks tyrosine residues. This alternative activation of STAT3 is a good target to partially block IL-22 activities
In vivo experiments performed in the lab with mice truncated for the C-terminal part of the IL-22R (Cter-/- mice) have demonstrated that this alternative mechanism is involved in the development of imiquimod-induced psoriasis, where IL-22 is known to be deleterious. Indeed, cutaneous lesions were less important in Cter-/- mice compared to WT mice. On the contrary, the Cter doesn't seem to play an important role in the *C. Rodentium*-induced colitis model, where IL-22 is described as beneficial. Regarding these results, it seems to be important to study the impact of this Cter region in other inflammatory disorders such as pancreatitis and cancer but also to define the molecular mechanisms that explain the interaction between the Cter and STAT3. Altogether, these results suggest that specific targeting of this alternative activation of STAT3 could serve to treat psoriasis without affecting IL-22 dependent tissue regeneration.

#47. Proteasome antigen processing under oxidative stress.

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Cytolytic T lymphocytes recognize peptides presented at the cell surface by MHC class I molecules. These peptides originate from the degradation of cellular proteins by the proteasome, a large protease complex mainly located in the cytoplasm. Four subtypes of proteasome exist, which differ in their catalytic subunits and display different proteolytic activities: the standard proteasome (ß1ß2ß5), two intermediate proteasomes (ß1ß2ß5i and ß1iß2ß5i) and immunoproteasome (ß1iß2iß5i).
Previously, by performing in vitro digestion experiments with purified proteasomes, our lab has shown that oxidized proteins are more efficiently degraded by ß5i-containing proteasome. This suggests that cells exposed to oxidative stress might produce a different peptide repertoire, which originate from the degradation of these oxidized proteins.
This is of particular interest in the field of cancer because the tumor environment is often low in oxygen, and this state of hypoxia has been shown to increase the production of ROS. We therefore plan to analyze the peptide repertoire of tumors cells grown in normoxia and in hypoxia using a mass-spectrometry-based approach, which was recently set up in our laboratory. Analysis if the peptidome will be coupled to a more general analysis of the proteome and the transcriptome to understand whether and how hypoxia induces a change in the peptidome of various tumor cells. This study might lead to the identification of new peptides, which could represent potential targets for anti-tumor CTL and could therefore be used as immunotherapeutic cancer vaccines.

#48. The specific history of viral infections may pave the way for tumor development.

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Research in recent decades has highlighted the importance of environmental signals in many diseases, including cancer. Respiratory viruses are among the most common human pathogens and, beyond their pathogenic features, they have the potential to induce lasting changes in the host immunity. While these changes have been shown to alter responses to heterologous infections and immune tolerance in the context of asthma, it is still unknown their potential impact on anti-tumor responses. We hypothesized that changes imprinted by different viruses could affect anti-tumor immunity and play a major role in determining tumor establishment and progression. To address this question, we used a Lewis lung carcinoma (LLC) murine model and two different viruses: Murine herpesvirus 4 (MuHV-4) and Pneumonia virus of mice (PVM), which are homologs of two highly prevalent human viruses, Epstein-Barr virus and respiratory syncytial virus, respectively. Our results show that MuHV-4 and PVM infection affect oppositely the development of lung tumors one month after infection. While pre-infection with MuHV-4 reduces tumor establishment, pre-infection with PVM promotes early tumor establishment and enhances tumor growth. Interestingly, we observe a strong effector T-cell response under the control of imprinted regulatory macrophages after MuHV4 infection, which may enhance anti-tumor responses while preventing deleterious inflammation. On the other hand, impaired T-cell responses and uncontrolled inflammation in mice pre-infected with PVM may favor a pro-tumor response. Overall, our work highlights that the specific history of infections may have unexpected long-term training effects that profoundly shape anti-tumor immune responses.

#49. Atypical myeloid cells orchestrate epithelial repair by promoting AT2 expansion following respiratory viral infection.

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Lung tissue repair mechanisms are critical components of the host immune response to respiratory viral infections in order to restore tissue integrity and homeostasis. As a corollary, dysregulated immune responses can be deleterious and lead to severe disease phenotypes. While myeloid cells are thought to substantially contribute to tissue repair, their complexity, diversity and fine-tuned functions remain under-investigated. Here, we used a sublethal mouse model of influenza virus infection (strain PR8) to investigate the mechanisms underlying lung tissue recovery. By analyzing lung immune cell dynamics post-infection, we observed the emergence of an as-yet-unknown atypical myeloid cell population peaking during the early recovery phase of infection, which shared expression of macrophage markers such as CD11b, F4/80 and CD64 and of neutrophil markers like Ly-6G and CXCR4. Electron microscopy analyses highlighted unique ultrastructural features, including a multilobular nucleus, granules, lysosomes and autophagy vacuoles, all delimited by a microvilli-rich cell membrane. These hybrid cells arose in the lung in a CCR2-dependent manner, and their presence and unique ability to produce high levels of Arginase-1 were dependent on IL-4 receptor signaling, a feature shared with alternatively activated macrophages. Furthermore, we found that they were located in alveolar spaces, at the edges of injured, hepatized areas, in the vicinity of alveolar epithelial cells. We have accumulated evidence *in vivo* and *in vitro* that one critical function of such atypical myeloid cells is to promote epithelial repair and lung re-epithelialization by physically interacting with alveolar type 2 epithelial cells. Ongoing work is investigating this myeloid-epithelial cell axis and the mechanisms behind their central role in tissue healing and lung function recovery.

#50. *Staphylococcus aureus* is capable of inducing a neutrophilic inflammation in a surgical mouse model of CRS, in a more prominent way than *Pseudomonas aeruginosa* or *Streptococcus pneumonia.*

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Introduction: Chronic rhinosinusitis (CRS) is an inflammatory respiratory disease with symptoms of nasal blockade and secretions, headache and loss of smell for at least 12 weeks. It affects 11% of Europeans and is a global health burden. We can find T2 eosinophilic or non-T2 neutrophilic CRS and currently, its pathogenesis is not fully understood, especially regarding non-T2 CRS, and no validated mouse models are available to study disease mechanisms. We aimed at establishing a neutrophilic mouse model of bacterial-induced CRS.
Methods: a nasal tampon was surgically inserted in the nasal cavity of mice and inoculated with three different bacteria: *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. Inflammatory features in nasal mucosa were evaluated after 4, 8 and 12 weeks on decalcified skulls by histology, antibodies and cytokines were measured in nasal and bronchoalveolar lavages by ELISA and differential cell-counts were performed.
Results: post-operative mortality was more important for *S. pneumoniae* than for *P. aeruginosa*. While *S. aureus* and *P. aeruginosa* were still detectable in the nasal lavage after 4, 8 and 12 weeks post-surgery, *S. pneumoniae* seemed to be cleared. Mice with *S. aureus*-induced CRS showed a significant increase in epithelial thickness (4w, 8w, 12w,), subepithelial fibrosis (12w), and neutrophilic infiltration (4w, 8w) at the nasal mucosa. Mice with *P. aeruginosa*-induced CRS showed significantly increased epithelial thickness (12w) and subepithelial fibrosis (12w). Mice with *S. pneumoniae*-induced CRS did not show any changes at any timepoint.
Conclusion: *S. aureus* is the most potent inducer of neutrophilic non-T2 CRS in a mouse model of bacterial-induced CRS, which allows us to investigate the pathogenesis of non-T2 CRS.

#51. Single-cell analysis of human fetal and pediatric γδ thymocytes reveals distinct functional thymic programming.

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Developmental thymic waves of innate-like and adaptive-like γδ T cells have been described, but the current understanding of γδ T cell development is mainly limited to mouse models. Here, we combined single cell (sc) RNA gene expression and sc γδ T cell receptor (TCR) sequencing on fetal and pediatric γδ thymocytes in order to understand the ontogeny of human γδ T cells. Mature fetal γδ thymocytes were committed to either a type 1, a type 3 or a type 2-like effector fate, independent from γδ T cell subsets (Vγ9Vδ2, nonVγ9Vδ2), and were enriched for public CDR3 features upon maturation. Strikingly, these three effector modules expressed different CDR3 sequences and followed distinct developmental trajectories. In contrast, the pediatric thymus generated a small effector subset that was highly biased towards Vγ9Vδ2 TCR usage and showed a mixed type 1/type 3 effector profile. Finally, by performing multiparametric flow cytometry experiments, we validated at protein level the described effector γδ thymocyte modules and could track them in distinct fetal peripheral tissues.
Thus, our combined dataset of gene expression and detailed TCR information reveals distinct and age-dependent functional thymic programming of γδ T cell immunity in human and provides a resource for further study.

#52. *Plasmodium*-induced immunomodulation in cancer immunosurveillance and septic shock.

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Introduction: *Plasmodium* infection is a life-threatening illness, but it can also be a mild infection with little symptoms. The pathology of the host is a result of the balance between anti-parasite immunity and immunomodulatory mechanisms induced by the parasite which can modulate concomitant immune-mediated disorders.
Methods: We addressed the role of *Plasmodium* in the modulation of the host-microbial interaction in mouse models of cancer immunosurveillance and septic shock.
Results: Infection with *Plasmodium yoeli 265 BY* significantly protected BALB/c mice from the early development of plasmacytoma. The protective effect of plasmodium was lost by in vivo depletion of NK cells. On the other hand, the susceptibility of 129 Sv mice to endotoxin shock was substantially increased after infection with *Plasmodium*. The increased susceptibility was linked to the strong production of proinflammatory cytokine TNFα and neutralization of this cytokine abolished the exacerbating effect.
Conclusion: Immune changes induced by *Plasmodium* infection can improve or exacerbate the outcomes of coexisting illness. Examining the processes behind these impacts could lead to the development of newer therapeutic approaches to these pathologies.

#53. Investigating the formation of active tertiary lymphoid structures and their anti-tumor immune functions in human breast cancer

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The clinical relevance of tumor infiltrating lymphocytes (TIL) in breast cancer (BC) is now widely accepted and starting to be implemented in clinical practice. Our laboratory demonstrated that 60% of BC organize some of their TIL in tertiary lymphoid structures (TLS). TLS have been detected in a wide range of solid tumors with evidence supporting their ability to function as mini-lymph nodes at these chronic inflammatory sites. Although their prognostic value is increasingly accepted, reliable markers that define and characterize a TLS and its activities are currently challenging, limiting their effectiveness as a reliable biomarker. A comprehensive characterization of TLS would likely help identify and define the spectrum of "TLS states", based on characteristics such as cellular composition, location, maturation and functionality.
The specific aim of this project is to identify the genes and pathways that are associated with anti-tumor activities generated in TLS and reflect improved responses to treatment and long-term survival. Our study involves the analysis of tissues from 38 primary HER2+ and triple negative (TN) breast cancer (BC) patients diagnosed and treated at the Institut Jules Bordet between 2015 and 2020. All the FFPE blocks from each of the 38 untreated breast tumors were examined and selected by an experienced pathologist. Using dual CD3/CD20 and Ki-67/PD-1 immunohistochemical (IHC) staining, TLS defined as B-cell follicle surrounded or adjacent to a T-cell region were scored. Patients were divided into 2 groups based on their TLS scores: A) BC with active TLS defined by the presence of a germinal center (GC^{pos} ; $Ki67^{+}$ follicular B cells), B) BC with inactive TLS (GC^{neg} ; $Ki67^{-}$ follicular B cells). Subsequently, RNA from the microdissected TLS were extracted and sequenced. The deconvolution of RNA-seq data from BC patients samples with active TLS and to inactive TLS was done using CIBERSORTx, in order to estimate the abundance of the different immune cell population, showed that patient with active TLS had higher levels of naïve memory and plasma B cells compared to patients with inactive TLS. Furthermore, a preliminary analysis of B cell maturation states and gene expression in active vs inactive TLS reveals their potentially important roles in TLS functionality.

#54. Shaping neonatal immunity by innate γδ T cells via maternal administration of probiotics.

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Background and aim: The early-life period is increasingly being recognized as a window of opportunity to shape innate and adaptive immunity that will influence life-long immune homeostasis. Gut microbiota and related probiotics have an important impact on immune cell development and function. However, while γδ T cells are the first T cells generated in early life, it is not clear how microbiota and probiotics can influence the development of this innate T cell population. γδ T cells are lymphocytes that express a rearranged TCR γ and TCR δ chains in their T cell receptor (TCR). Innate γδ T cells are the first T lymphocytes to arise from the fetal thymus and populate epithelial tissues such as in lung, where γδ T cell-derived IL-17 has been shown to play an important protective role against influenza infection in early life in a mouse model. In this study, we aim to investigate the influence of maternal microbiota and probiotics supplementation on the development and function of innate γδ T cells in the newborns.
Methods: Pregnant mice received a probiotic strain or a cocktail of antibiotics in drinking water. The effect of maternal microbiota modulation on the development and functional differentiation of γδ T cells was investigated in the thymus and lungs of newborns.
Results: On the first day of life, maternal probiotic administration resulted in a shift towards IL-17 effector committed γδ T cells in the lung. Therefore, maternal microbiota modulation via probiotics can be a potential strategy for protecting newborns against early life respiratory tract infections, which is currently under investigation.

#55. Single cell RNA sequencing to uncover intestinal cell-type specific cis-eQTL driving inherited predisposition to IBD.

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IBD is characterized by a chronic idiopathic inflammation of the gastrointestinal (GI) tract and consist of two main forms: ulcerative colitis and Crohn's disease. The importance of genetic susceptibility has been well established through Genome Wide Association Studies (GWAS), which have identified over 200 risk loci for IBD. However, the « true causative » genes in these loci have been identified for only few on the basis of independently associated coding variants. Fine-mapping studies suggested that most risk variants cause "cis"-eQTL, but recent post-GWAS studies could not find matching cis-eQTLs for the majority of risk loci (137/200). This indicates that the relevant cell types were either not present or under-represented.
In this study, we performed cis-eQTL analysis with single cell RNA-seq of human gut biopsies to uncover the truly relevant cell types by unbiased approach. Biopsies were collected from three GI locations (ileum, transverse colon, rectum) from the same individuals. Cell suspensions were prepared, tagged by location and cell fraction using TotalseqB hashtag antibodies and processed to the 10X Genomics Chromium. Data were analyzed using Cellranger and Seurat to identify the cell clusters and marker genes. In total, 57 individuals' biopsies data were integrated. Simultaneously, genotype was analyzed with Infinium OmniExpress-24v1 chip from 1 ml blood and imputed. Both scRNA-seq data and imputed genotypes were input to qtltools for cis-eQTL anlaysis. Analysis are actually ongoing and will certainly generate new set of cell-based eQTL and determine whether some of these drive inherited predisposition to IBD by comparing the corresponding expression association patterns with disease association patterns using methods developed in our laboratory.

#56. Maternal probiotics administration modulates the neonatal immune response against Influenza A infection.

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Background: Composition of the gut microbiota plays an important role in the education of the neonatal immune system and could influence the susceptibility to viral infection such as the Influenza A virus (IAV) that causes the seasonal flu and can lead to high rate of mortality in the high-risk population including the neonates. We started to investigate the impacts of probiotic treatment through the maternal supplementation with *Lactobacillus rhamnosus* (*L.rhamnosus*) and *Bifidobacterium lactis* (*B.lactis*) on the protection against IAV infection in the offspring.
Methods: Pregnant/breastfeeding mice were fed with *L.rhamnosus* or *B.lactis* and IAV infection was induced in their 3-days-old neonates with the IAV PR8 strain. Parameters of the IAV infection were assessed by flow cytometry and PCR in wild-type mice. Cytokines and chemokines status of the neonatal lungs and spleen were assessed also before the IAV infection. The $CD8^+$ T-cell activation was deeply assessed.
Results: *L.rhamnosus* initiated an inflammatory reaction in the neonatal spleen (TNFα, IFNγ) and in the lungs (IFNγ, Il6) while *B.lactis* initiated only an inflammatory reaction in the neonatal spleen (TNFα, IFNγ). In the IAV model, *L.rhamnosus* and *B.lactis* treatment decreased the viral loads in the neonatal lungs and during the first days of infection. During the active phase of infection, *L.rhamnosus* and *B.lactis* increased the production of IFNγ and downregulated the pro-inflammatory chemokines like CXCL9 and CXCL10. The activated-effectors $CD8^+$ T cells produced more IFNγ after the infection with IAV in the *L.rhamnosus* and *B.lactis* groups. Moreover, the $CD31^+$ $CD8^+$T cells increased their production of IFNγ in the *B.lactis* treated group.

#57. Role of both innate and adaptive immunity in the exacerbation of experimental autoimmune encephalomyelitis by Murid Herpesvirus 4; towards an explanation on how EBV increases multiple sclerosis risk.

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Multiple sclerosis (MS) is a multifactorial inflammatory disease of the central nervous system caused by a combination of genetic, immunological and environmental factors. The risk factor most strongly associated with MS is Epstein-Barr virus (EBV), but it is not yet known how EBV contributes to the development of MS. Here, we compared the development of experimental autoimmune encephalomyelitis (EAE), an animal model of MS, in mock mice and in mice infected with Murid Herpesvirus 4 (MuHV-4), an EBV related mouse virus. We showed that MuHV-4 infection prior to EAE induction leads to a significantly worse clinical outcome compared to mock infected mice. Moreover, we observed a major change in the phenotype of infiltrating monocytes after MuHV-4 infection in EAE induced mice; higher expression of MHC-II, CD86 and Sca-1, as well as Saa3 and CXCL9 related to a pathogenic phenotype in EAE development. Similar phenotypic alterations were also observed in the microglia of EAE mice infected with MuHV-4. In addition, we observed major changes in CNS infiltrating T cells in MuHV-4 infected mice compared to mock infected mice; more $CD8^+$ T cells, less regulatory $CD4^+CD25^+$ T cells and a higher expression of Ly6C on both $CD4^+$ and $CD8^+$ T cells. Surprisingly, the increase in clinical symptoms was not dependent on MOG_{35-55} autoantibodies or MOG_{35-55} specific $CD4^+$ T cells as significantly fewer of these antibodies and CD4+ T cells were present in EAE mice infected with MuHV-4 compared to mock infected EAE mice. Finally, we showed that all of the observed results were dependent on latency. Based on this initial characterization, we aim to understand how EBV increases the overall risk of MS, potentially opening up perspectives for the development of specific EBV-targeted therapies for MS.

#58. Restriction of IL-5 has minimal on the transcriptomic profile of residual eosinophils in murine and human hosts.

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Interleukin (IL)-5 is a cytokine almost exclusively devoted to regulating the biology of eosinophils. It is essential to the development of eosinophilia in severe eosinophilic asthma and other eosinophil-associated diseases. IL-5 has therefore become a therapeutic target of monoclonal antibody-based anti-eosinophil therapies, such as mepolizumab. This anti-IL-5 therapy alleviates eosinophilia and provides clinical benefits in the management of severe eosinophilic asthma. Besides its role in eosinophilia, IL-5 has been attributed a multitude of activities on eosinophils, from maturation of eosinophil progenitors to activation and survival of eosinophils in tissue. Yet the exact roles of IL-5 on eosinophils *in vivo* are not fully understood. We aimed to determine the exact impact of IL-5 on the development of eosinophils *in vivo* using $Il5^{-/-}$ mice and blood from mepolizumab-treated severe asthma patients. We compared the gene expression program of eosinophils in IL-5-depleted and IL-5-replete human and murine hosts, at steady state *in vivo* and following stimulation by the eosinophil-activating alarmin IL-33 *ex vivo*. Restriction of IL-5 availability did not elicit any demonstrable transcriptional response in residual eosinophils from mepolizumab treated patients or $Il5^{-/-}$ mice, and only affected 14 genes in their response to IL-33. From a clinical perspective, these results provide a reassuring message that long-term IL-5 restriction spares a pool of residual eosinophils that closely resemble those of healthy controls. Fundamentally, however, these results seem to contradict a long-held belief that IL-5 is required for the maturation of eosinophils. Our results suggest a major role for IL-5 in eosinophil expansion rather than influencing their maturation and differentiation.

#59. Identifying the sources and targets of TGF-β1 in human tumors.

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TGF-β1 plays a deleterious role in cancer, notably by inhibiting anti-tumor immunity. Which GARP-expressing cells among Tregs and/or blood endothelial cells produce TGF-β1 and which immune cells are suppressed by TGF-β1 within human tumors is not known. To address these questions, we analyzed, by multiplexed immunofluorescence (mIF), 87 samples of several cancer types (i.e. cutaneous melanoma metastases (CMM), colon adenocarcinoma (CC), lung carcinoma (LC), urothelial adenocarcinoma (UC), breast adenocarcinoma (BC)) and 10 samples of inflamed tonsils. We quantified and located cells and vessels (i) of the whole tumoral region of the mIF scans, and (ii) in 0.5*0.5 mm^2-squares virtually defined by dividing the region into a grid of squares, in order to identify the distribution of each element.
Majority of tonsil fragments were infiltrated by Tregs, with at least 0.35 % of Tregs among all cells, and at least 1/4 of tumor fragments were infiltrated with more than 0.35 % of Tregs among all cells. T cells represented 23 to 50% of all cells in tonsil fragments. In tumors, T cell infiltration varied greatly, ranging from 0 to 37% of all cells. We observed a higher Treg/T cells ratio in tumor fragments (0.13 on average) than in tonsil fragments (0.03 on average).
The abundance of cells under the influence of TGF-β1 (i.e. $pSMAD2^+$ cells) ranged from 0 to 10% of $pSMAD2^+$ cells among all cells. $pSMAD2^+$ cell abundance didn't correlate with the abundance Tregs or T cells.
Concerning the spatial distribution, Tregs, T cells and $pSMAD2^+$ cells appeared to be spatially heterogeneous. We discovered that Treg and T cells colocalized in most of the tumors, while it was not the case in the tonsil fragments. For the moment, no clear colocation between $pSMAD2^+$ cells and Tregs was established.

#60. MafB-restricted local monocyte proliferation precedes lung interstitial macrophage differentiation.

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Resident tissue macrophages (RTM) are differentiated immune cells populating distinct niches and exhibiting important tissue-supportive functions. RTM maintenance is thought to rely on either monocyte engraftment and differentiation, or RTM self-renewal. Two main RTM populations have been identified in the lung: alveolar macrophages (AM) and interstitial macrophages (IM). Previously, we have demonstrated that lung IM exhibit a tolerogenic profile and are able to limit the development of aberrant immune responses against allergens, thus underscoring their role as crucial regulators of lung homeostasis. To further understand the contribution of IM subpopulations to lung development, homeostasis, inflammation and repair, it is fundamental to investigate the mechanisms underlying IM development and differentiation. To this end, we developed an inducible mouse model of IM niche depletion and repopulation to investigate IM development *in vivo*. Using time-course single-cell RNA-sequencing analyses, bone marrow chimeras and gene targeting, we generated a real-time transcriptional atlas of monocyte-to-IM differentiation and found that engrafted Ly6C$^+$ classical monocytes could proliferate locally in a CSF1R-dependent manner before their differentiation into RTM. We further showed that the switch from monocyte proliferation towards IM subset specification was controlled by MafB, while c-Maf specifically regulated the identity of the CD206$^+$ IM subset. Our data shed new light on the transcriptional regulation of IM development and provide evidence that, in the mononuclear phagocyte system, cell proliferation is not merely restricted to myeloid progenitor cells and mature macrophages, but is also a tightly regulated capability of mature monocytes developing into RTM *in vivo*.

#61. Spectral Cytometry technology: High dimensional immunophenotyping with 22 colors panel to identify activation markers on T cells.

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Spectral flow cytometry is an innovative system that enables user-friendly multiparametric detection with several key advantages, including flexibility in panel design, ability to re-use spectral references, and tools to manage autofluorescence.
The SONY ID7000™ Spectral Cell Analyzer supports high-parameter flow cytometry by providing comprehensive information on heterogeneous cell populations, with high sensitivity, to detect dim and rare populations. The greatest advantage of spectral flow cytometry is the ability to measure the full fluorescence spectrum for each individual fluorophore in the sample.
In addition, cellular autofluorescence can be used as a parameter, providing an additional powerful approach to cell characterization. The channel signals are used to create a single spectral emission signal, regardless of the number of fluorochromes analyzed. Unmixing, a powerful capability, then separates the fluorophores into pure signals that measure the intensity of each fluorochrome at each wavelength to more accurately measure the data being analyzed.
In collaboration with the GIGA Hematology laboratory, a 22 colors full spectrum flow cytometry panel was designed and optimized to identify activated/exhausted T cells. Development and optimization of this panel was performed on freshly isolated T cells from buffy coat and *ex vivo* stimulation with interleukin -2 and CD3/CD28 beads. In the future, this panel will allow GIGA Hematology laboratory researchers to characterize T cells in humanized NSG murine models of GVHD.

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#62. Th2-skewing of human circulating iNKT cells in the context of obesity.

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Systemic metabolic disorders associated with obesity have been proposed to negatively impact both intrinsic metabolism and function of circulating immune cells. To further investigate this question, we analyzed the peripheral invariant Natural Killer T (iNKT) cells phenotype and function in lean and obese patients. iNKT cells are unconventional T cells that express semi-invariant TCR. iNKT cells recognize through their TCR lipid antigens presented by CD1d on antigen presenting cells, causing the rapid release of Th1 and/or Th2 and/or Th17 cytokines.
Because the frequency of iNKT cells is downmodulated in obesity, we hypothesized that a chronic and/or excessive stimulation of iNKT cells could modulate their activity and contribute to the loss of immunosurveillance. We have highlighted a disruption of peripheral circulating peripheral iNKT cells in obese patients compared to lean individuals: 1) iNKT cells show an activated profile; 2) The percentage of anti-inflammatory $CD4^+$ iNKT cells subpopulation is increased to the detriment of pro-inflammatory subpopulation; 3) iNKT cells produce significantly less IFN□ in response to ex vivo PMA-Ionomycin stimulation and the ratio (% of IFN-γ^+ cells /% of IL-4^+ cells) decreases. Taking together, these results indicate a shift towards anti-inflammatory Th2-phenotype with obesity. This strong alteration of iNKT cells activity seems to be correlated to plasma lipid content and fasting insulin levels but not with glycemic status.
As iNKT cells mediate various immune responses in peripheral organs, such impact of obesity on iNKT cells function could participate to impaired immunosurveillance against microbial infections and tumor in obese patient.

#63. CD22: a specific surface marker of IL-4-dependent virtual memory CD8+ T cells in the periphery.

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During persistent helminth infection, IL-4 drives the expansion of antigen (Ag)-inexperienced CD49d^{low}CD44^{high} virtual memory CD8 T cells (T_{VM}), resulting in a raised CD8 T cell activation and could be associated with enhanced control of viral coinfection. However, the regulation of IL-4-driven expansion of peripheral T_{VM} remains unknown. Here, single-cell RNA sequencing has been used to study IL-4-driven T_{VM} expansion during helminth infection. Using mice conditionally deficient for IL-4Rα in peripheral CD8$^+$ T lymphocytes, we could identify a cluster of cells that upregulated signature genes of T_{VM} cells in response to IL-4 during worm infection. Among upregulated genes, we identified *Cd22* that encodes the inhibitory receptor sialic acid-binding immunoglobulin-type lectin 2. CD22 expression could be detected by flow cytometry and was restricted to IL-4-responsive T_{VM} cells in the spleen, mesenteric LN but also non-draining lymphoid tissues during helmith infection. *In vivo*, IL-4c treatment did not induce CD22 expression in thymic T cells, and while IL-4c-induced expansion of T_{VM} cells was maintained over time, CD22 surface detection was however transient. In addition, *in vivo* IL-4c treatment upregulated IFNγ expression specifically in CD22$^+$ T_{VM} cells. Bulk RNAseq experiment revealed that CD22$^+$ T_{VM} in response to IL-4c displayed an activated phenotype with high expression of effector molecules like *Gzma, Gzmm, Ctla2a, Ccl4, Xcl1,* as well as inhibitory receptors *Klra5*, *Klrc2, Klrd1, Entpd1* (CD39) or CD160, and proliferation marker *Mki67*. While further investigation of the functional role of CD22 in regulating the T_{VM} response to IL-4 and in response to viral infection are on-going using CD22$^{-/-}$ mice. These data demonstrated that CD22 is a surface marker that further defines IL-4-induced T_{VM} cells and is likely involved in the regulation of T_{VM} responses during helminth infection.

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