UNBIASED APPROACH TO DISSECT *EX-VIVO* HUMAN CD8 T CELL RESPONSES AGAINST SARS-COV-2

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Cytotoxic T lymphocytes with high avidity T cell receptors (TCR) kill targets displaying a single peptide-major histocompatibility complex-I complex. CD8 T cell responses are mainly studied with an educated-guess approach that is based on the usage of algorithm-predicted peptides binding to a given human leukocyte antigen (HLA) molecule to isolate specific T cells. This strategy is limited because it does not take into account differences in the abundance of naturally processed peptides. We developed a target-agnostic method for the unbiased stimulation and cloning of pathogen-specific CD8 T cells, exploring the entire response of the individual taking into account antigen processing. We analysed TCRVB sequences and specificity of polyclonal T cell lines and clones compared to donor-matched single cell sequencing of total CD8. To prove that our method outperforms the educated-guess approach, we focused on CD8 T cell response to SARS-CoV-2. We also isolated directly ex-vivo and cloned early activated CD8 HLA-DR+ CD38+ T cells 6 days post SARS-CoV-2 vaccination. Compared to the target-agnostic method, we obtained more clonotypes covering a broader specificity in shorter time. Overall, the data delineate an improved pipeline to study CD8 T cell responses and TCR repertoire diversity that will be of interest for the rational design of novel vaccines and adaptive T cell therapy.

DEVELOPING DNA-BARCODED MHCII MULTIMER TECHNOLOGY FOR HIGH-THROUGHPUT DETECTION OF ANTIGEN-SPECIFIC CD4 T CELLS

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The role of antigen specific T cells responding to antigen challenge is a topic of intense studies, and critical for development and mechanistic insight to therapeutic strategies and disease development. Major histocompatibility complex (MHC) I and II are the key to understanding T cell recognition in health and disease. Studied despite the genetic heterogeneity in antigen recognition, MHCI has been well adopted for T cell assays to determine the recognition of disease-specific antigenic peptides and studying functional behavior of CD8 T cells. The same is not true for CD4 T-cells as MHCII is more challenging to produce than MHCI. The current aim of the project is to establish a platform for high-throughput detection of antigen-specific CD4 T cells using DNA-barcoded MHC II multimers. The MHCII is loaded with a relevant peptide of interest, and multimerized as dextramer along with DNA barcodes. This would allow for in-depth analyses of the specificity of immune interactions driven by CD4 T cells, and provide better understanding of the antigen driven association between CD4 and CD8 T cell responses. We also aim to use the DNA barcode-tagged MHC multimer technology for single cell interrogation of antigen specificity combined with T cell receptor usage among CD4 T cells.

UNRAVELLING THE METHOD-OF-ACTION OF THE INNATE IMMUNE CHECKPOINT CD47-SIRPA

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In the last decades, different methods have been developed to harness the immune system for the treatment of cancer. One method is the use of tumor-specific monoclonal antibodies (mAbs). These mAbs specifically bind to tumor antigens and are recognized by different immune cells. Neutrophils are able to kill antibody-opsonized tumor cells through a process called trogocytosis. During this process, neutrophils phagocytose small pieces of the tumor cell cytosol, eventually inducing a necrotic type of cancer cell death, named trogoptosis. A way for tumor cells to evade the immune system is through the expression of checkpoint molecules. CD47-SIRPα is an innate immune checkpoint axis, where CD47 is expressed on virtually all cells, while SIRPα is expressed on myeloid cells, such as neutrophils and macrophages. Tumor cells often overexpress CD47 and thereby prevent tumor elimination by myeloid cells. Blocking the interaction between CD47 and SIRPα has been shown to potentiate tumor cell killing of antibody-opsonized tumor cells. However, downstream signaling of SIRPα in neutrophils remains to be elucidated. During my PhD I will investigate the method-of-action of CD47-SIRPα signaling in neutrophils. In addition, I will study the effect of blocking the CD47-SIRPα axis in combination with other immunotherapies to potentiate tumor cell killing.

ACTIVATION OF T-CELLS VIA METABOLIC LABELING

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Immunotherapy has become a well-established tool in cancer treatment. Although it can be an extremely powerful therapy with long-term positive outcomes, there are still many hurdles to overcome.

In our research, we introduce an original, chemistry-based approach to targeted anti-tumor T-cell activation. Using new methodology, the surface of the immune cells is modified via metabolic labeling in combination with bioorthogonal click chemistry and the T-cells are then guided to and activated against cancer cells via synthetic antibody mimetcs called iBodies. iBodies are biocompatible polymer conjugates consisting of N-(2-Hydroxypropyl)methacrylamide (HPMA) backbone decorated with small-molecule ligands which serve several purposes such as e.g. targeting, immobilization or bioconjugation. By 'clicking' iBodies carrying multiple prostate specific membrane antigen (PSMA) ligands to T-cells which were metabolically modified for this purpose, we were able to guide the immune cells to PSMA expressing cancer cells and induce their activation upon biding. Moreover, we were able to do so without prior antigen-specific sensitization of the immune cells.

By utilizing the 'chemical' approach to immune cell activation, we developed a tool which is flexible, requires no genetic intervention and might have promising potential in immunotherapy.

IDENTIFYING DIFFERENCES IN IMMUNE RESPONSE ACCORDING TO SOCIOECONOMIC STATUS (SES) USING THE *MILIEU INTÉRIEUR* COHORT

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Individuals with a low socioeconomic status (SES) as defined by educational attainment, household income and other demographic factors are at greater risk of both contracting and developing severe disease compared to people from a higher SES. Factors such as age, sex and Cytomegalovirus (CMV) serostatus are known to have a major impact on the human immune response, however the impact of SES on immune response variability is not well understood. To better understand the impact of SES on immune variability we used data from the Milieu Intérieur project, a large 1,000-person cohort of healthy individuals with extensive demographic, genetic and biological data. Using an Elo-rating system based on demographic features such as education, income, and household, we classified donors as low, middle, or high SES. Using linear models and machine learning techniques we assessed potential differences in gene expression following whole blood stimulation between SES groups. We found, for men, differentially expressed immune genes between groups of SES under stimulations specific to adaptative response. We found that females from a low SES have a significantly higher CMV seropositivity than those from a high SES. The CMV serostatus between low and high males was similar. Finally, application of random forests models reveal that the gene expression profile of the individuals seems to be highly explained by SES features such as Elo, incomes and housing. These findings suggest a disparity between males and females in the variation of the immune response regarding SES. The results presented here show further our understanding of how sociological factors can influence the immune response and begin to unpick the role of the immune system in mediating differential susceptibility to infection between low and high SES.

HUMAN MONOCLONAL ANTIBODIES TO THE SPIKE SUBDOMAIN 1 NEUTRALIZE SARS-COV-2 AND ITS VARIANTS OF CONCERN

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Progress in the fight against COVID-19 is jeopardized by the emergence of SARS-CoV-2 variants that diminish or abolish the efficacy of vaccines and antiviral monoclonal antibodies. Novel immune therapies are therefore needed, that are broadly effective against present and future coronavirus threats. In principle, this could be achieved by focusing on portions of the virus that are both functionally relevant and averse to change. The Subdomain 1 (SD1) of SARS-CoV-2 Spike protein is adjacent to the Receptor Binding Domain (RBD) and its sequence is conserved across SARS-CoV-2 variants. In order to specifically identify and study human antibodies targeting SD1, we designed a flow cytometry-based strategy that combines negative selection of B cells binding to the RBD with positive selection of those binding to SD1-RBD fusion protein. Among the 62 produced human monoclonal antibodies, 16 are SD1-specific and 6 of them cross-react with SD1-RBDs corresponding to all twelve main variants of concern. Antibody sd1.040 also neutralizes SARS-CoV-2 pseudovirus variants, likely stabilizing the Spike trimer. sd1.040 synergizes with an antibody to the RBD for neutralization, and protects mice when present in a bispecific antibody. Thus, naturally occurring antibodies can neutralize SARS-CoV-2 variants by binding to SD1 and can act synergistically against SARS-CoV-2 in preclinical models.

LOW GLYCOSYLATION OF THE VARIABLE DOMAIN OF IGG AUTOANTIBODIES IN RHEUMATOID ARTHRITIS IS ASSOCIATED WITH INCREASED INFLAMMATION

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The disease-specific IgG autoantibodies of rheumatoid arthritis (RA), anti-citrullinated protein antibodies (ACPA), are featured by the presence of N-linked variable domain glycosylation (VDG). Although it has been shown that N-glycan changes in the Fc region can be employed as biomarkers for immune activation, little is known about whether IgG-VDG changes are associated with inflammation. Here, we investigated the relationship between ACPA-IgG VDG and inflammation marker C-reactive protein (CRP). ACPA-IgG isolated from plasma of RA patients with low or high CRP levels were digested with a cysteine protease. N-linked glycosylation of total IgG, F(ab)2 and Fc was analyzed by LC-MS. We determined the percentage of ACPA-IgG VDG and analyzed changes in glycan composition. The results revealed a negative correlation between presence of VDG and CRP. ACPA-IgG VDG was abundant in the low CRP group (median of 106%), while individuals with high CRP levels had significantly lower VDG (median of 39%). No major differences in variable domain N-glycan composition were observed. In conclusion, RA patients with ACPA-IgG with high VDG exhibit less of the inflammation marker CRP compared to those with low VDG. It can be hypothesized that under high inflammatory conditions, new ACPA-IgG clones are generated with lower VDG or B cells secreting ACPA-IgG with low VDG are preferentially selected.

CD4 T CELL HELP EXPANDS A MODC POPULATION INVOLVED IN T CELL PRIMING

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Monocyte derived dendritic cells (MoDCs) have been shown to be recruited to draining lymph nodes (dLN) in an adjuvant or pathogen dependent manner. Here, they do not prime T cells but skew their differentiation by cytokine secretion. Several groups have shown that MoDCs are critical for CD4 T cell differentiation towards T helper 1(Th1) or T follicular helper cells (Tfh) and CD8 T cell differentiation to cytotoxic T cells. The influx of MoDCs in these contexts is dependent on adjuvant, but not antigen. We study T cell differentiation in mice by making using of a DNA tattoo vaccination model using plasmid DNA that either encodes only a CD8 T cell epitope (NoHelp) or a CD8 T cell epitope with CD4 T cell epitopes (Help). Using this model, we show infiltration of MoDCs in the dLN which is much more pronounced and persists longer in Help than NoHelp. Additionally, MoDCs in Help have an increased expression of costimulatory molecules such as CD40. Thus, our data suggests that engagement of CD4 T cells enhances the recruitment, persistence and activation of MoDCs. We hypothesize that CD4 T cells can organize their own differentiation into Th1 or Tfh and improve the CD8 T cell response via MoDCs. By making use of monocyte depletion with an anti-CCR2 antibody, we have shown that removal of MoDCs reduces the frequency and effector differentiation of antigen specific CD8 T cells in Help.

TARGETING CD169+ ANTIGEN PRESENTING CELLS WITH NANOBODY-LIPOSOMES RESULTS IN INCREASED CYTOTOXIC T CELL ACTIVATION

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The introduction of checkpoint inhibitors to treat cancer has greatly improved the prognosis of a group of patients distinguished by high immune cell infiltration within the tumor microenvironment. Vaccination strategies with tumor-associated or specific neo-antigens can be used to induce immune cell activation and tumor infiltration and thereby enhance the effect of checkpoint inhibitors. We have previously observed that splenic CD169+ macrophages, which in humans also express DC-SIGN, efficiently transfer antigens to dendritic cells which subsequently activate cytotoxic T cells. In this project, we have developed a vaccination strategy that consists of cancer-antigen containing liposomes to which CD169- or DC-SIGN-targeting single domain antibodies from camelids (nanobodies) are conjugated. Both in vitro and in vivo, we demonstrated increased binding and uptake of CD169 and DC-SIGN nanobody (Nb) liposomes by CD169- and/or DC-SIGN expressing human and murine cell subsets. After incorporating tumor antigens into these liposomes, we showed CD169 Nb liposomes could elicit increased antigen-specific immune response as compared to non-targeted control liposomes in vivo. To conclude, Nb liposomes are a novel vaccination strategy that can be extended to different targets on immune cells in order to elicit a versatile immune response against specific tumor antigens.

REGULATORY T-CELLS ARE VERY SHORT-LIVED AND THEIR MAINTENANCE IS LARGELY INDEPENDENT OF THYMIC OUTPUT

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Regulatory T-cells (Tregs) are a key factor in maintaining immune homeostasis, though many aspects about their maintenance mechanisms and lifespan estimates are inconsistent between studies.

We used 2D2O labelling in humans to estimate the Treg lifespan in vivo. In addition, we studied T-cell receptor (TCR) repertoire diversity of Tregs. To investigate the contribution of the thymus in the maintenance of Tregs, we examined how disturbed thymic situations affect the dynamics and TCR diversity. Here, we compared the TCR repertoire of Tregs in young individuals (20-37 years), individuals who underwent thymectomy early in life (ITECs, 23-33 years) and older individuals (69-91 years).

We found that Tregs have a shorter lifespan than CD4+ memory T-cells. Additionally, we found that the population size and the fact that Tregs express consistently higher levels of proliferation marker Ki-67 than memory T-cells, were independent of the age or the presence of the thymus. Nevertheless, we observed a lower level of TCR diversity of Tregs among older individuals compared to young individuals, but it remains similar in ITECs compared to young individuals.

Altogether, we show that the Treg population is maintained dynamically, and mostly independent of thymic output. In addition, the decreased TCR diversity of Tregs in older individuals is assigned to other factors than thymic involution.

REVEALING UNKNOWN EVASION MECHANISMS OF LEUKEMIC CELLS FROM NATURAL KILLER CELLS

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Immunoediting describes the dual function of the immune system in tumor development that can be tumor-suppressive and/or -promoting. Whereas the importance of adaptive immune cells in this process is well known, the role of innate lymphocytes like natural killer (NK) cells remains enigmatic. We hypothesize that besides killing, NK cells put selective pressure on leukemic cells causing tumor editing characterized by recurrent changes in key immune surveillance-related pathways.

To test our hypothesis, we co-cultured DNA barcoded mouse B-ALL cell lines with NK cells for 20 days in vitro to generate an NK cell-resistant tumor cell population. Tumor cells were analyzed for their barcode diversity and epigenetic and transcriptomic changes. Quantitative analysis of the barcode abundance in NK cell-resistant and -naïve cells showed that most tumor cells were either intrinsically sensitive or resistant towards NK cells, whereas very few tumor cell clones showed an acquired resistance. Transcriptome analysis revealed a novel set of differentially regulated genes in NK cell-resistant tumor cells, which are currently validated functionally. Our results demonstrate that tumor cells are sculpted by NK cells and use different avenues to escape from eradication. These findings will help to uncover novel evasion mechanisms and may provide new strategies for future cancer therapies.

THE DNA SENSING PLATFORM CGAS/STING IS CRITICALLY NEEDED FOR THE CONTROL OF RNA VIRAL ENCEPHALITIS

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Infections of the central nervous system (CNS) can lead to detrimental inflammation, which may result in severe neurological manifestations and even death. As functional therapeutics are still very limited, it is important to find new ways to combat viral encephalitis. The early sensing of pathogens by pattern recognition receptors is of high relevance for the survival of an infected individual. Since the cGAS/STING pathway has mainly been studied in the context of DNA virus infections, it remains unclear whether this primarily DNA-sensing platform is also involved in the control of RNA virus infections of the CNS. In order to address this question, we intranasally infected wild type (WT) and cGAS/STING deficient mice with vesicular stomatitis virus (VSV). Our experiments showed that STING is critically needed to support survival upon VSV infection. Using the ribosomal tagging approach (RiboTag), we identified microglia as the predominant CNS cell type expressing cGAS and STING in the homeostatic as well as the infected olfactory bulb. Furthermore, histological analyses revealed that infected cGAS/STING deficient mice exhibit higher infiltrations of myeloid cells than infected WT mice. Overall, our data suggests an important role of the cGAS/STING axis in the control of RNA viral encephalitis.

PD-1^{+}TCF-1^{+} STEM-LIKE CD8^{+} T CELLS RESULT FROM DEFICIENT CD4^{+} T-CELL HELP DURING PRIMING

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In chronic infection and cancer, "stem-like" PD-1^{+}TCF-1^{+} CD8^{+} T cells are recognized as the virus- or tumor antigen-specific precursors of terminally exhausted cells and the responders to PD-(L)1-targeting cancer immunotherapy, but their origin is unclear. Using a mouse vaccination model and high-dimensional flow cytometry, we demonstrated that the stem-like PD-1^{+}TCF-1^{+} CD8^{+} T cell population is raised by "helpless" priming, as result of an incomplete trajectory towards terminally differentiated effector CTL. In presence of CD4^{+} T-cell help, the primed CD8^{+} T-cell pool initially gives rise to stem-like PD-1^{+}TCF-1^{+} cells that are gradually replaced by KLRG1^{+}CX3CR1^{+} terminally differentiated effector CTLs. In absence of CD4^{+} T-cell help, stem-like CD8^{+} T cells accumulate in lymph nodes and no terminally differentiated CTLs are formed. Helpless CD8^{+} T cells expand and complete full CTL effector differentiation if CD4^{+} T-cell help is provided, indicating that these cells can are not fated for terminal exhaustion. These results argue that providing CD4^{+} T-cell help signals or their mimic can rescue stem-like cells from terminal exhaustion and should be incorporated in immunotherapies of chronic infection and cancer.

DIRECT CELL REPROGRAMMING OF FIBROBLASTS TO NATURAL KILLER CELLS

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Natural Killer (NK) cells are key innate lymphocytes that control cancer development. NK-based immunotherapies emerged as promising treatments against hematological and solid tumors. However, limited persistence in vivo and complexity of differentiation protocol, pose roadblocks to widespread NK-based therapeutics. Direct cell fate reprogramming was successfully applied to reprogram myeloid immune cells, but instructor transcription factors (TFs) of lymphoid cells are not known. We hypothesize that NKs can be efficiently generated by direct cell fate reprogramming. To induce NK identity, we overexpressed NK canonical TFs – TBET, ETS1, NFIL3, EOMES, (TENE) – in mouse fibroblasts and profiled induced cells by scRNA-seq. TENE induced downregulation of fibroblast signature, with upregulation of NK genes illustrated by expression of CD34, CD38 and Itga2. In human fibroblasts TENE induced surface expression of CD34 and CD56, supporting the species conservation of the minimal transcriptional network for NK reprogramming. To identify candidates that support NK reprogramming, we screened 48 TFs employing a barcoded TF approach coupled with scRNA-seq and identified RUNX, IKZF and STAT families as potential novel regulators. Overall, our findings contribute to understand the role of TFs in NK specification and present evidence for an alternative platform to generate patient-specific NK.

MECHANISMS OF REGULATION OF ANTI-TUMOR RESPONSE IMPLEMENTED IN TERTIARY LYMPHOID STRUCTURES IN CLEAR CELL RENAL CELL CARCINOMA AND SOFT TISSUE SARCOMA

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Immune tumor microenvironment has been shown to control cancer progression. These immune cells, especially T and B cells, can be organized in tertiary lymphoid structures (TLS), found in tissues with chronic inflammation and antigen persistence. How these structures shape the anti-tumor response has been studied in clear cell renal cell carcinoma (ccRCC). Using spatial transcriptomic analysis, we showed that plasma cells differentiated in TLS disseminate into tumor beds and produce anti-tumor antibodies. IgG-labeled tumor cells undergo apoptosis when close to macrophages, suggesting that antibody-dependent cellular cytotoxicity may occur and support the anti-tumor immune response. TLS presence was indeed associated with positive clinical outcomes and responses to immunotherapy in ccRCC. In an heterogeneous type of cancer, soft tissue sarcoma (STS), selecting patients for the presence of TLS increases response rates to immunotherapy. I am involved in a study to build a sarcoma immune atlas to characterize anti-tumor responses in 12 histological subtypes of STS. Interestingly, not all lymphocytes perform anti-tumor function in TLS. For example, Tregs in TLS are associated with poor responses to immunotherapy in STS, suggesting that there may be mechanisms involved in the inhibition of the anti-tumor activity implemented in TLS. This issue will be studied during this PhD project.

ENGINEERING A HIGH-THROUGHPUT PLATFORM FOR SCREENING T CELL RECEPTORS FOR SPECIFICITY AND FUNCTIONALITY

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While T cell receptor recognition plays a central role in regulating human disease, there are few high-throughput tools for screening T cell receptor libraries for specificity and functionality. Human T cell lines have been engineered to express recombinant T cell receptors. However, this has mostly been attempted using viral transduction. Viral integration of recombinant T cell receptors is random and may occur at multiple sites in the genome. Instead, using CRISPR/Cas9 to insert T cell receptors in the endogenous T cell receptor locus ensures single-site integration and expression from the endogenous promoter. We have been engineering a human T cell line for high-throughput screening of specificity and functionality of CD8 co-receptor-dependent T cell receptors. The cell line will be engineered to express Cas9 enzyme allowing for low-cost downstream knock-in of recombinant T cell receptor libraries. In addition, we will knock out the endogenous T cell receptor and knock-in CD8. This T cell receptor screening platform will be applied for validating 40 novel T cell receptors identified as persistent T cell clones in patients receiving adoptive cell therapy with tumor-infiltrating lymphocytes. Re-expression of these T cell receptors will confirm their tumor-reactivity and antigen-specificity *in vitro* and may contribute to identifying T cell receptors for therapeutic use.

INTEGRATIVE APPROACH TO ELUCIDATE B-CELL LYMPHOMA MECHANISMS

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The complexity of biological systems requires orchestrated analysis of incongruous data to better understand diseases. We developed an approach to re-purpose datasets, add statistical power to the data, and contextualize significant molecules across multiple studies to generate novel hypotheses. Our study model is Diffuse Large B-cell Lymphoma (DLBCL), an heterogeneous cancer segregated in subtypes by their gene expression profile. However, there is a need to understand the uniqueness of their etiology to propose novel strategies for the most aggressive cases. Through our approach, we identified molecules across DLBCL subtypes associated with DNA-repair mechanisms, crucial and tightly regulated processes in B-cells for antibody development and affinity maturation. Our hypothesis proposes the reliance of the aggressive DLBCL cases on the aberrant function of B-cells class switching recombination (CSR) to promote hallmark translocations in DLBCL and survival of cancer cells. The expression of these molecules has been validated and can segregate DLBCL-subtype lines. We also evaluated and validated the biological implication of these molecules in CSR through knockouts in a murine CSR model. As an outlook, we aim to dissect the molecular mechanisms these molecules are involved in, assess their clinical significance and the pathological implication of CSR in DLBCL lines.

TISSUE-RECRUITED INFLAMMATORY MACROPHAGES HAVE ENHANCED NLRP3 INFLAMMASOME PATHWAY ACTIVITY IN RHEUMATOID ARTHRITIS

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The NLRP3 inflammasome is a sensor of homeostasis activated in myeloid cells by pathogen- or tissue-derived danger signals. Activated NLRP3 drives the secretion of proinflammatory cytokines IL-1b and IL-18 to initiate the inflammation, and simultaneously induces pyroptosis to eliminate compromised cells. While activation of NLRP3 is protective in infection, excessive NLRP3 activity contributes to chronic inflammatory diseases such as neurodegeneration, colitis, and rheumatoid arthritis (RA). In which cells NLRP3 becomes activated in diseased tissue is unknown. Further, what keeps NLRP3 activity off in health and resolution is poorly understood but could be harnessed for therapeutic interventions.

Resident synovial macrophages express efferocytosis receptors including MerTK. MerTK is expressed in health and resolution but is lost in active RA. MerTK is a known inducer of IL-10, a negative regulator of NLRP3. Recent studies have demonstrated inflammatory macrophages are recruited to the synovium during active RA. We characterized the expression of inflammasomes in macrophages in health and disease of human and mouse RA. We demonstrate low NLRP3 and IL-1b expression in resident synovial macrophages, but high in recruited MerTK- GM-CSFR+ expressing macrophages in active RA. We also show *in vitro* that GM-CSF promotes enhanced and sustained NLRP3 activity in bone marrow progenitors.

MACROPHAGES REGULATE ILC2 IMMUNE CHECKPOINTS EXPRESSION AND RESPONSIVENESS TO TISSUE- DERIVED SIGNALS

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ILC2s are a subset of innate lymphoid cells involved in the type 2 immunity and are specialized in the secretion of IL-4, IL-5, and IL-13 in response to IL-25, TSLP, and IL-33. ILC2s were discovered recently, therefore, little is known about how other immune cells regulate their response. ILC2s are mainly tissue resident cells and they are known to interact with macrophages. We wanted to investigate how macrophages regulate ILC2 functions and what are the molecular mechanisms involved. To study this, we performed co-culture experiments between ILC2 from healthy donors' peripheral blood and monocytes-derived macrophages (MoM). We found that co-culture results in a reduction of PD-1 expression and an increase of CTLA4 expression, compared to ILC2s cultured alone. This is associated with a higher ability of co-culture-derived ILC2s to secrete type 2 cytokines in response to IL33+IL25 stimulation. Moreover, these experiments indicate that immune checkpoints PD1 and CTLA4 are regulated by different mechanisms. These results suggest that MoM limit ILC2 activation, thus preventing their exhaustion and rendering them more responsive to signals from the tissue. This study would help us understand how ILC2 function is regulated in pathological conditions in which they are known to play an important role, such as cancer and allergic diseases.

A FEEDBACK LOOP BETWEEN EPITHELIUM AND INNATE LYMPHOID CELLS CONTROLS INTESTINAL TGF-B1 DYNAMICS

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Innate Lymphoid Cells (ILCs) are involved in intestinal homeostasis and pathogenesis. Type-1, 2 and 3 ILCs transdifferentiate into one another leading to their altered distribution in intestinal inflammation. ILCs express Transforming Growth Factor-beta 1 (TGF-β1), a pleiotropic cytokine secreted in an inactive form, leading to modulation of intestinal and matrix remodelling and contributing to the development of fibrosis. We aimed to determine how ILC-produced TGF-β1 is activated and how its levels are regulated in the intestine. Lamina propria ILCs and intestinal organoids were cultured with Transformed Mink Lung Cells to detect bioactive TGF-β1. To identify TGF-β1 activators, publicly available data sets were screened, and target genes validated by RT-qPCR. ILC-organoid co-cultures were used to study the immune-epithelial interactions. In addition to possessing the capacity for Tgfb1 expression, ILCs respond to, produce and activate TGF-β1. When stimulated with recombinant TGF-β1, organoids increase their production of this cytokine. The expression of TGF-β1 activators changed when ILCs and organoids were co-cultured or stimulated with TGF-β1. Collectively, these data demonstrate the ability of ILCs and organoids to produce and activate TGF-β1, reveal possible activators and suggest that TGF-β1 levels in the intestine are controlled by an ILC-epithelial feedback loop.

UNRAVELLING THE B CELL IMMUNE SYNAPSE: A PROTEOMIC APPROACH TO DISSECT B CELL RECEPTOR SIGNALLING THROUGH SURFACE-BOUND ANTIGEN

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The immune synapse (IS) is a cell-cell interaction platform critical in lymphocyte activation by specific antigens. Despite of B cells being able to also respond to soluble antigens, in particular the in vivo importance of the IS and surface-tethered antigen recognition has strongly emerged in the recent years. The IS serves as a dynamic hub for multiple cellular actions but the molecular details of these functions, especially beyond the B cell antigen receptor (BCR) signalling, remain poorly understood. Here, to address the lack in the systems level understanding of the IS, we setup methodology for comprehensive investigation of the composition of the primary mouse B cells' IS at proteome level. Utilizing functionalized magnetic beads to mimic antigen presenting cells and trigger IS formation on them, we developed a method to specifically and robustly extract the cell adhesions on the beads, namely the IS or transferrin receptor mediated adhesion as a control. Our data revealed 661 proteins exclusively present in the IS at 15 minutes after BCR engagement, 13 exclusively in the control adhesions and 365 proteins shared between the samples. We got strong coverage of the known components of the IS as well as identified a plethora of unknown proteins and functional pathways with hitherto unknown roles in B cell IS.

IL-1B$^+$ MACROPHAGES SPATIALLY SEGREGATE WITHIN INFLAMED TISSUE NICHES IN PANCREATIC CANCER

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Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease, where inflammation cooperates with altered tissue architecture to foster tumor growth. While being the most abundant leukocytes in PDAC, tumor-associated macrophages (TAMs) have heterogeneous ontogeny, gene expression profile and spatial location. As a result, TAMs might exert diverse activities within the tumor microenvironment, posing a challenge to their therapeutic targeting. We identified interleukin (IL)-1b$^+$ TAMs, a macrophage population that correlates with inflammation and poor prognosis of PDAC patients. Building on spatial transcriptomics, we showed that IL-1b$^+$ TAMs occupy functionally discrete niches in the mouse PDAC stroma, which are distinct from those occupied by other TAM subsets and correspond to areas of inflammation, angiogenesis and hypoxia. We applied Molecular Cartography to map clusters of TAMs with single-cell resolution, confirming the presence of IL-1b$^+$ TAM niches in human PDAC. Notably, dissection of cellular interactors within areas of IL-1b$^+$ TAMs uncovered co-localization with populations of tumor cells and fibroblasts that selectively express an inflammatory gene program. Collectively, our findings revealed segregation of IL-1b$^+$ TAMs within inflamed tissue niches in PDAC, prompting further investigations aimed at harnessing their local interactions to modulate immunotherapy efficacy.

DENDRITIC CELLS, BRIDGING NEUROMODULATION AND IMMUNITY

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The immune and nervous system have co-evolved to modulate each other to provide quicker, anticipatory, and more effective immune responses to different challenges, including cancer. Interestingly, tumour innervation is often correlated with poorer disease outcome. Nonetheless, anti-tumor immunity is mainly driven through the integration of different environmental signals by Dendritic Cells (DC), a population of the immune system. However, how DC integrate neuronal cues and how this axis could be harnessed to improve tissue health and disease, has not yet been explored. We hypothesise that DC are at the interface of the neuroimmune axis by integrating, among other signals, neuronal-derived cues that will modulate the immune response. Our preliminary data indicate that DC have a specific set of neuronal receptors through which they can incorporate different neuronal signals that impact their function in vitro and in vivo. Additionally, blocking neuronal sensing in DC leads to improved prognosis in a mouse model of melanoma supporting the idea that this novel neuroimmune axis plays a critical role in the regulation of anti-tumour immunity. We foresee that unravelling the molecular dialog between neurons and DC and its effects on DC-mediated immune modulation will serve as a cornerstone for comprehending how neuroimmune circuits influence a wide range of conditions beyond cancer.

PROTEASOME ACTIVATION PREVENTS T CELL EXHAUSTION AND PROMOTES ANTI-TUMOR IMMUNITY

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Immunotherapy has been a breakthrough in cancer control. Nevertheless, few patients can benefit from it and a high fraction relapses. T cell exhaustion limits the efficacy of some of these treatments. Tumor-infiltrating lymphocytes (TILs) suffer chronic stimulation, which together with features of the tumor microenvironment (TME), leads to exhaustion generation, characterised by poor anti-tumor responses. Single-cell transcriptomics of TILs from 19 different cancer types revealed proteasome as a key factor in exhaustion. Simulating features of the TME, we have developed an *in vitro* model of exhausted T cells (iT_{EX}). iT_{EX} mimicked typical hallmarks of exhaustion and accumulated dysfunctional proteins. In response to this, iT_{EX} upregulate proteasome genes and present augmented proteasome activity. However, the capacity of the proteasome is not enough to cope with such proteotoxicity, leading to exhaustion. By inducing NRF1, a key transcription factor of proteasome genes, T cells upregulate proteasome genes and increase the activity of the proteasome. The enhancement of the proteasome induces the downregulation of exhaustion markers, as well as a higher cytotoxicity and anti-tumor activity of T cells *in vitro* and *in vivo*. In summary, here we present the proteasome as a potential new therapeutic target to generate resistance to TME stressors to induce superior effector functions.

DYNAMIC MAINTENANCE OF MEMORY T-CELLS IN TISSUE: CHALLENGING THE STATIC PARADIGM

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Memory T-cells play a major pathogenic role in autoimmune diseases, where they can persist in tissue for years. The persistence of these cells is often taken as evidence for longevity, but could equally well be explained by continuous cell division. Understanding how memory T-cells are maintained offers opportunities to interfere with the local persistence of these cells.

To investigate the *in vivo* lifespan of memory T-cells in different tissues, we followed the incorporation of deuterium in the DNA of memory T cells in tissues of mice and humans over time. We obtained blood, bone marrow and skin from otherwise healthy humans undergoing elective surgeries. Complementarily, we used wildling mice, which mirror humans better in the amount of memory T-cells in tissues than specific-pathogen free mice.

In mice, we found that memory T-cells lived considerably shorter in non-lymphoid than in lymphoid tissues. This tissue specialization was equally prominent in humans. Memory T-cells from skin lived *at least* as short as those from peripheral blood. Conserved across species and tissues, we found memory T-cells to be maintained in a dynamic way, with the longest-lived memory T-cells found in the bone marrow. We found no evidence for long-lived memory T-cells in barrier tissues, which challenges the widely-held view that tissue-resident memory T-cells are maintained in a static way.

IMMUNOSUPPRESSIVE HUMAN LOW-DENSITY NEUTROPHILS IN PATIENTS WITH LUNG CANCER DISPLAY MATURE PHENOTYPES

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The presence of neutrophils in the tumor microenvironment is correlated to poor therapeutic outcomes and overall survival. Targeting neutrophils is thus an interesting potential strategy to enhance and support current approaches to cancer immunotherapies. Most studies on the immunosuppressive capacities of human neutrophils focus on low-density neutrophils (LDN), which are elevated in number in the blood of cancer patients. We isolated LDN and normal-density neutrophils from the blood, and tumor-associated neutrophils (TAN) from the tumors of non-small cell lung cancer patients (NSCLC). We compared the cells' phenotypes, suppression of T-cell proliferation, and transcriptomes. In the blood of cancer patients, we observed two types of LDN of different maturity and function. The immature did not suppress T-cell proliferation but expressed the highest levels of the Arginase-1- and NOX2-coding genes. The suppressive neutrophils in the blood of cancer patients displayed a mature morphology and were found in both light and high-density fractions. Normal-density neutrophils from healthy donors did not suppress T-cell proliferation in vitro, despite few transcriptomic differences to immunosuppressive LDN and normal-density neutrophils from NSCLC patients. Thus, immunosuppression by neutrophils in cancer patients does not depend on the cells' density but is associated to their maturity.

HOW LONG DO LONG-LIVED PLASMA CELLS LIVE? INSIGHTS FROM DEUTERIUM LABELING IN MICE AND MEN

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Long-term immunity is crucially dependent upon the long-term production of antibodies. Both in mice and men, the main producers of antibodies are plasma cells. Certain subsets of plasma cells are thought to be long-lived, dependent upon survival niches and the survival cues given therein. Here, we sought to determine how long "long-lived" plasma cells live and how the population of plasma cells is maintained over time.

To study the lifespan of plasma cells, we used *in vivo* labelling with deuterium – a stable isotope that is incorporated into the DNA of dividing cells and can be measured using gas chromatography/mass spectrometry. We FACS-sorted plasma cells from the spleen and bone marrow of wildling mice, i.e. C57Bl/6 mice born to wild mice, and from the bone marrow of humans undergoing an elective hip surgery.

In mice, we found that labeled plasma cells were gradually lost over time, with a half-life of 28 days in the spleen and 35 days in bone marrow. In contrast, we found that nerve cells from the brain had a half-life of 384 days. Although we found a lower uptake of deuterium in "long-lived" CD19- compared to "shorter-lived" CD19+ plasma cells in humans, both subsets still incorporated more deuterium than naïve T cells did—which have an estimated lifespan between 4-9 years.

Together, these results indicate that even "long-lived" plasma cells maintain themselves dynamically.

ENDOSOMAL REMODELLING DURING TOLL-LIKE RECEPTOR CROSS-TOLERANCE

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Toll-like receptors (TLRs) are conserved pattern recognition receptors (PRRs) that play a crucial role in innate defense processes. Upon bacterial or viral infection, TLRs expressed by innate immune cells recognize pathogen-associated molecular patterns (PAMPs) and elicit a pro-inflammatory immune response. Unlike the adaptive immune system, innate cells have generally been considered devoid of any memory function. However, in recent years, many studies have shown that innate cells such as macrophages can acquire a memory-like phenotype after being exposed to different stimuli. We have identified a stimulation condition in which TLR2 tolerization leads to suppression of subsequent endosomal TLR9 activation in response to DNA ligands. In addition, we observed rearrangement of the endosomal network in response to various TLR ligands via super-resolution structured illumination microscopy (SIM). As we are particularly interested in how signals from the cell surface modulate organelle physiology and thereby affect TLR responses, we would like to further investigate endosomal mechanisms that mediate this cross-tolerance between surface and endosomal TLRs.

CHRONIC HELMINTH INFECTIONS MODULATE THE IMMUNE RESPONSE TO AIRWAY ALLERGENS

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The prevalence of chronic inflammatory conditions such as asthma have experienced a notable increase in the last 60 years in high-income countries. This increase has been associated with a reduction in infectious diseases, parasitic infections, and dietary changes. Experimental evidence suggests that helminth infections directly protect the host against allergic airway inflammation, although the mechanism remains poorly understood. Using *Trichuris Muris* (*T. muris*) a natural murine intestinal parasite, we found that chronic helminth infections confer protection against allergen-induced airway inflammation, reducing the number and function of ILC2 and Th2 cells in the airways. As helminth infections can alter host metabolism, we explored metabolic regulation as a potential mechanism of immune suppression. Performing metabolomic analysis, we identified changes in host metabolites but also bacterial-derived metabolites that are upregulated during *T. Muris* infection. Our data show that the administration of metabolites found during helminth infections ameliorates airway inflammation by reducing eosinophilia and lymphocyte infiltration in the context of fungal and protease allergens. Further investigation is required to understand the mechanism of action, but these preliminary findings offer a potential therapeutic strategy in the treatment of allergen-induced airway inflammation.

ROLE OF IL-12 SIGNALLING IN TYPE 1 REGULATORY T CELL SPECIALIZATION

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Regulatory T cells (Tregs) play an important role in dampening excessive immune responses in the periphery. Their activity can be beneficial in the context of autoimmunity, but also compromise pathogen clearance in e.g. strong viral infections. Since Treg discovery 25 years ago, their main characteristics and functions have been described and well-studied. Nevertheless, deeper characterization and mechanisms of action in specific immune contexts remain unclear. We investigate the specialization of Tregs during type 1 immune responses in vivo. We observed that IL12Rβ2 is up-regulated on antigen-specific Tregs upon infection and its expression takes part in the acquisition of a suppressor phenotype (determined by the up-regulation of co-inhibitory receptors) after several viral infections. Moreover, antigen-specific effector cells transferred in IL12Rβ2 conditional KO animals proliferated more upon infection than in WT controls, suggesting a decreased ability of the IL12Rβ2 KO Tregs to dampen effector T cell proliferation.

IN DEPTH B CELL IMMUNE PROFILING AFTER SARS-COV-2 VACCINATION IN PATIENTS TREATED WITH IMMUNOSUPPRESSANTS

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T cell-dependent B cell responses are crucial for formation of long-lasting high affinity antibodies after vaccination. Patients with auto-inflammatory disorders treated with specific immunosuppressants can present a reduced humoral response after SARS-CoV-2 vaccination. Our studies revealed that methotrexate (MTX) and TNFα inhibitors (iTNFα) showed lower induction (MTX) or maintenance (iTNFα) of antibody titres after SARS-CoV-2 vaccination in patients with rheumatoid arthritis or inflammatory bowel disease [1]. To assess how these immunosuppressants affect the dynamics of B cell differentiation after vaccination, we used multiparameter high-dimensional spectral cytometry to elucidate longitudinal antigen-specific B cell responses in a cohort of vaccinated patients treated with different immunosuppressants. Our data showed relatively more cells in the naïve state early after vaccination and a reduction in spike-specific B cells 3-6 months after SARS-CoV-2 second vaccination in patients treated with iTNFα. Currently, analysis of more in depth immune profiling of antigen-specific B cells at different time points after vaccination is ongoing. In conclusion, this study shows how immunosuppressants can affect the SARS-CoV-2 specific B cell response after vaccination and can help us identify cues that may predict duration of humoral immune protection upon use of immunosuppressants.

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ENHANCING T CELLS EFFECTOR FUNCTIONS THROUGH CRISPR/CAS9 GENE KNOCK-OUT

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Chronic antigen exposure occurring in cancer or persistent infections causes T cells to enter into a dysfunctional state called exhaustion. Exhausted T cells progressively lose their effector functions, proliferation potential and upregulate inhibitory receptors on their surface[1]. This condition is not only responsible of defective pathogen clearance, but can also reduce the efficacy of cancer immunotherapy treatments based on redirecting T cells to kill tumour cells. Therefore, there is much interest in understanding the key molecular mediators driving T cell exhaustion. Recently, CRISPR based screening approaches revealed several genes involved in the control of T cell dysfunctional state, among which RAS GTPase-activating protein (RASA2)[2]. Our aim is to study the role of candidate proteins involved in exhaustion, including RASA2, by applying CRISPR/Cas9 mediated gene knock-out in the respective coding genes. In particular, we started targeting human CD8 and CD4 T cell clones with known specificity for viral pathogens, and also *ex vivo* isolated naïve T cells. The phenotype of edited T cells will be characterized in terms of their specific functions including proliferation, killing activity, and cytokine production. The results of these studies may open the possibility to establish gene editing strategies to boost the efficacy of pathogen or tumor-specific T cell therapies.

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EPCAM EXPRESSION ON ALVEOLAR MACROPHAGES IS CRUCIAL FOR HOST DEFENSE AGAINST BACTERIAL INFECTIONS

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Alveolar macrophages (AMs) are tissue-resident innate immune cells that play a critical role in maintaining lung homeostasis. Residing in the terminal air sacs of the lungs, they are the initial responders to environmental insults and respiratory pathogens, including viral pathogens such as influenza virus or coronavirus and bacterial infections, e.g. *L. pneumophilia*. EpCAM (epithelial cell adhesion molecule, CD326), initially describes as a tumor antigen, is a cell surface glycoprotein that is highly expressed in epithelial cancers, epithelial cells, and dendritic cells. Recently, it became evident that EpCAM is a signature gene of fetal-derived AMs with yet unknown function.

Using EpCAM-floxed; CD11c-Cre mice, we show that EpCAM expression does not affect AM numbers and function in steady-state conditions and inflammation. We demonstrate that during infection with influenza A virus, EpCAM is downregulated on AMs; however, presence of EpCAM on AMs does not influence survival or cell numbers during infection. In contrast, we show that mice lacking EpCAM on AMs are more susceptible to *L. pneumophilia* infection and have impaired bacterial clearance. We obtained similar results from mice lacking EpCAM on the epithelial compartment in the lungs, suggesting that homophilic EpCAM interactions between AMs and alveolar epithelial cells are crucial for antibacterial responses.

INVESTIGATING THE RELATIONSHIP BETWEEN THE GUT MICROBIOME, UNCONVENTIONAL T CELLS, AND RESPONSES TO CHADOX1-VECTORED VACCINATION

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Emerging evidence has shown that the gut microbiome influences vaccine responses, but the exact mechanism by which this is achieved is unknown. Unconventional T cells such as mucosal-associated invariant T (MAIT) cells offer a potential explanation due to their ability to both augment vaccine responses and respond to microbiome-derived metabolites. The abundance and phenotype of circulating MAIT cells varies widely between individuals, but it is not known whether microbiome differences are responsible for this variation, and whether this impacts on vaccine responses. To address this, we are conducting shotgun metagenomic sequencing analysis on stool samples and spectral flow cytometry on blood samples from ChAdOx1-vectored vaccine trials in the UK and sub-Saharan Africa. In parallel, we are utilising gnotobiotic mouse models to explore the relationship between the microbiota present from birth, the unconventional T cell compartment and vaccine responses, and understand whether MAIT cells require stimulation from the microbiome to carry out their signal amplification role in vaccination. These experiments may ultimately shed light on the reasons behind the great disparity in vaccine responses across individuals and populations.

THE IMPACT OF SARS-COV-2 INFECTION DURING PREGNANCY

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Physiologically, the immune system of pregnant women undergoes several alterations, which can be disadvantageous upon infection [1,2]. The COVID-19 pandemic posed a new challenge for pregnant women, who are considered at higher risk of developing severe COVID-19 and pregnancy complications [3]. The precise differences in the immune response to SARS-CoV-2 infection during pregnancy, as compared to non-pregnant females, is not well understood, as are potential impacts on the offspring.

Here, using an established model of murine COVID-19 (maVie16) [4] we infected timed-pregnant dams and observed a dampened inflammatory response and a delayed viral clearance, without detrimental effects on disease severity or outcome. The infection remained maternally restricted, and offspring did not show overt signs of disease. However, upon a subsequent challenge with *Streptococcus pneumoniae*, offspring of infected dams (at a young adult age) showed a faster upregulation of pro-inflammatory genes and more potent cytokine production, which resulted in a faster neutrophil recruitment to the lung.

Our data suggest that pregnancy modulates the immune response to SARS-CoV-2 and that a maternal viral infection has an imprinting effect on the immune system of the offspring.

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EMPLOYING SCRNASEQ AND MULTIPLEXED IMAGING TO DISSECT MECHANISMS OF RESISTANCE TO CANCER IMMUNOTHERAPY

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Cancer immunotherapy has revolutionized cancer therapy. In particular, immune checkpoint inhibitors (ICIs) demonstrated durable anti-tumour efficacy. Still, the majority of patients are resistant to ICIs and develop disease progression. Therefore, it is of the utmost importance to understand the mechanisms underlying therapy resistance.

To understand mechanisms of resistance to anti-PD-1/CTLA-4 combination therapy, we developed a bilateral syngeneic, orthotopic breast cancer model that results in a fraction of animals who initially respond to the therapy, others who do not respond, and those that relapse after initially responding. The introduction of a second contra-lateral tumour allows us to study the tumour microenvironment (TME) at an early timepoint of response or resistance and follow the fate of the second tumour in the opposite flank of the mice. By doing this, we will obtain a longitudinal study of the tumour response after checkpoint blockade, which allows us to address mechanisms behind ICI resistance.

To obtain a comprehensive view of the immune infiltrate inside the TME in responding and non-responding/relapsing mice we will perform single-cell RNA sequencing and TCR sequencing on intratumoral CD45+ cells, combined with multiplexed imaging. Ongoing analysis will be presented during the meeting.

FOXP3, CD83, CD138, AND IL-15: VALUABLE PROGNOSTIC IMMUNE MARKERS IN RESIDUAL TUMORS OF TRIPLE-NEGATIVE BREAST CANCER PATIENTS WITH INCOMPLETE RESPONSE TO NEOADJUVANT CHEMOTHERAPY

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Between 60 and 70% of triple-negative breast cancer (TNBC) patients fail to achieve a pathological complete response (pCR) to neoadjuvant chemotherapy (NAC), resulting in shortened overall survival (OS) and relapse-free survival (RFS) within five years after surgery [1]. Prior studies suggest that patient outcome and response effectiveness to neoadjuvant chemotherapy (NAC) are strongly influenced by changes in the elements infiltrating the tumor microenvironment (TME) [2]. Hence, this study aims to identify the immune constituents of the residual TME of patients unable to achieve a pCR and assess their impact on the patient prognosis depending on the type of response. For this purpose, the cellular and molecular content in the residual TME of post-NAC biopsies from 96 partial vs. non-responders TNBC patients was determined using immunohistochemical staining and chromogenic in situ hybridization, respectively. Compared to non-responders, Kaplan-Meier analysis revealed that partial responders exhibiting higher levels of FOXP3+ Treg cells, CD83+ dendritic cells and IL-15 expression but lower levels of CD138+ plasma cells in the residual TME presented an improved OS and RFS. Overall, this study highlights the relevance of identifying immune targets within the residual TME to progress in the search for immunotherapies for TNBC patients with the most unfavorable outcomes.

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ROLE OF PLASMA CELLS IN MCMV INFECTION

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Murine cytomegalovirus (MCMV) is a well-known pathogen which establishes persistent infection in salivary glands to facilitate horizontal transmission. Early experiments described that although B cells do not seem to be crucial for the control of viral titers during primary infection, antibodies are critical in confining viral reactivation and preventing inter-organ spread. Research has so far primarily focussed on neutralizing serum IgG, where titers have been demonstrated to inflate during the latent phase of infection. However, the antibody response in mucosal tissues is skewed toward IgA production, where little is known about the role of MCMV-specific IgA in mucosal tissues. In preliminary experiments, we identified B cell accumulations in the persistently infected salivary gland as well as IgA-producing plasma cells. To assess inter-organ differences in B cell accumulations and the viral antibody repertoire, we are using a combination of single-cell RNA sequencing analysis, spectral flow cytometry and novel multispectral imaging techniques. Furthermore, to elucidate the role of IgA-producing plasma cells in protection of mucosal tissues, we will apply these techniques to assess their priming sites and effector niches and we will establish specific perturbations to modify the development and maintenance of mucosal B cell niches and their impact on local MCMV control.

DIFFERENTIATING HEMATOPOIETIC PROGENITOR STEM CELLS INTO NEUTROPHILS TO UNDERSTAND NUCLEAR LOBULATION

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Neutrophils are the most abundant immune cells in circulation and the first line of defense against infections. Their nuclear morphology – consisting in three to four lobes – distinguishes them from any other cell, but its function remains unknown. Obtaining experimental proofs remains challenging: Neutrophils are terminally differentiated cells which live only up to 6-8 hours outside the body; this prohibits gene editing. Other models (cell lines or mice) fail to imitate lobulation patterns and functions such as phagocytosis or chromatin release. We work on a model to overcome these difficulties: Hematopoietic Progenitor Stem Cells (HPSC). HPSC can be expanded, which opens a window to deliver CRISPR/Cas machinery, and we can differentiate them into neutrophil-like cells with similar morphology and physiology. Our model offers a promising alternative to the field and may shed light on the underlying mechanisms of their nuclear morphology.

ANTIBIOTIC TREATMENT INCREASES DISSEMINATION OF A MDR-KLEBSIELLA PNEUMONIAE STRAIN FROM THE GUT IN A GNOTOBIOTIC MURINE MODEL

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Klebsiella pneumoniae (*Kp*) is the third leading cause of deaths directly attributed to or associated with antimicrobial resistance (AMR). *Kp* strains exhibit increasing AMR, including to carbapenems, a last line therapeutic. *Kp* colonises mucosal surfaces such as the gut, with high hospital acquired colonisation rates. Enrichment of enteric *Kp* is associated with multiple inflammatory diseases, like inflammatory bowel diseases (IBDs[1]) and can regulate immune profiles in secondary sites such as the lung[2]. We aim to study the role of *Kp* gut colonisation on gut microbiome changes and host immune responses using a gnotobiotic mouse model with a known microbiome (Oligo-MM12).

A single dose of an AMR *Kp* strain (KP35) was gavaged into MM12 mice and colonisation was monitored based on faeces colony forming units (CFUs). We found that KP35 can colonise the gut for over two months without disruption to the abundance of MM12 microbiome members. We then investigated the effect of antibiotics on KP35 colonisation and expansion. Meropenem was added to the mouse drinking water for 1 week. Significantly increased KP35 CFUs were observed at secondary sites (e.g. kidney, lung and liver) compared to controls. Further work is underway to characterise how KP35 colonisation and antibiotic treatment influences gut microbiome and intestinal immunity, like barrier integrity and inflammatory profiles.

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THE IMPACT OF *HELICOBACTER HEPATICUS* INFECTION ON HOST INNATE IMMUNITY

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Infection of mice with the intestinal bacterium *Helicobacter hepaticus* (*Hh*) has been used to model human inflammatory bowel disease (IBD) and provided insights into pathogenetic mechanisms. However, mice with intact immune regulatory pathways do not develop intestinal pathology following *Hh* colonization, as the bacteria induce dominant tolerance that allows persistent colonization without pathology. We hypothesise that chronic *Hh* infection may confer host benefits by modulating host immune functions. Using *in vivo* infection models, we found that *Hh* colonization was protective against chemically induced colitis, including reducing disease severity and decreasing myeloid infiltration to the colon. We next isolated colonic macrophages from *Hh*-infected mice and functionally compared them to those from uninfected donors. Macrophages from infected mice showed reduced phagocytosis and suppressed cytokine responses when stimulated *ex vivo*. Finally, we are using an intestinal epithelial organoid system to study the effects of *Hh* on epithelial cells. We are investigating its effect on proliferation and differentiation, and testing whether organoids from *Hh*-infected mice show altered responses when stimulated *ex vivo*. Together this work aims to contribute to our better understanding of the mechanisms through which the host is influenced by its resident immunomodulatory microbiota.

MAINTENANCE OF TISSUE-RESIDENT MEMORY T LYMPHOCYTES BY STROMAL CELL-INDUCED PI3K SIGNALING

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We have identified previously a prominent bone marrow-resident memory T (Tm) lymphocyte population, that is maintained individually as resting cells in contact to bone marrow stromal cells. These cells provide local and systemic immunity to systemic challenges. Their localization resembles the organization of memory B cells and memory plasma cells in the bone marrow. For the latter we had shown before that they are maintained by integrin-induced PI3K signaling, making them resilient to apoptosis. Here we demonstrate that the PI3K inhibitor Wortmannin selectively ablates $CD69^+$ $CD44^{high}$ Tm from the bone marrow of mice, too. $CD69^-$ $CD44^{high}$ Tm of the bone marrow, about 30 to 60% of the $CD44^{high}$ Tm in the bone marrow, are not ablated, suggesting that they may persist by alternative mechanisms. This is the first demonstration that $CD69^+$ tissue-resident and $CD69^-$ Tm of the bone marrow use different molecular mechanisms to persist. It adds to our previous observation, that maintenance of tissue-resident Tm of the bone marrow is not dependent on homeostatic proliferation. Their dependency on integrin-mediated contact to stromal cells is corroborated by our finding that blocking antibodies against VLA-4 (CD49d) & LFA-1 (CD11a) on Tm, the ligands of VCAM and ICAM of the stromal cells, ablates both $CD4^+CD44^{high}$ and $CD8^+CD44^{high}$ from the bone marrow, interestingly both $CD69^+$ and $CD69^-$ Tm.

CHARACTERIZATION OF A PANEL OF NOVEL HUMAN FCGRI-SPECIFIC ANTIBODIES, WITH FOCUS ON TWO BLOCKING ANTIBODIES

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Fc gamma receptor I (FcγRI) binds IgG antibodies with high affinity. FcγRI overactivation via immune complexes (IC) and auto-antibodies has been linked to several autoimmune disorders. Antibodies that specifically inhibit IC binding could prove useful in these diseases. Commercial antibodies block FcγRI via their unspecific Fc tail. Hence, we developed Fab-mediated FcγRI blocking antibodies.

Mice were immunized, and a single-chain variable fragment (scFv)-expressing-phage display library was used to ensure Fab-mediated selection of anti-FcγRI binders. Seven FcγRI-specific antibodies were generated and cloned into Fc-silent IgG1 backbone. Functional characterization revealed two distinct antibodies that bind to FcγRI in the same region as IgG (C01 and C04) and could prevent 90% of human IgG and IC binding. Overnight incubation with C01 and C04 led to 60% displacement of prebound IC. Commercial antibody 10.1 was outperformed by C01 and C04 in both blocking and displacing hIgG and IC. Furthermore, no receptor activation occurs upon binding and crosslinking of these two antibodies.

In conclusion, C01 and C04 can inhibit IC binding and displace bound IC without activating the receptor. They have the potential to replace clone 10.1 as the most effective FcγRI blocking antibody in further research. Future research will focus on their applicability in several autoimmune disorders.

THE INTERACTION BETWEEN *K. PNEUMONIAE* AND THE INNATE IMMUNE SYSTEM

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Klebsiella pneumoniae is gram-negative bacterium that can cause opportunistic infections e.g., pneumonia, urinary tract infection and bacteremia. In Denmark the number of invasive cases of K. pneumoniae has been increasing over the last decade. Furthermore, a rise in isolates positive for Extended Spectrum β-lactamase is observed. Therefore, the aim of this study was to elucidate the interaction between *K. pneumoniae* and the innate immune system including to which extent the complement system affects the interaction.

Two serum resistant *K. pneumoniae* isolates were analyzed by flow cytometry and by immunofluorescence microscopy. The flow cytometry was used to evaluate the effect of complement deposition in relation to phagocytoses and immunofluorescence microscopy was preformed to visualize the interaction between PMNs and *K. pneumoniae*.

Both assays show distinct difference in the interaction when the complement system was removed by heat inactivation of serum. The flow cytometry assay showed that the killing of *K. pneumoniae* happens through opsonophagocytosis, with a phagocytose percent at 50% higher when complement was present. When visualizing the PMNs by immunofluorescence microscopy, PMNs incubated in normal human serum had intact nuclei and close interaction with the bacteria whereas the PMNs incubated in heat inactivated serum were undergoing NETosis. The results show the importance of complement for uptake of gram-negative bacteria in PMNs.

IMMUNE-GUT INTERACTIONS IN ORAL TOLERANCE AND FOOD ALLERGY

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Early oral introduction of peanut can prevent the development of peanut allergy yet the mechanisms driving an allergen-specific induction of oral tolerance in the gut are largely unknown. Innate lymphoid cells (ILC) are amongst the first responders to food allergens and were recently shown to mediate T cell responses in an antigen-specific manner via the major histocompatibility complex II (MHCII). We assessed phenotype and frequencies of ILC subsets in the peripheral blood of peanut allergic, non-atopic children and those who resolved peanut allergy. An *in vitro* co-culture system of blood human ILC precursors (ILCPs) alongside human intestinal organoids has been used to generate mature ILCs and investigate their role in response to food allergens in the gut. Preliminary data show that there were no significant differences in ILC distributions between patient groups. However, expression of MHCII on ILC differed between the various patient groups. Expanded ILCs were able to take up and degrade Dye Quenched-Bovine Serum Albumin. Allergen presentation is a potential mechanism for an allergen specific ILC-mediated pathway. Understanding the role of ILC subsets may lead to the identification of novel therapeutic targets for food allergy that promote protective ILC populations.

IDENTIFICATION OF GENES REQUIRED FOR ALVEOLAR MACROPHAGE DEVELOPMENT USING CRISPR/CAS9

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Alveolar macrophages (AMs) are innate immune cells residing in the terminal air sacs of the respiratory tract, where they remove inhaled particles, clear airborne pathogens, and metabolize pulmonary surfactant to ensure functional gas exchange. Perinatal AM development depends on the induction of the transcription factor PPARg by the lung epithelial-derived cytokine GM-CSF. To better understand the transcriptional programs instructed by the GM-CSFR/PPARg-axis, we performed RNA-sequencing of fetal lung AM precursors and identified several candidate genes that are deregulated in the absence of the GM-CSF receptor a chain and PPARg. Missing niche signals and consequently altered transcriptomes and epigenomes pose obstacles for studying the role of these candidate genes *in vitro*. Here, we present a novel method that allows to test for their requirement *in vivo* by CRISPR/Cas9-mediated engineering of cultured AM precursors. Following genome editing, cultured fetal liver monocytes (cFLiMos) retain the capacity to differentiate into *bona fide* AMs upon intranasal transfer into neonates lacking endogenous AMs. Transplanted cFLiMo-derived AMs are fully functional indicated by efficient clearance of pulmonary surfactant. Harnessing this approach, we currently target several of the identified genes to unveil the genetic signature governing the development of functional AMs.

DYNAMIC ADOPTION OF ANERGY BY ANTIGEN-EXHAUSTED CD4+ T CELLS

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Exhausted immune responses to chronic diseases represent a major challenge to global health. We study $CD4^+$ T cells in a mouse model with regulatable antigen presentation. When the cells are driven through the effector phase and are then exposed to different levels of persistent antigen, they lose their T helper 1 (Th1) functions, upregulate exhaustion markers, resemble naturally anergic cells, and modulate their MAPK, mTORC1, and Ca^{2+} /calcineurin signaling pathways with increasing dose and time. They also become unable to help B cells and, at the highest dose, undergo apoptosis. Transcriptomic analyses show the dy-namic adjustment of gene expression and the accumulation of T cell receptor (TCR) signals over a period of weeks. Upon antigen removal, the cells recover their functionality while losing exhaustion and anergy markers. Our data suggest an adjustable response of $CD4^+$ T cells to different levels of persisting antigen and contribute to a better understanding of chronic disease.

MODELLING INFLAMMATION-DRIVEN FIBROSIS USING HUMAN LUNG ORGANOID MODEL

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The formation of fibrotic tissue is a key mechanism of tissue repair, but when dysregulated, it can result in progressive accumulation of extracellular matrix components leading eventually to tissue disruption, loss of function and even organ failure. Pathologic fibrosis manifests as a deregulated proliferation and activation of fibroblasts, upregulated production of extracellular matrix, which outpaces its degradation, and stiffening of surrounding tissue. Fibrosis is also linked to inflammation and immune response, with several cytokines such as TGF-β, TNFα, IL-6, and IL-1β being implicated in fibrosis development. 3D human lung organoid models provide a tool to study the induction and progression of fibrosis in an environment akin to real human tissue, including natural cell organisation and function. Our laboratory has shown that these organoids are able to form an immunocompetent environment in response to pathogen stimulation, as seen by the upregulation of inflammatory markers[1]. They contain epithelial as well as mesenchymal cells which makes them a great model for studying the initiation and progression of fibrotic changes in tissue in response to exogenous stimulation. Here we aim to develop and characterize human lung organoids as a model for studying inflammation-driven fibrosis and to use that model to study fibrosis development and progression.

[1] Jose S.S, *et. al*., Comparison of two human organoid models of lung and intestinal inflammation reveals Toll-like receptor signalling activation and monocyte recruitment, 2020

MURINE GBP7 AND ITS INTERACTION WITH TOM1 AND *T. GONDII* SURFACE PROTEIN SRS29C IN PATHOGEN DEFENCE

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Toxoplasma gondii is an obligate intracellular parasite infecting about a third of the world population with high clinical relevance, especially for immunocompromised patients. Also, acute toxoplasmosis during pregnancy is a considerable health risk for the unborn. Murine GBPs (mGBPs) have been shown to play an important role in the immune defence against *T. gondii* as shown in infected knock-out mice, with mGBP7 knock-out mice succumbing as fast to the infection as IFN-γ receptor deficient mice. Previously, using mouse embryonic fibroblasts (MEFs), several proteins have been identified, that interact with mGBP7 after *T. gondii* infection. Among these proteins, the endosomal trafficking adaptor protein TOM1is of special interest. TOM1 and mGBP7 have been shown to colocalize at the *T. gondii* parasitophorous vacuole (PV), the intracellular replicative niche of the parasite. Investigating the recruitment hierarchy using confocal microscopy, we could determine that mGBP7 is not essential for the recruitment of TOM1 to the PV. Since TOM1 is known to interact with various proteins we are using co-immunoprecipitation to investigate potential interaction partners after a *T. gondii* infection. Furthermore, we are in the process of establishing a TOM1 knock-out cell line to investigate the effect of TOM1 deletion in the defence against *T. gondii*.

TGF-B1, TGF-B2 AND TGF-B3 IN SYSTEMIC SCLEROSIS (AND OTHER FIBROTIC/AUTOIMMUNE DISEASES)

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Systemic sclerosis (SSc) is a rare fibrotic autoimmune disease with heterogenous clinical manifestations and limited treatment options. TGF-β1, β2 and β3 are pro-fibrotic and immunosuppressive cytokines thought to be involved in SSc pathogenesis. Unpublished genetic analysis revealed the presence of a rare *TGFB2* variant in both affected members of a family suffering from SSc. The variant encodes a Val to Met (VnM) mutation at a position conserved in the 3 TGF-β isoforms. We hypothesized that the VnM variant increases TGF-β activity and contributes to SSc pathogenesis. Our first in vitro results across multiple cell lines confirm that human and murine TGF-β1, β2 and β3 VnM mutants are more activated than their WT counterparts. We thus sought to exploit these VnM mutant strains as tools in vivo, to study the pathological consequences of excessive TGF-β activation. We have generated 3 knock-in mice strains by introducing the VnM mutation in the *Tgfb1*, *b2* or *b3* genes. We are currently comparing the phenotypes of the mutant mice to their WT controls to determine if an excessive activation of TGF-β could lead to the spontaneous development of an SSc like disease or could increase susceptibility to experimentally induced fibrotic diseases. We hope to decipher the role of the TGF-β isoforms in the pathogenesis of these diseases that could aid in developing novel therapeutic strategies.

BROADENING OF SARS-COV-2 ANTIBODY REPERTOIRE AFTER SUCCESSIVE VACCINATIONS IN PATIENTS TREATED WITH IMMUNOSUPPRESSIVE MEDICATION

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The mechanisms of antibody repertoire evolution upon repeated SARS-CoV-2 mRNA vaccination remain incompletely understood. We previously established a nation-wide prospective cohort study using at-home sampling from patients with various immune-mediated inflammatory diseases (IMID) on various immunosuppressants (ISP) to identify ISP that might seriously impair vaccine-induced humoral immunity. We found that with the exception of several "poor responder" ISP, most patients mounted protective anti-spike receptor-binding domain (RBD) IgG responses largely irrespective of particular IMIDs. Here we present ongoing work aimed at elucidating antibody repertoire evolution by comparing serum IgG reactivity against different domains of the original Wuhan-Hu-1 spike protein as well as that of the Omicron (BA.1) variant. We find that reactivity against non-RBD portions of the spike protein proportionally increases over time after 2nd vaccination and rapidly after 3rd vaccination, and that these trends align with an emerging anti-Omicron RBD reactivity. Furthermore, we show that affinity against both spike variants increases concurrently, and these changes occur similarly in patients on moderate ISP (methotrexate) but not on poor-responder ISP (anti-CD20). Our data supports the growing body of evidence that successive mRNA vaccination drives a shift of the anti-spike repertoire.

PHENOTYPIC AND FUNCTIONAL CHARACTERISATION OF CONVENTIONAL DENDRITIC CELLS IN CANCER

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Conventional dendritic cells (cDCs) constitute key orchestrators of T cell-mediated tumour control. The general intratumoural paucity of cDCs has limited our ability to explore their biology. Recent transcriptomic analyses of tumour-infiltrating immune cells have uncovered a cDC population that clusters independently of the typical cDC1 and cDC2 subsets. This cluster is characterised by selective expression of T cell-instructive cytokines, co-stimulatory molecules, and chemokine receptors essential for cDC migration. Concomitantly, they show high PD-L1 expression, among other inhibitory ligands. Based on these features, this population has been named mDCs (mature or migratory DCs, a.k.a. mregDCs or DC3) and is believed to represent a convergent activation state of cDC1 or cDC2. Due to a lack of research tools, the contribution of mDCs to cancer immunity remains largely speculative. Here, we sought to characterise mDC function through deep immune profiling of mouse tumours. We generated a new genetically-engineered mouse strain that allows mDC-specific tracking and isolation. We also optimised primary cell cultures to generate mDCs *in vitro* that are reminiscent of their tumour-infiltrating *in vivo* counterpart. Using these new experimental systems, we are currently performing an in-depth investigation of the immunobiology of this enigmatic intratumoural cDC population.

ROLE OF INTRACELLULAR NA^+ ACCUMULATION ON MACROPHAGE ACTIVATION AND FUNCTION

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Inflammation and infection can increase local sodium levels. Macrophages play an important role in tissue immunity. In macrophages, high salt environments (HS) induce osmoprotective signaling via p38/MAP kinase, NFAT5, and Nos2 expression, leading to a boosted proinflammatory and antibacterial activity.

We investigated if we could mimic HS conditions by increasing intracellular Na^+ levels pharmacologically. The Na^+/K^+-ATPase maintains macrophages' sodium balance. We applied cardiac glycosides to inhibit NKA activity. Similar to HS, cardiac glycosides induce intracellular sodium accumulation and p38/MAP kinase phosphorylation. Contrary to HS, NKA inhibition did not induce NFAT5 or nitric oxide production and showed heterogeneous effects on potassium and calcium levels, as well as antibacterial activity.

While HS environments cause osmotic stress and ionic perturbations, cardiac glycosides only induce the latter. Thus, we applied a combination treatment of mannitol, a non-ionic osmolyte, and ouabain, to mimic HS conditions better. Mannitol induces osmotic stress, while ouabain causes intracellular sodium accumulation in macrophages. However, we could only partially imitate HS conditions. We concluded that intracellular sodium accumulation and osmotic stress alone are required, but not sufficient to mimic the previously described sodium-boosted macrophage activation and function.

IMMUNE-COMPETENT HUMAN (MULTI)ORGAN-ON-A-CHIP MODELS

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Due to technological breakthroughs and impressive initial clinical results, innovative immunotherapies represent a focus of current drug developments. Mouse models and 2D immune cell cultures are poorly suited for testing innovative T cell-based immunotherapies since they do not adequately represent the important human tissue immunity component. Thus, there is a huge need to build a human dynamic test platform for qualifying innovative T cell-based immunotherapies. Our research addresses current scientific challenges such as T cell migration in organoids. For this, we work on vascularization models, test different biomaterials or scaffolds and work on new-designed organ-on-chips. Our group is part of different cooperation projects as the PACE study (vascularization), BCRT NewCrossfields (vascularization), TReAT (new testing model system for ATMPS) and geneTIGA (multi-organ testing model system for ATMPs) to reach the same aim of T cell migration into Organoids.

DEEP-PHENOTYPING OF ANTIGEN-SPECIFIC CD4+ T CELLS USING A NOVEL MHC CLASS II MULTIMER PLATFORM

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Monitoring of antigen (Ag)-specific T cells is important to gain insight in responses to vaccination or immunomodulatory therapies. MHC class II tetramers might be used to measure Ag-specific CD4 T cell responses directly *ex vivo*. However, current reagents are often limited in signal intensity and binding affinity. We developed novel peptide-specific MHC class II multimers based on an Ig Fc-fusion protein framework to optimize recognition of Ag-specific CD4 T cells. Multimers recognizing hemagglutinin-specific CD4 T cells stained the Ag-specific cells with much higher signal intensity and specificity than commercial reagents. We have shown that multiple sclerosis (MS) patients treated with anti-CD20 therapies had lower seroconversion rates after COVID-19 vaccination than healthy controls [1]. CD4 Tfh–B-cell interaction is necessary to induce affinity-matured B-cell responses. Besides, B cells might also affect the phenotypic differentiation of CD4 T cells. In this study, we aim to investigate the effect of the depletion of B cells by anti-CD20 therapy on the spike-specific CD4 T cell response. Using spike-peptide loaded MHC class II multimers, spike-specific CD4 T cells will be sorted from MS patients, either untreated or on anti-CD20 therapy. By performing scRNA-sequencing, we aim to obtain insight in the effect of lack of B cell interaction on the CD4 T cell phenotype.

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INVESTIGATING THE ROLE OF INNATE LYMPHOID CELLS IN BENEFICIAL AND PATHOLOGICAL INTESTINAL MATRIX REMODELLING

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Intestinal homeostasis is governed by bidirectional communication between the epithelium, underlying fibroblasts and the immune compartment. In chronic inflammation, a defining feature of inflammatory bowel disease (IBD), aberrant interactions between these populations underlie matrix-remodelling which contributes to IBD pathogenesis. We seek to address what role IBD-associated innate lymphoid cell (ILC) subsets play in intestinal matrix remodelling and how the extracellular environment conversely impacts ILC function. This will be investigated using a fully human *in vitro* system co-culturing intestinal ILC with pluripotent stem cell derived intestinal organoids embedded in modifiable gel matrices to mimic the extracellular stroma. Following co-culture, the phenotype of epithelial and stromal cells will be determined using flow cytometry, immunofluorescence microscopy and RT-qPCR and matrix changes will be quantified using atomic force microscopy and microrheology. The impact of pathologic matrix remodelling on ILCs will be assessed by growing ILC on matrix components or in gels of varying stiffness to mimic areas of matrix deposition and degradation. Tissue remodelling is a main source of complications in IBD patients and better understanding of the interplay between ILC, stromal cells and matrix cues can direct future therapies.

DEEP IMMUNE PROFILING AFTER PRIMARY SARS-COV-2 INFECTION IDENTIFIES NOVEL ANTIGEN-SPECIFIC IGG+ ACTIVATED AND RESTING MEMORY B CELL POPULATIONS

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T cell-driven B cell responses in the Germinal Center (GC) are needed to induce plasma cells (PCs) that secrete protective antibodies, but how long-lived PC formation is regulated is unknown. It remains to be elucidated how B cell differentiation pathways and dynamics relate to formation of antibody responses against SARS-CoV-2. We characterized the SARS-CoV-2-specific B cell responses in patients with different COVID-19 disease severity, early (45 days PSO) and later (100 days PSO) after recovery using spectral flow cytometry. We identified SARS-CoV-2-specific IgG+ activated B cells, described as GC derived and PC precursors, indicating an ongoing GC response at both timepoints post infection. Interestingly, the antigen-specific B cells targeting a recently encountered SARS-CoV-2 antigen were clustered differently to resting memory B cell populations, specific for previously cleared infections or vaccination. Deep profiling of these cells resulted in a combination of novel markers to discriminate differentiation states in IgG+ activated B cell and IgG+ memory B cell populations. Further cell culture of sorted subpopulations will provide more information into their differentiation potentials towards memory or plasmablasts to delineate when this decision point occurs. Our results will help identifying and targeting specific B cell subsets for future therapies and vaccinations.

METHOTREXATE IMPAIRS INDUCTION OF SARS-COV-2 VACCINATION-SPECIFIC CD4 T CELLS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Patients with auto-inflammatory diseases often use immunomodulating drugs. Because of the immunosuppressive nature of these drugs, these patients may mount insufficient immune responses after vaccination[1,2]. Methotrexate (MTX) and TNFα inhibitors (iTNFα) indeed resulted in attenuated antibody titers after SARS-CoV-2 mRNA vaccination in patients with rheumatoid arthritis and inflammatory bowel disease[3]. Besides antibody formation, cellular immune responses are important for future protection; CD4 T cells are a key player in the formation of high affinity antibodies. Therefore, we aimed to investigate the effect of MTX and iTNFα on vaccine-induced SARS-CoV-2 CD4 T cell responses. Use of an activation-induced marker assay revealed that MTX suppressed Spike-specific CD4+ T cell responses, most notably the T(f)helper 1 subset. This decrease was positively correlated to IgG antibody titers. Interestingly, a third vaccination increased the number of Spike-specific CD4 T cells as compared to after two vaccinations. Further deep phenotyping of these Spike-specific CD4 T cells might give us insight into how MTX affects the CD4 T cell compartment after antigenic stimulation. These results may not only have implications on future vaccination strategies but also improve our knowledge on how these immunosuppressive drugs modulate our immune system.

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T CELL-MEDIATED MODULATION OF MONOCYTE FUNCTION IN ATHEROSCLEROSIS

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Atherosclerosis is defined by the accumulation of plaques in the arterial wall. A driver of atherosclerosis is chronic inflammation, with monocytes being a key inflammatory cell type found in the plaque. Studies have shown that treatment with regulatory T (Treg) cells can reduce the atherosclerotic plaque whereas effector T (Teff) cells are both pro- and anti-atherogenic. The aim of this project is to determine how Treg and Teff cells modulate monocytes in atherosclerosis.

Previous work showed that CD4+CD25+ Treg cells steer monocytes towards an anti-inflammatory phenotype and function whereas CD4+CD25- Teff cells induce a pro-inflammatory monocyte profile. To understand the molecular mechanisms behind this T cell-facilitated modulation of monocytes, we performed bulk RNAseq of monocytes that were modulated by Teff vs Treg cells which revealed 2,587 differentially expressed genes with key pathways and genes involved in phagocytosis, efferocytosis and ox-LDL uptake. Current work is aimed at mining this RNAseq dataset to identify specific genes in T cell-modulated monocytes that are relevant to atherosclerosis. We will validate these genes at the protein level and assess the functional relevance via several atherosclerosis-specific functional assays. We will also investigate the ability of Treg vs. Teff cells to modulate monocyte function in patients with early atherosclerosis.

SINGLE-CELL ANALYSIS OF THE HUMAN SPLEEN ENDOTHELIAL CELLS IDENTIFIES LYMPATIC VESSELS IN THE RED PULP

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Spleen is the largest secondary lymphoid organ, which is important for immune response and for clearing antigens from blood circulation. Although recent single-cell technologies identified heterogeneous blood and lymphatic endothelial cell subsets and revealed their roles in murine and human lymph nodes, their heterogeneity in the human spleen is largely unknown. Here we used single-cell RNA sequencing to characterize non-hematopoietic cell heterogeneity in the human spleen. 17628 cells from 6 human spleens were sequenced. Unbiased clustering revealed 10 non-endothelial stromal and 12 endothelial cell clusters. Interestingly, we found a rare lymphatic endothelial cells-like subset that was expressing same markers that were previously shown to be expressed by a lymphatic subset in human lymph nodes. Immunohistochemical and three-dimensional immunofluorescence imaging of this subset indicates morphological similarity with typical lymphatic vessels and reveals their location within the red pulp in the vicinity of the T cell zone. The gene expression profile of this subset suggests its crucial role in regulating immune cell migration. Our study provides a single-cell transcriptomic atlas for comprehensive characterization of non-hematopoietic cell subsets and identifies new-type of lymphatic vessels in the human spleen.

CD4$^+$ HELPER T CELLS ENDOW CDC1 WITH CANCER-IMPEDING FUNCTIONS IN THE HUMAN TUMOR MICRO-ENVIRONMENT

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Despite their low abundance in the tumor microenvironment (TME), classical type 1 dendritic cells (cDC1) play a pivotal role in anti-cancer immunity, and their abundance positively correlates with patient survival. However, their interaction with CD4$^+$ T cells to potentially enable the cytotoxic T lymphocyte (CTL) response has not been elucidated. Here we show that contact with activated CD4$^+$ T-cells enables human ex vivo cDC1, but no other DC types, to induce a CTL response to cell-associated tumor antigens. Single cell transcriptomics reveals that CD4$^+$ T-cell help uniquely optimizes cDC1 in many functions that support antigen cross-presentation and T-cell priming, while these changes don't apply to other DC types. We robustly identify "helped" cDC1 in the TME of a multitude of human cancer types by the overlap in their transcriptomic signature with that of recently defined, tumor-infiltrating DC states that prove to be positively prognostic. As predicted from the functional effects of CD4$^+$ T-cell help, the transcriptomic signature of "helped" cDC1s correlates with tumor infiltration by CTLs and Thelper(h)-1 cells, overall survival and response to PD-1-targeting immunotherapy. These findings reveal a critical role for CD4$^+$ T-cell help in enabling cDC1 function in the TME and may establish the helped cDC1 transcriptomic signature as diagnostic marker in cancer.

ELUCIDATING THE ROLE OF HELICOBACTER HEPATICUS EXOPOLYSACCHARIDES IN INFLAMMATORY DISEASES

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Host-microbe interactions in the intestine promote health and when deranged can lead to chronic diseases such as inflammatory bowel disease (IBD). *Helicobacter hepaticus* (*H. hepaticus*) does not induce immune pathology in normal mice. However, mice develop colitis when the interleukin-10 receptor (IL-10R) is blocked or genetically deficient. This suggests a role for both the microbe factors and host in driving disease pathogenesis. We have shown that *H. hepaticus* secrets an exopolysaccharide (EPS) that activates CREB-dependent anti-inflammatory gene signature via the TLR2 receptor in intestinal macrophages. The identification of the EPS and its role in inflammatory diseases remain elusive. We stimulated macrophages with purified EPS to assess cytokines responses. The IL-10 production was dose-dependent increased, whereas TNFa and IL-6 were dose-dependent decreased, suggesting an anti-inflammatory property of the EPS. To determine the molecular size of the EPS, size-fractionation using spin filters was performed. Both the 100-300 KDa and the larger than 300 kDa fractions can increase IL-10 and decrease TNFa or IL-6, suggesting the EPS might be heterogenous. Work is ongoing to further explore the properties of the EPS, the molecular mechanisms in immunoregulation, and the role in inflammatory diseases. Ultimately, this work may provide prevention and treatment strategies for IBD.

IT TAKES TWO TO TANGO: MULTIPLE MYELOMA AND BONE MARROW IMMUNE CELLS

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Multiple myeloma (MM) is a plasma cell malignancy mainly occurring in the bone marrow (BM) and represents the 3rd most frequent hematological cancer worldwide. Hallmarks of MM include a permissive BM immune niche and despite the introduction of more effective treatments, MM remains incurable. Thus, a better understanding of this tumour microenvironment is essential to identify the rightest targets to develop a better and well-tolerated immunotherapy. To achieve this, we performed a detailed characterization of the BM immunome, using the murine MOPC315.BM cell line. This MM cell line induces paralysis in a median of 25 days post-injection due to spinal cord compression. Upon tumor-induced symptoms, we found a decrease in the expression of SIRPα, NDUFC2 along with an increase of IL-1β by macrophages in MM-bearing mice. In parallel, MM-bearing mice revealed an increase of both anti- and pro-tumoral features of T lymphocytes, including higher expression of granzyme b and PD-1, respectively. Even with this anti-tumor signature there was no prevention of MM development. These results are being used as rationale to develop a novel combinatorial nanotherapeutic strategy that aims to augment the anti-tumor effect of both arms of immunity: myeloid cells and lymphocytes. In the long run, we expect that this treatment may be included in the armamentarium of MM therapies.

SPATIAL ANALYSIS OF GARP DEPENDENT TGF-ß1 ACTIVITY IN HUMAN NSCLC

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TGF-β1 favors cancer progression by inhibiting anti-tumor immune responses. The precise cellular sources of TGF-β1 in human tumors, its mechanisms of activation and its cellular targets are only partially characterized. GARP, a cell surface protein expressed by regulatory T cells (Tregs) and blood endothelial cells (BECs), controls TGF-β1 release. Our lab showed that antibodies directed against GARP:TGF-β1 complexes block TGF-β1 production by Tregs and improve anti-tumor responses in combination with anti-PD-(L)1 blockade in mice [1]. We will study a series of non-small cell lung carcinomas (NSCLC) samples to further characterize GARP-TGF-β1 immunosuppression. Frozen tumor samples will be analyzed by multiplexed immunofluorescence. We will stain single tissue cryosections with antibodies directed at CD3 (T cells), FoxP3 (Tregs), CD34 (BECs), pSMAD2 (TGF-ß1 responding cells) and GARP. The localization, abundance, and spatial interactions of these cells (e.g. distances between cell types) will be quantified by computerized image analysis. For example, GARP+FoxP3+ cells close to CD3+pSMAD2+ cells would represent GARP-dependent, TGF-β1-producing Tregs that act on effector T cells. Bulk RNA-seq and spatial transcriptomic analysis of the same samples will help identify gene expression signatures (e.g. TGF-β1-induced genes, T-cell activation) correlated with the histological data.

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INVESTIGATING THE MECHANISM OF FERROPTOSIS IN T CELLS

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Regulated cell death is a crucial process during embryonic development, aging, immune responses, or clearance of infected cells. Several distinct cell death pathways have been identified and characterized. Ferroptosis is described as a regulated form of cell death and is induced by the peroxidation of polyunsaturated fatty acids (PL-PUFAs) in the phospholipid bilayer of the plasma membrane eventually leading to cell death. To cope with increased lipid peroxides levels, cells have implemented antioxidant mechanisms. Glutathione peroxidase 4 (Gpx4) is one of the most-studied lipid peroxidation scavengers. Previously, our laboratory reported that Gpx4-deficient T cells are very susceptible to ferroptosis in vitro and in vivo upon activation by viral or parasitic infection. However, regulation of ferroptosis and the players involved in this pathway are still poorly characterized. To gain more insight in the mechanism inducing ferroptosis in T cells, we performed a genome-wide CRISPR knock-out screen. The screen revealed that T cells heavily rely on acetyl-CoA synthetase long-chain family member 4 (Acsl4) to facilitate the incorporation of PUFAs in the lipid bilayer and induce ferroptosis. Additionally, genes involved in iron uptake, trafficking and storage seem to be potent inducers of ferroptosis indicating that iron metabolism may be a key regulator of ferroptosis in T cells.

THE T CELL LANDSCAPE OF GASTROESOPHAGEAL ADENOCARCINOMA IN TREATMENT NAÏVE PATIENTS

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Gastroesophageal adenocarcinoma (GEAC) incidence has significantly increased worldwide over the last 40 years and remains the third-leading cause of mortality worldwide. The high resistance to chemo- and radiotherapy as well as the overall poor clinical outcome make GEAC a largely unmet clinical need. A thorough definition of the immune infiltrate is crucial to understand the pathogenesis of GEAC. However, a detailed map of the immune composition and its dynamic variation is currently missing. Our aim is to provide a comprehensive view of the global immune landscape in GEAC patients, and to specifically focus on the composition and function of the T cell compartment. Using high dimensional flow cytometry, we quantified the infiltration of the main immune populations in matched healthy and tumor biopsies from 15 treatment naïve GEAC patients. Focusing on the T cell compartment we observed an increased CD4/CD8 ratio in the tumor tissue as compared to healthy tissue. Interestingly, we detected a trend toward a larger proportion of $CD4^+CD8^+$ T-cells within the tumor. We observed no significant changes to the myeloid or B-cell compartment. Integration of datasets including proteomic data, bulk RNA-seq and clinical data is currently ongoing. The role of $CD4^+CD8^+$ T-cells GEAC tumor progression as well as the contribution of other T cell subsets still needs to be defined.

EXPLORING PERIPHERAL TOLERANCE TO NON-SELF ANTIGENS: UNDERSTANDING MECHANISMS AND IMPROVING CLINICAL APPLICATIONS

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Immune tolerance is an antigen specific process, defined as "unresponsiveness", induced by lymphocytes exposure to that antigen. Understanding the mechanisms involved is crucial to develop effective therapies for autoimmune diseases and improve tolerance in transplantation and gene therapy [1].

One of our interests is to understand how peripheral tolerance works in healthy donors in response to non-self, harmless antigens such as commensal microbes and food. Through plasma screenings, AMBRA [2], and our optimized state-of-the-art pipeline for antibody discovery and expression, we observed the presence of IgA and IgG antibodies for common food antigens. Memory B cells and plasma cells are generated through different rounds of somatic mutations and selection occurring in germinal centers, where they interact with CD4 T cells that are also key players in this process. Thus, we are developing an ex vivo model that mimics B and T cells interactions in a germinal center-like system. By doing so, we are investigating gene expression, transcriptomics and phenotype of the different populations by flow cytometry analysis and 10X Genomics [3].

These data will help understand the immunological equilibrium and could provide insights to improve prevention and treatment of autoimmune diseases, to reduce rejection in transplant recipients and to develop vaccines for both cancer and viral infections.

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ANTIBODY-INDEPENDENT PROTECTION TOWARD SARS-COV-2 INFECTION OR VACCINATION

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Although the available vaccines are able to reduce the morbidity and mortality caused by SARS-CoV-2 infection, the emergence of new variants able to escape the humoral response threatens their efficacy. A deep study on the immune determinants that confer a memory protection, not only based on humoral, but also on cellular immunity, is needed. It is thought that SARS-CoV-2 specific memory T cells might play a role in modulating disease severity upon infection, contributing to ameliorate the COVID-19 clinical outcome. However, definitive demonstration is lacking.

To evaluate the antibody (Ab) independent protection against SARS-CoV-2 infection or vaccination, we took advantage of mice lacking both surface and circulating immunoglobulins. We crossed them with the newly generated hybrid human/mouse ACE2 transgenic mouse (expressing physiologically the humanized form of ACE2) or with the K18-hACE2 transgenic mouse. Ab-deficient mice exposed to aerosolized SARS-CoV-2 or immunized intramuscularly with SARS-CoV-2 mRNA vaccines were protected from the heterologous re-infection with SARS-CoV-2 variant, as observed in Ab-competent mice.

These results reveal the presence of cross protective T cell response upon both natural infection and vaccination even in the absence of the humoral response, paving the way for the design of new effective vaccines aimed to activate the cellular immunity.

ANTIGEN-DEPENDENT REGULATION OF THE EARLY STAGES OF UNWANTED HUMAN B CELL DIFFERENTIATION AND ANTIBODY FORMATION

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Blood transfusion is sometimes required in patients undergoing surgery or having specific diseases. Some patients form antibodies against the transfused red blood cells (RBCs) or platelets, a process called alloimmunization. These antibodies form a problem upon additional transfusion. Once formed, antibody-producing plasma cells (PCs) may survive for decades and challenging to eradicate. Preventing of formation of long-lived PCs is thus highly desired. The underlying mechanism of alloimmunization and, more generally, the differentiation of B cells into long-lived PCs remains largely unknown. Limited data show that high antigen density on RBCs promotes alloimmunization, while low antigen density on RBCs induces tolerance[1]. In this project, we will investigate how antigenic context regulates the early steps of human T cell dependent B cell differentiation into antibody-producing PCs. To investigate this, we will compare antigen as soluble protein, antigen complexes (e.g. by mulitmerisation or nanoparticle incorporation), and antigen incorporated into eukaryotic membranes (e.g. RBC with different amounts of antigen). With this study, we intend to find key regulators of the pathways leading to wanted and unwanted antibody formation and thereby identify potential targets for intervention.

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AN INFLAMMATORY LOOP BETWEEN MACROPHAGES AND CANCER CELLS PROMOTES PDAC PROGRESSION

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Pancreatic ductaladenocarcinoma (PDA) is a deadly disease characterized by a cancer promoting inflammation, where abundance of myeloid infiltrate, and in particular tumor associated macrophages (TAM), correlates with bad prognosis. This inflamed context influences cancer cell behavior with mechanisms yet to be investigated. We previously identified a population of $IL1B^+$ TAM, enriched in inflammatory response genes and conserved in human and mouse PDA. Their identity is promoted by tumor derived PGE_2 and targeting either IL1b or PGE_2 strongly impact on PDA growth *in vivo*. Here we show that cancer cells are the main target of IL1β since the lack of IL1R1 in tumors (but not in the stromal or hematopoietic-derived cells) dampens PDA growth. *In vitro* transcriptomic analysis revealed that IL1b triggered inflammatory genes (including *Tnfa*), myeloid cell recruiting chemokines and PGE_2-producing enzymes both in murine PDA cell lines and organoids; and longitudinal scRNA-seq analysis of tumors identified an inflammatory module stably expressed during tumor progression by PDA cells. Moreover, relying on a synergistic effect between PGE_2 and TNFa, IL1b-conditioned media from PDA cells rewired BMDM transcriptional state, strongly inducing *Il1b* transcription. In conclusion our study unveils a self-sustaining loop between cancer cells and $IL1B^+$ TAMs that fuels a pathogenic inflammation in PDA.

AN ORGANOTYPIC HUMAN MELANOMA-IN-SKIN MODEL AS *IN VITRO* TOOL FOR TESTING CELL-BASED THERAPIES

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Despite recent extraordinary clinical success in treating melanoma with immune checkpoint blockade, most patients do not respond or develop adaptive resistance. Thus, a clear need exists for additional therapy options. The ability of Vγ9Vδ2-T-cells to recognize and kill transformed cells independently of HLA-matching makes them a promising candidate for immunotherapy. *In vitro* melanoma reconstructed human skin (Mel-RhS) has been developed to mimic features of tumor progression and has an attractive potential to test melanoma-targeted therapies in preclinical studies.

Here, we investigated the capacity of expanded peripheral blood-derived Vγ9Vδ2-T-cells to target tumors in a Mel-RhS model. Vγ9Vδ2-T-cells were viable up to three days and cells in contact with the tumor acquired the melanoma-associated MCSP antigen from the melanoma cells. $MCSP^+$ Vγ9Vδ2-T-cells expressed higher levels of the activation markers 4-1BB and NKp44 compared to those from the healthy control RhS or their $MCSP^-$ counterparts. CXCL10 secretion was upregulated in supernatants from Vγ9Vδ2-T-cell-containing Mel-RhS, compared to without Vγ9Vδ2-T-cells.

In conclusion, a fraction of Vγ9Vδ2-T-cells came into close contact with the melanoma cells, acquired melanoma-associated membrane antigens, and became selectively activated. The novel Mel-RhS may present a promising tool to test T-cell-based therapies.

ACTIVATED PHOSPHOINOSITIDE 3-KINASE Δ (PI3KΔ) IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

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Systemic lupus erythematosus (SLE) is a common autoimmune disease which is difficult to treat due to high heterogeneity. Therefore, there is an urgent need to identify biomarkers that can help classify patients. Phosphoinositide 3-kinase δ (PI3Kδ) is a lipid kinase that regulates various functions of immune cells. Previous studies have shown increased PI3Kδ activity in SLE patients. Transitional B cells (TrB) and follicular helper T cells (Tfh) are the two known biomarkers of chronically activated PI3Kδ, as they are increased in Activated PI3Kδ Syndrome- a rare monogenic disorder caused by mutations in PI3Kδ. To study biomarkers of PI3Kδ activation in SLE, we designed an 18-marker flow cytometry panel and characterised lymphocyte subpopulations in the PBMC of 150 adult SLE patients and 30 healthy controls. The flow cytometry data revealed 12 and 5 SLE patients with unusually high numbers of circulating TrB and Tfh cells respectively (ROUT method, Q=10%), indicating that the level of TrB and Tfh cells are increased in a subset of patients with SLE. Our analysis also showed CD19+CD27-IgD- cells, known as double-negative B cells, are significantly increased in SLE patients compared to healthy controls (Padj <0.001). Further experiments are ongoing to investigate the association between the level of PI3Kδ activity and the frequency of T and B cell subpopulations in SLE patients.

A SUBSET OF ANTIBODIES TARGETING CITRULLINATED PROTEINS CONFERS PROTECTION FROM RHEUMATOID ARTHRITIS

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The presence of elevated levels of anti-citrullinated protein antibodies (ACPAs) is a hallmark of rheumatoid arthritis (RA). Although ACPAs have been always associated with RA pathogenesis and severity, the in vivo mechanism of action is still unclear. In this study, we have expressed monoclonal ACPAs derived from patients with RA, and analyzed their functions in mice, as well as their specificities. None of the ACPAs showed arthritogenicity but there was one (clone E4) that showed an outstanding protection in different models of rheumatoid arthritis in mice. E4 showed a binding pattern restricted to skin, macrophages and dendritic cells in lymphoid tissue, and cartilage derived from mouse and human arthritic joints. Proteomic analysis confirmed that E4 strongly binds to macrophages and RA synovial fluid proteins such as α-enolase. The protective effect of the antibody is dependent on the interaction with α-enolase, resulting in the formation of immune complexes able to stimulate FcγRIIB on synovial macrophages, leading to increased IL-10 secretion and reduced osteoclastogenesis. This study provides evidence for the first time that questions the central dogma that ACPA autoantibodies can only contribute to rheumatoid arthritis pathogenesis, but also be protective. Therefore, this data suggests that this subset of ACPAs could have a therapeutic potential for the treatment of RA.

GENERATION OF 3D HUMAN LYMPH NODE ORGANOIDS TO STUDY IMMUNE RESPONSES IN MULTI-ORGAN-ON-CHIP MODELS

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Lymph nodes & tonsils are secondary lymphoid organs that orchestrate the adaptive immune response. Their highly specialized architecture is regulated Fibroblast Reticular Cells (FRCs), which support immune cell functioning. The study of human tissue-specific antigen immune responses lack robust organoids and multi-organ-on-chip models due to their complex immune cell diversity. Here, we aim to create 3D human lymph node organoid models containing FRCs that can be implemented into microfluidic devices. Primary human FRCs and immune cells were isolated from lymph node or tonsil biopsies. FRCs were co-cultured with immune cells or the DC-like cell line MUTZ-3 DC in 3D hydrogels. Experimental readouts included flow cytometry, cytokine/chemokine analysis, histology & 3D imaging. The presence of FRCs in the hydrogel resulted in the survival of namely B cells in the immune cell co-cultures, which coincided with higher levels of stromal-secreted CXCL12 and BAFF. FRCs improved the viability and influenced the development of MUTZ-3 DC cells under cytokine stimuli into a lymph node-resident DC-like phenotype. Imaging revealed direct cell to cell contact between FRCs and immune cells. Such a platform presents an opportunity to further study antigen-specific immune responses, ultimately in a microfluidic setting, highlighting the importance of FRCs for immune cells in organoid models.

PREDICTING OUTCOMES FOR CROHN'S DISEASE USING A MOLECULAR BIOMARKER: PROFILE TRIAL

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The course of Inflammatory Bowel Disease (IBD) varies substantially between individuals, but there are limited, reliable, predictors to guide clinical practice. This has become an increasing problem given the rising prevalence of IBD, and an increasing recognition for the profound health and socio-economic impact on patients, affecting education, relationships, and employment. The majority of impacts from IBD have been ascribed to poor disease control, which in turn leads to repeated symptomatic flares and development of complications. If by early identification and effective management, disease modification could occur, whereby 'severe cases' could be turned into 'mild cases', this could substantially ameliorate the impact of IBD on the health and quality of life for patients.

Previously, our group has described a transcriptional signature detectable within peripheral-blood CD8 T-cells at diagnosis, identifying two subgroups, correlating with subsequent disease course. We sought to translate this biomarker to re-capitulate the prognostic CD8 subgroups and to assess whether this can improve clinical outcomes by appropriately matching therapy to disease course.

From a training cohort of 69 treatment-naïve patients, newly-diagnosed with IBD, whole-blood PAXgene RNA and peripheral-blood CD8 T-cell samples were obtained. Gene expression in both samples was measured by microarray. Statistical modelling identified a transcriptional classifier in whole-blood gene expression data re-capitulating CD8 findings and was optimised into a multi- gene qPCR assay with validation in a further independent cohort of 123 patients newly-diagnosed with IBD.

The PROFILE trial has incorporated this classifier to compare relative efficacy of 'top- down' and 'accelerated step-up' therapy between biomarker-defined subgroups in up to 400 patients with newly-diagnosed Crohn's disease.

Using a 17-gene qPCR assay, 123 patients from the validation cohort could be classified into two distinct subgroups, IBD^{hi} and IBD^{lo}. Irrespective of the underlying diagnosis, IBD^{hi} patients experienced significantly more aggressive disease than IBD^{lo} patients, with earlier need for treatment escalation (hazard ratio=2.65 (CD), 3.12 (UC)). This biomarker has subsequently been used to stratify therapy in PROFILE with 390 participants enrolled – with recruitment now complete and follow-up due for completion in December 2022.

PROFILE will be assessing outcomes which have been demonstrated to be important to patients – namely clinical remission, avoidance of steroid medications and reduced need for surgery, especially stoma formation which is associated with significant stigma and pyschological morbidity. In addition, key secondary outcomes measures are being collected to assess the burden of disease and the impact of this on quality-of-life for patients. Alongside, the trial there will also be a health economic analysis conducted by the Cambridge Centre for Health Services

Research, as well as a national evaluation by the National Institute for Health and Clinical Excellence (NICE).

In summary, we have developed, optimised and validated a whole-blood qPCR classifier that predicts disease course from diagnosis in patients with IBD. This classifier is currently being assessed in the PROFILE trial, the first biomarker-stratified trial in Gastroenterology, and if clinical utility of a stratified treatment approach is demonstrated this would represent a major step towards personalised therapy in IBD.

THE ROLE OF MMP-12 IN NEUROINFLAMMATION

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Matrix Metalloproteinases (MMPs) are often considered to be involved in the turnover, catabolism and degradation of the extracellular matrix. However, in the past few years it has become clearer that MMPs also hold important roles in controlling aspects of inflammation and immunity by regulating the activity of chemokines, cytokines and growth factors. MMP-12 is expressed in a GM-CSF dependent manner. GM-CSF is a central mediator in neuroinflammation and mice lacking GM-CSFR signalling in the $CCR2^{+}Ly6C^{high}$ monocytes are resistant to EAE. In experimental autoimmune encephalomyelitis (EAE), a disease model resembling multiple sclerosis, MMP-12 has been conversely associated with disease driving functions. While one study showed that MMP-$12^{-/-}$ null mice have a poorer EAE outcome compared to their wild-type littermates, our single-cell and bulk RNA sequencing datasets revealed that MMP-12 is expressed in CNS invading monocyte-derived dendritic cells (moDCs) and macrophages (moMACs). Thus, using both a pharmacological blockade via highly specific small molecules, and conditional and inducible gene targeting approaches, we want to investigate the role of MMP-12 in monocytes and monocyte derived cells in neuroinflammation during the acute and chronic effector phases of EAE.

EXPLORING DYNAMICS OF B CELL MIGRATION IN THE SPLEEN

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Lymphocyte trafficking through secondary lymphoid organs (SLOs) is critical to activate rare, antigen-specific naïve lymphocytes. The spleen is the largest SLO and the only one to filter the blood, making it critical for protection from blood-borne pathogens. Despite its unique immunological role, the chemotactic cues and anatomical structures which support lymphocyte trafficking through the spleen are poorly understood. A major challenge in addressing these basic questions has been the difficulties to develop imaging procedures that allow the analysis of live spleens. This is essential because the blood flow plays an important role in regulating cell behaviour in this organ. By employing a live imaging approach, our lab recently uncovered a novel network of blood vessel-guided migratory pathways that facilitate T cell entry to the white pulp of the spleen, where adaptive immune responses are regulated. We showed that entry is mediated via 3 distinct regulated steps. Here, we test the role of these pathways and the mechanisms that underlie their interactions with naïve and memory B cells migrating within the spleen. Through this study, we aim to understand the fundamental mechanisms of lymphocyte migration within this immunological organ and shed a light on the potential that this organ may have for future therapeutics.

FLUOROPHORE-SPECIFIC B-CELL RECEPTORS MIGHT HINDER ANTIGEN-SPECIFIC B-CELL DETECTION IN FLOW CYTOMETRY

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Small antigen-specific B-cell populations can be detected using flow cytometry. However, background events can falsely be classified as antigen-specific B cells, thus obscuring analysis. We hypothesize that this background can partly be caused by the fluorophore-specific B-cell receptors.

To investigate the presence of fluorophore-specific B cells, 500 naïve or memory B cells from healthy adults were sorted per well in 96-well plates and cultured while stimulating antibody production. After 14 days, supernatant was harvested for use in ELISA, using plates coated with fluorophore-conjugated antibodies. A positive well indicated the presence of fluorophore-specific B-cell receptors in at least 1/500 sorted cells of that well. The tested fluorophores were PE-Cy7, AF700, PE, BV421, APC, APC-H7 and BV786.

Six donors were recruited: three for naïve and three for memory B-cell experiments. Minimum frequencies of fluorophore-specific B cells were higher in naïve B cells (range: 1.3 to 2.6/1000 cells) than in memory B cells (range: 0.8 to 1.8/1000 cells). There were small differences in frequency between different fluorophores, and AF700 had the highest frequencies.

High fluorophore-specific B-cell frequencies might hamper reliable detection of genuine antigen-specific B cells, but additional research is needed to determine the extent of this phenomenon and how it can be blocked.

DEVELOPMENT OF A HUMAN IMMUNOCOMPETENT LYMPH NODE-ON-CHIP

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Lymph nodes are tissue-draining secondary lymphoid organs essential for an adaptive immune response. The lymph node stromal cells provide unique structural microenvironments, thereby creating separate areas for B and T cells. In the T cell zone, fibroblastic reticular cells (FRCs) produce various extracellular matrix (ECM) components, forming a large reticular network. This FRC network facilitates the survival, migration and interaction of immune cells within the lymph node via the production of specific cytokines and chemokines. Here, we aim to develop a human immunocompetent lymph node-on-chip with a main focus on the T cell zone. First, we will select a suitable 3D material for the culture of human FRCs to mimic the lymph node T cell zone. We will compare different 3D biomaterials including functionalized peptide hydrogels, fibrous networks, porous network scaffolds and collagen beads. In these 3D cultures, FRC viability and specific surface marker expression will be tested. Furthermore, network formation and ECM production by FRCs will be investigated. Finally, cytokine and chemokine levels in the different 3D FRC cultures will be measured. Investigating different 3D biomaterials for the construction of the lymph node T cell zone will help the development of a human immunocompetent 3D lymph node which can ultimately be integrated in an organ-on-chip platform.

A TUMOR SIZE-DEPENDENT NEO-ANGIOGENIC SWITCH DETERMINES THE CAPACITY OF CD8+ T CELLS TO EXERT CANCER IMMUNE SURVEILLANCE WITHIN THE LIVER

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CD8+ T cells play a crucial role in controlling liver tumors, such as hepatocellular carcinoma (HCC), and their antitumor activity depends on their recruitment to the tumor site, antigen recognition and exertion of effector functions. However, the precise dynamics of their interactions within the tumor at single-cell level are poorly described. We generated a new mouse model of spontaneous HCC in which the expression of a nominal antigen, the oncogene SV40 large T antigen (TAg),and fluorescent protein is restricted to transformed hepatocytes. Upon adoptive transfer of *in vitro* differentiated TAg-specific T effectors (T_{eff}) in tumour-bearing mice, we observed that just some mice responded to the therapy. Thus, we developed a multiparametric mathematical approach that divided the HCC lesions, based on their volume, in Responders (R, volume <10 mm3) and Non-Responders (NR, volume >100 mm3). We found that the vessel phenotype dramatically changed between Rs and NRs: the HCC volume gain correlates with higher neovascularization, capillarization and reduction of liver sinusoidal endothelial cells. Our data suggest that some hemodynamical and environmental features of each HCC lesion can influence their responsiveness to the T_{eff} activity. The innovative nature of our work will elucidate new mechanisms whereby T_{eff} fail to exert their immune function and activity in tumorigenic liver.

THE NUMBER OF DONOR HLA-DERIVED T-CELL EPITOPES AVAILABLE FOR INDIRECT ANTIGEN PRESENTATION DETERMINES THE RISK FOR VASCULAR REJECTION AFTER KIDNEY TRANSPLANTATION

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Following a kidney transplantation, HLA mismatches between donor and recipient may lead to T-cell- or antibody-mediated rejection. Although it has been established that the indirect pathway of allorecognition plays an important role during antibody-mediated rejection, the role of this pathway in acute T cell-mediated rejection (aTCMR) is not well defined. In this study, we aimed to evaluate the role of the indirect pathway in aTCMR using the PIRCHE-II algorithm, which can quantify the amount of theoretical T-cell epitopes available for indirect allorecognition. As a measure for the potential indirect $CD4^+$ T-cell alloreactivity, the PIRCHE-II score was calculated for 688 donor kidney-recipient combinations. A diagnosis of aTCMR was made in 182 cases, 61 of which were vascular aTCMR. A strong association between the PIRCHE-II score and the incidence of first-time aTCMR was observed, in particular vascular rejection. This association was found mainly for the peptides derived from donor HLA-DR/DQ (PIRCHE-II DR/DQ) ($p<0.001$). Our results suggest that indirect antigen presentation of donor HLA-peptides may significantly contribute to the risk for acute vascular rejection. This finding increases our current understanding of the pathogenesis of aTCMR and may contribute to risk stratification following kidney transplantation.

NEUTROPHILS: THE IMMUNE CELL POPULATION DOMINATING MURINE PROSTATE CANCER

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Prostate cancer (PCa) is the most common diagnosed cancer among men of western societies, yet most immunotherapy trials were unsuccessful on PCa and the involvement of immune cells has been only recently addressed. The novelty of my PhD work lies on unravelling the heterogeneity and function of immune infiltrates in healthy murine prostate and during PCa progression in $PTEN^{(i)pe-/-}$ mice. In these mice, the PTEN gene is selectively ablated in prostatic luminal epithelial cells at adulthood, reproducing the occurrence and features of human PCa. The strict temporal control of PTEN depletion along with the slow tumour progression allows to characterize the immune cells in the tumour microenvironment and to test therapeutic strategies.

By combining state-of-the art techniques, I established a high-resolution immune atlas of murine PCa from neoplasia to adenocarcinoma. I highlighted a massive neutrophil influx harboring immunosuppressive signature, infiltrating the cancerous epithelium and lumen, along with an increase of exhausted $CD8^+$ T cells dominating the stromal area. Currently, I am testing immunotherapeutic approaches to control PCa by depleting the pro-tumoral neutrophils and reinvigorating the $CD8^+$ T cells in vivo. Thus, my study elucidates the complexity of immune cell behavior in PCa and unravels potential immunotherapeutic targets to benefit the tumor immunology field.

THE MUCOSAL HOST RESPONSE TO EARLY LIFE INFECTION WITH GIARDIA LAMBLIA

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The small intestinal epithelium in the adult host is in close contact and constant interplay, with the commensal bacteria and at the same time is facing the threat of a pathogenic insult. Intraepithelial lymphocytes (IELs), which reside between intestinal epithelial cells are a miscellaneous population of lymphoid cells that is involved in this immune homeostasis. The intestinal epithelium in the neonatal host is markedly different. The ongoing maturation of the mucosal immune system increases the threat by pathogenic insult. Aim of the present study was the analysis of the host response upon neonatal infection with the enteric pathogen *Giardia lamblia (G. lamblia)*. We have previously shown that while adult mice rapidly cleared the infection, mice infected as neonates remained chronically infected. TCR$\alpha\beta^+$ and TCR$\gamma\delta^+$ lymphocytes seem to play a crucial role in the clearance of *G. lamblia* infection in the adult host. Further, adult mice infected as neonates displayed enhanced levels of TCR$\alpha\beta^+$ CD8$\alpha\alpha^+$ and TCR$\gamma\delta^+$ CD8$\alpha\alpha^+$ IELs. IELs begin to accumulate in the gut epithelium during the third week of life and infection at this time led to a decreased parasite burden. compared to a first and second week of life infection. Our findings relate postnatal mucosal immune developmental to susceptibility to *G. lamblia* infection and suggest a possible role of intraepithelial lymphocytes.

EFFECT OF CHRONIC STRESS ON AGEING T CELL TRANSCRIPTOMICS IN 'DIRTY' MICE

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The booming rise of Silver Tsunami has highlighted the need to unravel the complexities of ageing. Chronic stress accelerates ageing and examining the subtleties of immune cells, specifically T cells that get compromised on ageing, under stress is warranted. In our study, we aim to analyse how chronic stress modulates ageing T cells using 'dirty' mice. 'Dirty' mice are a superior mouse model for immunological studies since they have an activated immune system like 'dirty' humans. We subjected aged 'dirty' mice to a 21-day chronic variable stress protocol and analysed various lymphoid and peripheral tissues by flow cytometry. Interestingly, there were no changes in the proportions of splenic T cells including various T cell subsets under stress. Further analysis of stressed splenic T cells by scRNA-seq showed an increase in homeostatic genes, Hsp90 and Il7r. Since T cell ageing has been associated with loss in proteostasis, the role of these homeostatic genes upregulated under stress in maintaining T cell proteostasis needs to be explored. Additionally, increased T cell proportions in bone marrow, liver, and salivary gland was observed with minute changes in specific T cell subsets. Understanding of the transcriptional dynamics of ageing T cells in different tissues is crucial for designing effective treatment regimens and vaccinations in the aged.

CX3CR1 IS A GRADED AND UNIVERSAL DIFFERENTIATION MARKER UNIFYING HUMAN AND MURINE T CELL DIFFERENTIATION

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T cells differentiate into a range of functionally distinct effector and memory states upon antigen-encounter. These states, or 'subsets', are delineated by different cell surface markers for murine and human T cells, which hampers cross-species translation of T cell properties. We identified that fine-graded expression of CX3CR1 distinguished functionally distinct states of antigen-experienced $CD8^+$ and $CD4^+$ T cells in both species. Increased CX3CR1 levels correlated with higher cytotoxic potential. In contrast, cells expressing intermediate CX3CR1 levels were the most potent effector cytokine producers. Consequently, the CX3CR1 gradient identified that T cell cytotoxicity and effector cytokine production are not correlated, but instead functionalities held by distinct T cells. CX3CR1 levels, refined with CD62L accurately captured the continuum of high-dimensional T cell differentiation states. Fine-graded stratification of $CD8^+$ T cells by CX3CR1 level delineated states with comparable functional properties in humans and mice. This applied to $CD8^+$ T cells in healthy humans and mice, and to virus-specific $CD8^+$ T cells that were tracked longitudinally in both species. In summary, measuring CX3CR1 expression levels provide a simple and practical strategy to translate the behavior of functionally distinct T cell differentiation states across species.

EFFECT OF MODIFIED BETA-2-MICROGLOBULIN ON NEUTROPHIL RESPIRATORY BURST

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Beta-2-microglobulin (β2m) is a small single chain protein expressed by all nucleated cells. It is a central component of the MHC class I complex, and as such, the main known function of the protein is peptide presentation to CD8 T-cells. A modified form of free β2m can be found in serum from patients undergoing chronic hemodialysis (dk58β2m). It is formed by proteolytic cleavage of β2m mediated by C1s complement followed by the removal of Lys^{58} by a carboxypeptidase B-like activity. The function of both dk58β2m and the cleaved intermediate (ck58β2m) remains unknown.

Studies have shown that ck58β2m binds neutrophil granulocytes with higher affinity than the native protein. We therefore wanted to investigate the effect of both native and modified β2m on the respiratory burst in human neutrophils, a process characterized by a rapid increase in the production of reactive oxygen species (ROS). Flow cytometric analyses showed that both ck58β2m and dk58β2m inhibit the production of ROS in neutrophils stimulated with either TNF or the bacterial peptide fMLF. Interestingly, ck58β2m and dk58β2m were also shown to inhibit spontaneous ROS production in neutrophils indicating that the effect of modified β2m on neutrophil respiratory burst is independent on direct stimulation. Further analyses are needed to determine how modified β2m affect the generation of ROS in neutrophils.

DECIPHERING PULMONARY NEURON-ILC CIRCUITS

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Crosstalk between neuronal and immune cells has been reported as a key player in maintenance of organ physiology and regulation of inflammation and infection. Previous work has depicted the complexity of neuroimmune communication and its potential to be harnessed for therapeutics. Our preliminary data shows Innate Lymphoid Cells (ILC) in close proximity with pulmonary neurons and, upon stimulation, ILC modulate their expression of synaptic proteins and neurotransmitter receptors, supporting that these cells engage in tight communication. However, the mechanisms underlying this crosstalk remain elusive. Thus, we propose to characterize and modulate ILC-neuron interactions *in vivo,* unraveling the nature of this communication. By using advanced microscopy, combined with genetic and molecular approaches and profiling of the immune compartment, we will dissect how pulmonary neuroimmune communication is orchestrated, and study its impact in maintenance of homeostasis and the onset and resolution of inflammation.Together, our strategy will transform our understanding of pulmonary infections, allowing to decipher neuroimmune language with unprecedented mechanistic and conceptual detail.

INVESTIGATING HOW HELIGMOSOMOIDES POLYGYRUS INFECTION AFFECTS GUT BARRIER INTEGRITY

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Helminth infection is a major public health concern across the globe, with 2 billion people infected. Bacterial co-infections are common, but little is understood about the interplay between the two pathogens and immune responses. Data shows that helminth infection can attenuate immunity to concurrent infections, but whether bacteria alter immunity against helminths is unclear.

Our research asks whether intestinal helminth infection causes breaches in the gut barrier that allow bacteria to cross. We show that entry and exit of the helminth *Heligmosomoides polygyrus* through the gut wall is accompanied by spikes in inflammation, tissue remodelling and expression of IFN-γ. Our data suggest that bacterial translocation does not occur, perhaps reflecting antimicrobial and tissue repair responses to the helminth infection. We are assessing whether the IFN-γ signature is initiated by bacterial or helminth antigens, and whether its presence regulates tissue repair, parasite clearance or bacterial spread. Current experiments suggest that IFN-γ plays a role in repair and antimicrobial processes during helminth infection, and continued research will allow further understanding of the regulation of immunity to helminth infection and the tissue-based interactions that determine the outcome of co-infection.

UNRAVELING FUNCTIONAL CONSEQUENCES OF TUMOUR-MEDIATED GALECTIN DOWNREGULATION IN DENDRITIC CELLS

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Tumour immune-evasion mechanisms remain poorly understood. Glycans and their interacting proteins, namely galectins (gals), pose another level of cellular organization instrumental in modulating immune-cell function and are considered as novel mechanisms of tumour immune-evasion. Gals are carbohydrate-binding lectins that modulate immune-cell function by organizing glycosylated cell-surface proteins into functional membrane-microdomains, ultimately influencing numerous immune-cellular processes. In dendritic cells (DCs), the role of gals has not been yet characterized. Our results show that human primary DCs knocked-down for the expression of gals are unable to form immune synapses with T-cells and ultimately promote T-cell effector responses. This highlights the role of galectins in initiating adaptive immunity. Interestingly, we found gals expression to be downregulated in DCs upon co-culture with 2D and 3D tumour-microenvironment models. Furthermore, cancer patients with low-galectin-DCs show a poor prognosis compared to those with high-galectin-DCs. Overall, our data suggests an essential role for galectins during DC-mediated anti-immunity. We hypothesise that disruption of the galectin-mediated interactions could be exploited by malignant cells, posing a novel melanoma immune-evasion mechanism.

DIVERSITY OF HUMAN LYMPH NODE STROMAL CELLS UPON ISOLATION AND CULTURING

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Lymph node stromal cells (LNSCs) play a pivotal role in adaptive immunity. By establishing a protocol with multiple rounds of enzymatic digestion, we are able to obtain human lymph node (LN) cell suspensions containing viable LNSCs. In addition, two main subtypes of LNSCs: fibroblastic reticular cells (FRCs) and lymphatic endothelial cells, can be cultured in our lab depending on culture conditions. Based on published single cell transcriptomics, LNSCs can be subtyped into different subsets, each with their own function. To identify the subsets of FRCs *in vitro* and their adaptation to culture over time, we performed spectral flow cytometry for different FRC markers (including VCAM1, ACKR4, CD146, BST1, CD90, CD21, CD271, CD34 and PDPN) on fresh LNs (n=5) and upon culture over multiple passages (n=5). In fresh LNs, 11 FRC subsets can be identified, of which 5 are still present *in vitro*. Interestingly, not all subsets express PDPN, previously described as the main marker for FRCs. However, all FRCs *in vitro* express CD90 and BST1, suggesting that these markers are more reliable to identify FRCs *in vitro*. In fresh LNs the potential precursor CD271+CD34+ subset can be found, while *in vitro* only CD271-CD34+ cells remain, independently of passage number. This demonstrates that culture does not induce FRC maturation and that several FRC subsets present in fresh LNs remain in culture.

ANTI-PD-1 PROMOTES CD8 T CELL RESPONSES IN TUMOR-DRAINING LYMPH NODES BY INDUCING FOLLICULAR HELPER T CELL-DEPENDENT IL-4 RELEASE

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While anti-PD-1 therapy targets intra-tumoral $CD8^+$ T cells to promote clinical responses in cancer patients, recent evidence suggests an additional activity in the periphery, in particular, in draining lymph node. However, the mechanism by which anti-PD-1 mAb act in lymphoid organs is unclear.

Here, we show that anti-PD-1 mAb enhances $CD8^+$ T cell responses in tumor-draining lymph nodes by stimulating cytokine production in follicular helper T cells (Tfh). In two different OVA-expressing tumor models, we observed that anti-PD-1 increased the proliferation and effector functions of tumor-specific $CD8^+$ T cells in the draining lymph node. Surprisingly, anti-PD-1 did not primarily target $CD8^+$ T cells but instead specifically bind Tfh cells *in vivo*, which are cells expressing the highest levels of PD-1 in lymph nodes. Anti-PD-1 acts on Tfh cells inducing their proliferation, their relocation outside germinal center and, most importantly, their interleukin 4 production. Blocking IL-4 or inhibiting the transcription factor Bcl6 abrogated anti-PD-1 activity lymph nodes while injection of IL-4 complex was sufficient to recapitulate the activity of anti-PD-1. Finally, a similar mechanism was observed in a vaccine setting.

We propose that Tfh cells and associated cytokines, in particular interleukin 4, play a key role in the peripheral activity of anti-PD-1 mAb.

BYSTANDER TH17 IMMUNITY AND ITS FUNCTIONAL IMPACT ON HETEROLOGOUS CHALLENGE

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Fungal infections trigger type 17 immune responses, where IL-17 is generated by CD4+ T cells, innate ILC3 and γδ T cells. It was discovered that even in animals that have never been exposed to the fungus previously, TCRαβ+CD4+ T cells that display hallmarks of memory T cells quickly produce IL-17. According to our hypothesis, they are memory Th17 cells that have undergone antigen independent activation, or bystander activation. Using fungal infection models *C. albicans* and *Malassezia*, we intend to extensively profile these bystander activated CD4+ T cell populations in terms of recruitment, expansion, and cues that can trigger memory Th17 cell bystander activation. Our recent results indicate that memory CD4+ T lymphocytes from the spleen of *C. albicans*-infected mice become activated when stimulated with IL-23, IL-6, IL-18 and IL-1β implying that cytokine signals alone have the ability to activate this population. Additionally, animals harboring memory cells from preceding *C. albicans* infections, displayed a higher Th17 response when challenged with the unrelated fungus *Malassezia* compared to control mice. Memory mice also cleared fungi more quickly, suggesting that this population may possibly have functional impact. This preliminary data points to possible bystander activation of Th17 cells, and a thorough investigation would be important to fully comprehend this population.

DEVELOPMENT OF MACHINE LEARNING METHODS FOR IDENTIFYING PROTEIN PATTERNS IN AUTOIMMUNITY

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An autoimmune disease is when the adaptive immune system starts recognising and attacking healthy tissue. The repercussions of this are often dire for the patient. Estimates suggest up to nine percent of the world population acquires an autoimmune disease, and that the prevalence is rising especially in developed countries [1]. Yet the ethiology of the disease has not been determined. Several risk factors have been demonstrated such as genetics [2], infections, and environmental factors. Especially, there have accumulated evidence suggesting a high correlation between patients and expression of certain major histocompatibility complex (MHC) alleles. However, none of these risks alone explains the onset of the disease.

This project will first examine if it is possible to cluster different autoimmune diseases based on common target-protein features and whether we can learn some of the properties that play a role in antigen processing and the breaking of immune tolerance. We will do this by collecting a wide range of protein features from expression levels, structure, physico-chemical properties to predicted epitopes and their binding affinity to MHCs. Interpretable machine learning algorithms will be used to learn common patterns across different autoimmune diseases and the importance of the different features.

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9.008.

THE NFATS AS NEW TOOLS FOR IMMUNOTHERAPIES

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The NFAT transcription factors NFATc1 and NFATc2 control the activity of adaptive immune system. Using *ex vivo* systems for T cell 'exhaustion' our data show that NFATc2 supports the expression of markers of exhausted $CD8^+T_{EX}$ cells, as Tim-3, Tigit and others and inhibits markers of progenitor $CD8^+T_{PEX}$ cells, as Tcf7, Id3 and others.

By contrast, the activity of NFATc1, the most prominent NFAT factor in nuclei of activated T cells, appears to be 'frozen' during chronic activation. These data are supported by *in vivo* data showing that ablation of NFATc2 supports the killing of tumor cells. While NFATc1 induction seems to play a minor role in chronic immune reactions, it orchestrates acute immune reactions. In addition, the first studies preliminary data of our current research suggest that NFATc1/αA differs markedly, from NFATc1/βC and NFATc2. The short isoform, NFATc1/αA, seems to counteract the exhaustion, and play a role on the survival of $CD8^+$ exhausted T cells under chronic conditions.

Currently, we are extending these findings by studying pre-clinical models of solid tumors and explore whether – as we assume – components of calcineurin/NFAT network can be used to improve the therapy of solid human tumors.

IMMUNOTHERAPY FOR CANCER: T-CELLS' JOURNEY TO BECOMING CAR-T CELLS

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Chimeric antigen receptors (CARs) are synthetic receptors comprised of the extracellular antigen recognition antibody-derived single chain variable fragment (scFv) and the intracellular T cell signaling domains. CARs endow T cells with the designed specificity and function. Since 2017, CAR-T cell immunotherapy is an approved cancer treatment approach for patients with certain types of leukemia and lymphoma. Here we present the general principles of CAR-T cell therapy, the CAR structure, the production of the cellular product, and some of the major challenges in existing therapies including the limited persistence of CAR-T cells and lack of tumor-specific targets. We aim to produce safer and more effective mouse CAR-T cells by improving the individual design steps. One such improvement is developing CAR-T cells with the knock-out (KO) of the endogenous T-cell receptor (TCR). TCR KO prior to the introduction of the CAR molecule to the T cells reduces alloreactivity and contributes to the production of a more homogenous, and therefore safer, cellular product. We tested the knock-out of the TCR with CRISPR/Cas9 system and designed sgRNAs targeting TCR α constant (TRAC) locus in Jurkat cell line and primary mouse $CD4^+$ and $CD8^+$ T cells. Flow cytometry analysis showed that the KO efficiency was high in both Jurkat cell line (up to 90 %), and mouse primary T cells (up to 60 %).

IMMUNE PROFILING OF THE METASTATIC LYMPH NODES ACCORDING TO MOLECULAR SUBTYPES FOR BREAST CANCER OUTCOME PREDICTION

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The immune response is known to be crucial in breast cancer (BC) progression [1]. At diagnosis, the immune elements infiltrating the primary tumour are among the most well-established prognostic factors with clear implications for treatment effectiveness and disease development [2]. However, their prognostic value in the axillary lymph nodes (ALNs) is poorly understood. Hence, this study aims to: (1) compare the immune cell concentrations in metastatic ALNs ($ALNs^+$) of 46 luminal A and 26 triple negative BC (TNBC) patients using immunohistochemistry, and (2) evaluate through Kaplan-Meier analysis its impact on patient outcomes according to BC subtype. On the one hand, we found a higher proportion of CD4, CD68 and CD1a but a lower proportion of CD57 in luminal A than in TNBC $ALNs^+$ samples. On the other hand, our results highlighted that patients with higher concentration of CD4, CD8 and CD83 in $ALNs^+$ have worse outcome in TNBC, and patients with higher concentration of CD21 have better outcome in luminal A. Therefore, we found a converse involvement of CD4, CD8 T cells and CD83 mature dendritic cells (DCs) vs. CD21 follicular DCs with BC patient outcomes. In conclusion, the immunologic components of the $ALNs^+$ according to BC subtypes might help refine therapeutic strategies and, ultimately, improve patient outcomes.

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LYMPHATICS ACT AS A SIGNALING HUB TO REGULATE INTESTINAL STEM CELL ACTIVITY

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Barrier epithelia depend on resident stem cells (SCs) to preserve and restore tissue integrity. In the small and large intestine, SCs are continuously active and respond to signals from their local microenvironment (niche) to ensure efficient tissue turnover. The cellular complexity and underlying communication pathways of the intestinal SC niche, however, are still unfolding. Pairing single cell with spatial transcriptomic analysis of the mouse intestine, we take advantage of BayesPrism to deconvolve gene expression programs and pioneer SpaceFold to computationally reconstruct the intestinal cellular landscape and map spatial, cell type-specific gene expression. In doing so, we identify lymphatics as a major signaling hub in SC proximity, secreting a variety of canonical SC-promoting factors (WNT2, R-SPONDIN-3) at the crypt base and uncover lymphatic-derived REELIN as a hitherto unappreciated signal that governs SC activity *in vitro* and *in vivo*. Besides directly modulating SC activity, lymphatics may indirectly impact SCs through modes of immune cell trafficking. Probing our transcriptomic atlas for spatially concentrated gene programs and complementing that with receptor:ligand analyses between lymphatics, SCs and immune cells, will yield additional insights into dynamic modes of SC regulation in the future.

FUNCTIONAL INTRACELLULAR AND EXTRACELLULAR ANALYSIS OF INDIVIDUAL B CELLS IN MURINE PANCREATIC CANCER USING SINGLE-CELL DROPLET MICROFLUIDICS

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In recent years, tumour-associated B cells have emerged as a key player in tumour immunity and lymphoid neogenesis [5]. In fact, B cells were shown to infiltrate tumors and exert various functions, most prominently antigen presentation and antibody secretion. B cells can undergo germinal center reactions to produce antibodies that target tumour antigens (Mazor et al. 2022). Such functionality is strongly impacted by intracellular enzymes such as Activation- Induced Cytosine deaminase (AID). Moreover, B cells are also capable of expressing and secreting other proteins which regulate tumour immunity, such as IL-35 [4][2]. Indeed, although different functions have been allocated to B cells in the tumour environment, it remains unclear which B cell subpopulations and level of functionalities support or hinder an effective anti-tumour immune response, making their role overall ambiguous [6].
Previously, our lab developed a platform to simultaneously detect and measure antibody secretion from individual B cells using droplet microfluidics, allowing a dynamic (over-time) and quantitative measurement [1]. In this project, we aim to implement and apply assays for single-cell droplet microfluidics to tumour-infiltrating B cells to *(i)* quantify and characterize secreted antibodies and other effector molecules, and *(ii)* to correlate quantitative secretory read-outs with additional intracellular effectors. For this purpose, we aim to encapsulate B cells from spleen, bone marrow and tumour infiltrates and to measure their functionality quantitatively and dynamically. We developed, calibrated and validated our assays using primary B cells to measure secretion in single droplets over time, allowing for read-outs from up to 40’000 cells simultaneously. To study intracellular markers, we further combined antibody/protein secretion with protocols for vector-free intracellular delivery of fluorophore-labeled probes targting AID and other proteins while maintaining viability and functionality [3]. Next in this project, we aim to apply these assays to study B cells from a murine pancreatic tumour model to evaluate the quality and quantity of the functionality of B cells and the impact of level and change in their functions on tumour progression and response specifically. Because secretion analysis allows for a much more precise functional classification of secreting and co-secreting subpopulations of B cells eliciting signals within their microenvironment, droplet microfluidics coupled with single-cell analysis and intracellular staining possesses a vast potential to disentangle the function of B cells and their products.

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SURVIVAL OF MEMORY T CELLS CIRCULATING IN BLOOD

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The molecular basis of how human immunological memory is maintained in the apparent absence of antigen is not yet completely understood. To address this fundamental question, we interrogate signal transduction pathways potentially regulating the survival of primary human memory T cells (Tm) circulating in blood or residing in bone marrow. Memory T cells are cultured *ex vivo* in the presence of autologous serum. Autologous serum supports survival of circulating Tm. This enhanced survival could be inhibited with FTY720, a drug blocking sphingosine-1-phosphate receptors (S1PRs), suggesting that S1PRs and its ligand sphingosine-1-phosphate, are relevant for the survival of circulating Tm. PI3K signaling, a critical signal for bone marrow resident Tm (see poster of L. Heiberger), is not crucial for survival of circulating Tm, it is not impaired by PI3K inhibitors. However, AKT may be involved. Apparently the lifestyle of Tm residing in bone marrow and those circulating in blood differs fundamentally. Clues on candidate genes involved come from comparing the *ex vivo* transcriptomes of resident and circulating Tm.

INTERSTITIAL SYNOVIAL MACROPHAGES: LOCATION DEFINES FUNCTION

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Synovial macrophages are joint tissue-resident immune cells contributing to its function both in steady-state and inflammation. Recent single-cell RNA sequencing revealed their substantial heterogeneity in both mice and humans [1,2]. Interestingly, a novel population marked by an increased expression of a transcription factor Crip2 was identified in the murine synovium [1]. This population did not have a previously defined function in the literature and our gene ontology re-analysis of the published scRNAseq dataset revealed enrichment in genes from homeostatic and metabolic pathways. Based on our trajectory inference analysis, this population represents a transitionary phase of cells developing into lining macrophages.

Utilising confocal microscopy and flow cytometry, we found that these macrophages are not largely derived from adult haematopoiesis and that their numbers change in inflammation and ageing. Interestingly, they occupy a restricted niche in the synovium in the interior of the joint, close to the menisci, indicative of a specialised function. We propose that these cells represent a mechano-osmotically active subset of tissue-resident cells capable of replenishing other populations of synovial macrophages. We envisage that a better understanding of the maintenance of synovial macrophages will reveal novel therapeutic strategies for joint inflammatory diseases.

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MODE OF ACTION OF NANOPARTICLES AS CARRIERS FOR BACTERIAL GLYCOCONJUGATE VACCINES

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Protein nanoparticles (NPs) are promising platforms for vaccine antigen presentation. Indeed, NPs show an enhanced lymphatic trafficking, an increased capture in lymph nodes by antigen-presenting cells (APCs) and improved cross-linking of antigen receptors on specific B cells [1,2]. However, the impact of physicochemical properties of these carriers on glycan-specific immunity is still poorly understood.

To dissect the glyco-NPs immune response, we have selected NPs with different shape, size, and valency. These constructs have been expressed in *E.coli* and conjugated to short oligo- and long polysaccharide of Group B *streptococcus* type II capsular saccharide.

Moreover, human peripheral blood mononuclear cells were stimulated by NPs naked or decorated with GBSII saccharides to determine the role of the glycan moiety on APCs uptake and activation. Flow cytometry results demonstrated that decorated NPs are less internalized by classical APCs and B cells than naked one. However, analysis of culture supernatant showed production of pro-inflammatory cytokines without any difference between naked and glyco-NPs stimulation.

Next, mice will be immunized with the set of glyco-NPs to evaluate the antigen-specific immune response.

In conclusion, *in vivo* and *in vitro* results could provide new insights into NPs cellular uptake allowing a better design of future glycoconjugate vaccines.

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NEUTROPHIL-MEDIATED TUMOR CELL KILLING INDUCES UPTAKE OF ANTIGENS AND DENDRITIC CELL MATURATION

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Immunotherapy is a promising strategy for cancer treatment. Unfortunately, many tumors have an immunosuppressive microenvironment, precluding the induction of long-term adaptive immune responses. This immunosuppressive tumor environment can be infiltrated with various immune cells secreting anti-inflammatory mediators thereby preventing tumor cell killing by other immune cells. Recently, we developed a bi-specific antibody called TrisomAb, consisting of three elements including tumor-associated antigens (tumor cell binding) and FcαRI (neutrophil binding) with a functional IgG Fc tail (NK cells and macrophages binding). Therefore, we want to investigate if adaptive immune responses can be induced using TrisomAb. Co-culture of tumor cells with dendritic cells, neutrophils and TrisomAb resulted in neutrophil activation, thereby release of tumor antigens by neutrophils and enhanced antigen uptake by dendritic cells. Uptake of tumor antigens by dendritic cells led to maturation as well as secretion of pro-inflammatory cytokines that are involved in induction of T cell responses. Taken together, neutrophil-mediated killing via TrisomAb leads to tumor cell antigen uptake by dendritic cells. Moreover, tumor antigen uptake by dendritic cells results in activation and subsequent secretion of factors that could lead to induction of adaptive immune responses after treatment with TrisomAb.

RNA-BASED *IN VIVO* EXPRESSION OF MONOCLONAL ANTIBODIES

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Monoclonal antibodies (mAb) have been in use for decades as therapeutic agents in medicine. However, development of recombinant mAbs for clinical application is costly and time-consuming and the worldwide production capacities are limited. Here, we aim to develop an approach that is based on the *in vivo* expression of mAbs encoded by *in vitro* transcribed (IVT) mRNA. Clinically, such an approach has the advantage of applying mAbs in a cost- and labor-effective manner, potentially for a prolonged period. Additionally, it is well suited for rapid response to pandemic situations where mAbs would be useful. In this project, we aim to study different liposomal formulations delivering mRNA encoding antibody *in vitro* and *in vivo* against SARS-CoV-2. Two mRNAs encoding the light and heavy chains of the potent SARS-CoV-2 neutralizing antibody E04L were successfully transcribed *in vitro* with high quality and purity. E04L antibody targets the receptor-binding domain (RBD) of spike (S) protein and has potent neutralizing activity against variants of concern, including Omicron. In addition, mRNA encoding GFP and gaussian luciferase (gLuc) were also transcribed as internal controls to check the transfection efficiency and secretion efficiency, respectively. IVT mRNAs showed efficient transfection in *in vitro* settings with high cell viability. Furthermore, IVT mRNA encoding the light and heavy chain of the antibody E04L led to promising expression levels upon delivery to mammalian cell lines. In addition to conventional antibodies, innovative antibody formats such as bispecific and single-chain antibodies will be expressed and evaluated in *in vivo* studies.

We would create and optimize a novel mAb delivery platform technology, in which IVT mRNA encodes the antibody. Our approach would reduce the costs and development times for antiviral antibody treatments to be better prepared for future pandemics.

STUDYING BRAIN MACROPHAGES POPULATIONS IN THE MOUSE

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Emerging evidence shows that tissue macrophages not only provide host defense against pathogens, but also critically contribute to development and the maintenance of homeostasis. Respective distinct tissue niches imprint macrophage heterogeneity which is lost when the cells are taken into culture. The study of functions of specific tissue macrophages hence warrants animal models that allow to investigate these cells in their physiological context.

The macrophage populations of the central nervous system (CNS) comprise parenchymal microglia and various border-associated macrophages (BAM). Here, we discuss our recent efforts to dissect functions of these cells by using newly established binary transgenic splitCre models [1], Specifically, we employ imaging, RiboTag-based translatome profiling and mutagenesis by Crispr/Cas9 manipulation in combination with AAV based strategies.

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THE ROLE OF GUT BACTERIA MEDIATED METABOLITES IN ANTI-VIRAL IMMUNE RESPONSES

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Metabolites and metabolic pathways play crucial roles in regulating immune cell function, as shown by the thriving field of immunometabolism. Understanding immune cell metabolism and its influence on immune regulation is essential to prevent and combat viral infections. The gut microbiome provides numerous metabolites to the host and has a vast impact on the regulation of immune responses in a variety of diseases. Some of these metabolites like short chain fatty acids (SCFAs) and bile acids and their impact on the hosts immune system have been well studied. Nevertheless, the impact of other gut microbial derived metabolites remains less well studied in the context of viral infections.

Our aim is to decipher the impact of less well studied and novel gut microbial derived metabolites in anti-viral immunity. For this, we are using an interdisciplinary approach, combining analysis of systemic metabolite changes and gut bacterial composition during anti-viral infection with high-throughput image-based in vitro screens and flow cytometry-based readouts and well-established perturbation mouse models.

UNCOVERING THE ORIGINS OF EMBRYONIC HEMATOPOIESIS

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The emergence of blood cells during mammalian embryonic development occurs in three independent waves. Yolk-sac (YS) derived hematopoiesis, providing the embryo with primitive erythrocytes (EryP, wave 1) and erythro-myeloid progenitors (EMPs, wave 2), is essential for embryonic development and early life. Of note, hematopoietic stem cells (HSCs, wave 3), dispensable for embryogenesis, emerge later in the embryo proper and supply blood cells during adulthood.

As hematopoietic cells of different waves share a lot in common, there is still a lack of genetic models that would enable tracing and genetic manipulation of YS-derived hematopoietic waves. While EMPs and HSCs are determined to originate from the hemogenic endothelium, EryPs allegedly arise directly from the mesoderm. Also, the existence of primitive macrophages (MF) and megakaryocytes derived from the first wave was described but remains controversial.

The goal of this project is to reveal the molecular mechanism behind specification of YS derived hematopoietic lineages. Using scRNA-Seq approach combined with flow cytometry and development of novel lineage-tracing mouse models, we aim to reveal the origin of EryPs and the first embryonic myeloid cells. Based on analysis of scRNA-Seq data of YS-derived progenitors, we suggest, that the first MFs derive from the EMP wave and that EryPs emerge from hemato-endothelial cells.

INVESTIGATING TERTIARY LYMPHOID STRUCTURES IN SOLID TUMORS USING SPATIAL TRANSCRIPTOMICS

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Immune cell infiltration plays a major role in progression of solid tumors and susceptibility to cancer immunotherapy. However, the bare abundance of infiltrating immune cells does not provide a sufficient explanation for the heterogeneity of anti-tumour immune responses. In fact, it has become more and more clear that the spatial organization of immune infiltrates within a tumor are crucial for tumor stratification.
The spatial architecture of immune infiltrates in solid tumors ranges from simple cell accumulations to highly organized cellular communities within tertiary lymphoid structures (TLS). In this project, we use spatial transcriptomics of different solid tumors to gain an unbiased overview of tissue organization and immune cell infiltration in combination with gene expression. We use cell-type specific transcriptional deconvolution and differential gene expression analysis to quantify the TLS cellular compositions and differences in tissue specific gene expression, respectively. When combining the spatial characteristics of immune infiltrates with clinical data, we are able to determine how it correlates to overall patient survival. Our aim is to identify molecular mechanisms that drive the heterogeneity of immune responses within the TLS and thereby pave ways for developing new immunotherapies.

ANALYSIS OF THE DYNAMICS AND TRAFFICKING OF MGBPS TO MEMBRANES IN PATHOGEN INFECTED CELLS

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Murine Guanylate-binding proteins (mGBPs) belong to the superfamily of dynamin-like proteins featuring an important role in the cell-autonomous immunity against intracellular pathogens. For example, mGBP2 and mGBP7 are essential effector proteins in the control of *Toxoplasma gondii* (*T. gondii*) replication. *T. gondii* is an apicomplexan parasite, which replicates within the parasitophorous vacuole (PV) in the host cell. MGBPs are induced by Interferon-γ (IFN-γ) and accumulate at the PV membrane (PVM) of *T. gondii* leading to its disruption or permeabilization. Eventually, the plasma membrane of *T. gondii* is targeted as well. However, the molecular processes behind these observations are yet to be understood. To this aim, we make use of the giant unilamellar vesicles (GUVs) technology in order to investigate the interactions of mGBPs with membranes of different compositions and to unravel the mode and consequences of mGBP-lipid binding. Additionally, the role and function of Interferon-stimulated gene product 15 (ISG15) in *T. gondii* infection is in the focus of our studies, as it plays an essential role in the host response against pathogenic stimuli. ISG15 is a member of the Ubiquitin-like protein family and targets proteins in a three-step enzymatic process called ISGylation. We aim at understanding the role of post-translational ISGylation on the outcome of *T. gondii* infection.

DISCOVERY OF A NEW CD70+ CD27- PLASMABLAST POPULATION IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND HEALTHY INDIVIDUALS

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The CD70 molecule is a ligand of the CD27 receptor and is expressed upon activation on the cell surface of B cells, T cells and DCs. Here, we describe a novel plasmablast (PB) subset expressing CD70, but lacking CD27. This finding was unexpected as human PB are usually characterized by high expression of CD27. Therefore, we aimed to investigate this new subset in detail.

We identified the CD70+CD27- subset using spectral flow cytometry as it clustered together with conventional PB. We confirmed that CD70+CD27- PB are bona-fide antibody-secreting cells by ELISPOT. The cells produced comparable numbers of spots as conventional PB. Furthermore, CD70+CD27- PB expressed transcription factors IRF4, PRDM1 and XPB1, indicating PB identity. Within plasma cells, protein expression of CD27 inversely correlated to CD70, a pattern that was also observed following in vitro B cell activation. The CD70+CD27 PB population was present in both healthy and Systemic lupus erythematosus (SLE) patients, and contained autoreactive cells in SLE patients.

In conclusion, these experiments indicate that our newly identified CD70+CD27- subset has a PB-like phenotype and is able to produce antibodies. This population is not included in current gating strategies for PCs. More research is needed to identify the function of this subset, its cellular precursors and its potential role in autoimmunity.

HIGH-THROUGHPUT SCREENING OF SMALL MOLECULAR COMPOUNDS TARGETING FOXP3 IDENTIFIES MAPK AND STAT3 INHIBITORS AS REGULATORY T CELL INHIBITORS

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Regulatory T cells (Tregs) have a central role in suppressing antitumor immunity and promoting tumor progression, and the Treg linage-defining transcription factor FoxP3 is key to maintaining the Treg suppressive functions. Thus, targeting FoxP3 expression in Tregs could be a promising anti-tumor approach. We have developed a unique, phenotypic, cell-based, high-throughput flow cytometry assay for screening a library of 1522 approved drugs to identify drugs targeting FoxP3 expression. Here we found drug candidates that downregulate FoxP3 expression in Tregs, show low T cell toxicity, downregulate expression of suppressive Treg markers like LAG-3, PD-1 and ICOS, and inhibit Treg suppressive functions; these drugs converge at inhibiting MAPK and STAT3 signaling. To further characterize these drugs and determine their structure-activity relationship, we built new sub-libraries and searched for analog compound structures by *in silico* prediction. We found that several of these drugs also inhibit Treg suppressive functions in a manner similar to- or better than the original compound. Although more studies are needed, these drug candidates could serve as probes for studying Treg functions, which could expand the repertoire of anti-tumor therapies. The high-throughput assay will also enable further discovery of drugs regulating Treg functions.

IDENTIFICATION OF ΓΔ T CELLS FROM SINGLE-CELL RNA SEQUENCING DATA USING AGGREGATED TCR GENE EXPRESSION ANALYSIS

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Gamma delta (γδ) T lymphocytes are a distinct subset of T cells that express a unique γδ T cell receptor (TCR) heterodimer. However, due to the low expression of TCR genes and the overlap in phenotype with some αβ T cells, accurately identifying γδ T cells from single-cell RNA sequencing (sc-RNAseq) datasets without sc-γδTCRseq or CITE-seq data can be challenging. To address this issue, we developed an aggregated TCR gene expression analysis method that calculates gene module scores for TCR-α/β and TCR-δ genes. We tested our method on reference 5' sc-RNAseq datasets that underwent both sc-αβTCRseq and sc-γδTCRseq, and found that it accurately identified γδ T cells with high sensitivity. Our analysis method also demonstrated stable performance across different tissue datasets and different subtypes of γδ T cells. Thus, we propose that the aggregated TCR gene expression analysis method be adopted as a standardized tool for identifying γδ T cells from 5' sc-RNAseq datasets. This will enable a better understanding of the precise roles of γδ T cells in various physiological and pathological processes.

REGULATORS OF CD8 T CELL FATE DETERMINATION IN CHRONIC LCMV INFECTION

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Antigen-specific CD8 T cells are driven to a state of exhaustion in the context of continuous antigen stimulation, such as chronic viral infections and cancers. Several subsets of exhausted CD8 T cells are described, which differ in their proliferative capacity, effector functions, expression of exhaustion markers and their transcriptomic, epigenetic and metabolic profile. However, only little is known about the molecular regulation of CD8 T cell differentiation towards these different cell subsets. To identify such potential regulators of CD8 T cell fate determination in chronic infections, we set up an *in vivo* CRISPR/Cas9-targeted screening approach. In principle, genes of interest, derived from previous studies of mRNA signatures of exhausted CD8 T cells, are disrupted by CRISPR/Cas9-targeting in primary murine CD8 T cells. The differentiation of these CD8 T cells containing single gene disruptions is then analysed in recipient mice chronically infected with Lymphocytic Choriomeningitis Virus (LCMV). With this approach we could knock-out the inhibitory receptor PD-1 in primary murine CD8 T cells and retrace alterations in differentiation by comparing their distribution in different exhausted cell subsets to control CD8 T cells. We will now use this screening system to reveal potential regulators of CD8 T cell fate determination in chronic LCMV infection.

EFFECT OF NON-ISOSTERIC ISOPOLAR PHOSPHONATE MODIFICATIONS OF CYTIDINE IN CPG MOTIVES ON THE ACTIVITY OF SELECTED TLR9 LIGANDS

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TLR9 can recognize nonmethylated bacterial DNA or synthetic oligodeoxynucleotides (ODN) with nonmethylated CpG sequences as the “danger signal”. For our study we have selected well-characterized human type B ODN 2006 which was applied in multiple clinical trials. We synthesized more than fifty different non-isosteric isopolar phosphonate analogues of cytidine in CpG motives of type B human ODN 2006. We have determined activity of the analogues using NF-κB/AP-1 reporter cell lines or in PBMC stimulatory assay. Most of the modifications abrogated the activity of the parental ODN. But interestingly, analogues #1 and #2 were superior in stimulation of NF-κB signaling pathway and especially (R) enantiomer #2 was potent stimulator ($EC50_{ODN2006}$=0.482±0.307μM, $EC50_{\#1}$=0.112±0.074 μM, $EC50_{\#2}$=0.015±0.008 μM). We have tested nuclease stability of two most potent analogues (#1, #2) in human plasma for 20h and both ODN were stable. Surprisingly we have not concluded any increase in activity by incorporation the most potent analogues #1 and #2 into mouse type B (ODN 1826) or human type A ODN 2216. The structure of human TLR9 has not been described but the result of our study contributes to understanding structure-activity relationships of the TLR9 ligands and could lead to design of more potent TLR9 activators.

A TRYPANOSOMA BRUCEI GAMBIENSE SURFACE RECEPTOR FOR HUMAN COMPLEMENT C3 AND C3B AND IT'S ROLE IN THE RESTRICTION OF THE ALTNERATIVE PATHWAY

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African Trypanosomes, the causative agents of human trypanosomiasis, a fatal disease commonly known as African sleeping sickness, have developed elaborate immune-evasion mechanisms to escape the adaptive immune response, many of which have been described in detail. On the contrary, very little is known about evasion of the human complement system, especially during the early stages of the infection.

The human-infective parasite *Trypanosoma brucei gambiense (Tbg)* specifically activates the alternative pathway in presence of human serum and deposits complement C3 on its surface, but the parasites do not succumb to cell lysis.

We have identified a *Tbg* protein that serves as a surface receptor for complement C3 and its proteolytic activation fragments and could demonstrate its role in selectively inhibiting the AP C5 convertase, restricting progression to the terminal pathway and thus preventing cell lysis. Based on the cryoEM structures of the *Tbg* surface receptor complexed both with native C3 and C3b, we propose a new model for receptor-ligand interactions as they may occur at the plasma membrane of blood-stage trypanosomes and likely facility innate immune escape of the parasite.

IMMUNOPHAGE: TARGETING RECURRENT URINARY TRACT INFECTIONS WITH GENETICALLY ENGINEERED IMMUNO-MODULATORY BACTERIOPHAGES

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Urinary tract infections (UTIs) are one of the leading causes for antibiotic prescription in primary healthcare. Recurrent UTIs affect up to 50% of female UTI patients, and current understanding suggests inadequate immune response to bladder pathogens is a contributing factor. Antibiotics are often ineffective at controlling recurrent infections as pathogenic communities can maintain themselves within the gut or intracellularly within the bladder epithelium ready to cause a relapse once treatment is terminated.

ImmunoPhage is a novel therapy that exploits the inherent bacteriolytic properties of bacteriophages (phages) to produce a unique antimicrobial and immunomodulatory combination therapy. To equip phages with immunomodulatory properties, we introduce heterologous genes, such as cytokines, into bacteriophage genomes. Upon ImmunoPhage infection of a bacterial host in a patient, cytokines are expressed and released alongside bacterial lysis. So far, we have engineered phages with an array of cytokines and have shown that clinical isolates of uropathogenic *E. coli* infected by ImmunoPhage release active payloads within the nanomolar range. To validate the ImmunoPhages, we are using *in vivo* mouse UTI models. The unique approach of ImmunoPhage aims at providing a lasting solution for patients suffering from recurrent UTIs.

T CELL INFILTRATION IN PRIMARY UVEAL MELANOMA CORRELATES WITH GENETIC PROFILE CLASSIFICATION

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Uveal melanoma (UM) is a type of cancer originating from melanocytes in the eye. Infiltration of immune cells is required to establish an effective anti-tumor response after treatment with immunotherapy. However, specifics of immune infiltration in UM are poorly understood.

We collected primary UM tissue from needle biopsies at time of enucleation or plaque placement. Genetic profile was assessed for most patients. PBMCs were isolated from blood at time of biopsy. Flow cytometry and multiplex immunohistochemistry, if available, was performed to study lymphocyte phenotypes.

A total of 41 patients were included in our study between July 2019 and January 2021. High-risk patients showed more T cell infiltration, while also being more CD8 dominated (average CD8/CD4 ratio 2.38 for high-risk and 1.08 for low-risk). Infiltrating T cells had a high expression of CD39, CD69 and PD-1. CD8 and CD4 T cells seem to be similarly spatially clustered, meaning the CD8/CD4 ratio correlates between biopsies and enucleations for many patients.

In many tumors high CD8 infiltration is considered beneficial for treatment response to immunotherapy. Here, we found that infiltration in primary UM is dependent on the genetic profile, with higher CD8/CD4 ratio and infiltration in high-risk patients. The infiltrate might be of value for the development of immunotherapeutic approaches in high-risk patients.

IMPACT OF INTRATUMORAL MICROBIOTA IN SOFT TISSUE SARCOMA TREATMENT EFFICACY

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Tumor resident microbiota is an emerging component of the tumor microenvironment and it is reported as composed of tumor type-specific bacteria which can affect tumor biology as well as play a role in response to therapy. Thus far, the contribution of intratumoral microbiota in the context of soft tissue sarcoma (STS) has been underestimated; therefore, this project is aimed to characterize the STS microbiota and understand if it can impair the outcome of chemotherapeutic regimens. STS are heterogeneous cancers and doxorubicin is the gold standard of chemotherapy, but it is effective for less than 50% of patients. Doxorubicin is naturally produced by *Streptomycetaceae*; hence the hypothesis that certain bacteria developed drug-inactivating mechanisms seems evolutionarily plausible. We performed the microbiome analysis on sarcoma tissues and adjacent healthy tissues showing that sarcomas arbor a distinct microbiome composition. Moreover, we isolate live bacteria from sarcomas to investigate their ability to interfere with chemotherapy. This study offers new insights about the relationship between the intratumoral microbiota and the occurrence of chemoresistance in cancer therapies, suggesting that microbial communities hosted within the tumor should be deeply investigated, and then manipulated, to improve therapeutic outcome.

METABOLISM AND TISSUE CONTEXT DEPENDENT DIFFERENTIATION PROGRAMS OF CD8 T CELL EXHAUSTION

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T cell exhaustion represents a differentiation state of CD8 T cells that develops in the context of sustained antigen exposure both during chronic viral infection and in cancer. This differentiation state is characterized by the upregulation of co-inhibitory receptors, reduced proliferative capacity, and declined production of effector cytokines. However, underlying mechanisms orchestrating T cell exhaustion are still incompletely understood. Importantly, T cell exhaustion represents a major challenge for effective immunotherapies. Recently, tumor infiltrating CD8 T cells were found to acquire a mitochondrial dysfunction phenotype, which was sufficient to drive epigenetic, transcriptomic, and functional alterations promoting T cell exhaustion.

In my project, I will use chronic lymphocytic choriomeningitis (LCMV) infection in mice as a model for chronic antigen exposure. The metabolic programs of virus-specific cells will be identified using SCENITH. In addition, virus-specific cells in different tissues will be profiled to assess tissue context dependent differences. By generating CRISPR/Cas9 single knock-out cells for candidate metabolic checkpoints, that were identified by our collaborators in CRISPR/Cas9 screens, we aim to identify metabolic targets and ultimately to understand metabolic regulations associated with microenvironmental cues that impinge on T cell exhaustion.

B CELL DEPLETION ATTENUATES CD27 SIGNALING OF T HELPER CELLS IN MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS). Traditionally, MS was held to be a T-cell mediated disease but accumulating evidence during the last decade also highlighted the crucial importance of B cells for the disease progression. Particularly, B cell depleting therapies (BCDTs), have demonstrated striking efficacy in suppressing inflammatory disease activity in relapsing-remitting MS. However, a detailed understanding of the role of B cells in the pathogenesis of MS is still lacking, and by extension also the mechanism of action of BCDTs. In this longitudinal multi-center study, we investigated the impact of BCDTs on the immune landscape in MS patients using high-dimensional single-cell immunophenotyping (cytometry by time-of-flight; CyTOF). Algorithm-guided analyses revealed a specific reduction of circulating T follicular helper (Tfh) cells with a concomitant upregulation of CD27 surface expression in memory T helper cells and Tfh cells. These findings indicate a costimulatory mechanism in the CD27/CD70 signaling pathway, through which B cells sustain the activation of pathogenic T cells. Disrupting the CD27/CD70 signaling axis via BCDTs provides a potential explanation for its clinical efficacy.

ASSESSING THE DISTRIBUTION AND FUNCTIONS OF PLASMACYTOID DENDRITIC CELLS IN THE HUMAN INTESTINE DURING HOMEOSTASIS AND INFLAMMATION

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Intestinal immune homeostasis relies on the balance between tolerating commensal microbiota and being able to mount protective immune responses to pathogens. This balance is disturbed in inflammatory bowel diseases (IBD), such as Crohn's disease (CD), where hyperactive immune response leads to uncontrolled inflammation. Plasmacytoid dendritic cells (pDC) are central in antiviral immunity by their rapid capacity to secrete type I interferons, tumor necrosis factor and interleukin-12. However, other roles of pDC are less understood, especially in peripheral tissues such as the intestine. We used our novel method to isolate human gut-associated lymphoid tissues (GALT) and GALT-free lamina propria LP (1, 2) to assess pDC location and transcriptional profile in healthy and CD intestine. We find pDC to be found within GALT, restricted to the T cell zone, in close contact with both CD4+ and CD8+ T cells. Further scRNA-seq analysis revealed pDC to express several factors suited for interactions with T cells, especially genes indicating potential roles in regulating T cell responses. Surprisingly, GALT pDC did not express type I interferon genes in health or CD. In contrast to blood derived pDC, GALT pDC did not express IFNA2 following in vitro stimulation with CpG. To conclude, our preliminary analysis suggests pDC to have more regulatory function in GALT in both health and CD.

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A POPULATION PHARMACOKINETIC MODEL OF NATALIZUMAB GUIDED BY THERAPEUTIC DRUG MONITORING IN MULTIPLE SCLEROSIS (MS) PATIENTS

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Personalized dosing guided by therapeutic drug monitoring (TDM) of treatments have shown to be beneficial. Our aim was personalized dosing of Natalizumab in multiple sclerosis (MS) patients, a humanized therapeutic monoclonal antibody IgG4κ targeting the α4 integrin on lymphocytes, which is key to endothelial cell trafficking into the central nervous system. Long-term treatment of Natalizumab is a known risk-factor of progressive multifocal leukoencephalopathy (PML). Previous research has shown that reducing the exposure of Natalizumab by extending the dosing interval can alleviate the risk of PML. Moreover, dosing interval extension can reduce healthcare expenses and minimizes the disease burden for patients. Currently, an ongoing Dutch multi-center MS study is extending based on trough concentrations and in agreement of both clinician and patient. However, a population pharmacokinetic (pop PK) model was constructed to complement and strengthen decisions of interval extension in this MS study. The pop PK model is trained an validated on data of previous trials MS studies in the Netherlands. Moreover, the final model shows adequate propagation of concentration-time profiles.

ROBUST SARS-COV-2 EPITOPES-SPECIFIC CD8 T CELLS RESPONSES IN MRNA-1273 VACCINATED INFLAMMATORY BOWEL DISEASE PATIENTS TREATED WITH TNFA INHIBITORS

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$CD8^+$ T cells recognize conserved SARS-CoV-2 peptides and ameliorate disease severity especially for novel virus variants. Healthy individuals (HI) generate robust spike (S)-specific antibodies and $CD8^+$ T cell responses upon mRNA vaccination. Whether autoimmune inflammatory bowel disease (IBD) patients treated with immunosuppressive anti-TNFα generate similar robust immunity upon SARS-CoV-2 vaccination is unknown. We compare 31 SARS-CoV-2-specific $CD8^+$ T cell populations, across 10 common HLAs using heterotetramer combinatorial coding, before and 10 days after second mRNA-1273 vaccination between SARS-CoV-2 (un)experienced anti-TNFα treated IBD patients and untreated and HIs. In addition, phenotypic and activation markers allowed in-depth *ex vivo* profiling of the SARS-CoV-2-specific $CD8^+$ T cell response. The vaccination induced robust S-specific $CD8^+$ T cells with a memory phenotype in both (un)treated IBD patients and HI. Robust S- and non-spike (NS)-specific $CD8^+$ T cell responses were observed in SARS-CoV-2 experienced donors prior to vaccination. While the frequencies of NS-specific $CD8^+$ T cells persisted, a more prominent S-specific response was identified after vaccination. These results indicate that treatment and previous SARS-CoV-2 infection did not affect the ability to mount a robust S-specific $CD8^+$ T cell response, indicating that IBD patients benefit from vaccination.

VISUALIZING COMPLEMENT ACTIVATION AND REGULATION IN THE TUMOR MICROENVIRONMENT

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Composition of the tumor microenvironment (TME) influences tumor progression. Complement activation in the TME has been reported to either lead to an anti- or pro-tumor response. These opposing effects of complement in the TME seem to be context and cancer type dependent. To better understand these opposing effects, we aim to determine where in the TME complement activation and regulation occurs.

Activation of the complement system results in the deposition on cell surfaces of complement protein C3 in its activated form C3b. C3b may then be regulated and broken down to its inactive fragments iC3b and C3dg. Thus determining the presence of C3b, iC3b and C3dg within the TME indicates sites of complement activation and regulation.

In order to distinguish the different C3 fragments deposited within tissue samples, well characterized anti-C3 antibodies (Abs) with known specificity for C3b, iC3b and C3dg are needed. Therefore a new anti-C3 Ab screening tool was developed using C3b, iC3b and C3dg linked to biotin, allowing physiological presentation of each fragment. Subsequently, biotinylated C3 fragments were bound to streptavidin coated surfaces to screen anti-C3 Abs for their selectivity and affinity.

This set-up enables us to identify specific Abs against different C3 fragments, which will help to shed light on activation and tissue regulation of the complement system in the TME.

IN RHEUMATOID ARTHRITIS, IGA AUTOANTIBODIES ARE PRESENT IN A POLYMERIC STATE; CLUES FOR A MUCOSAL ORIGIN OF THE AUTOIMMUNE RESPONSE?

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Rheumatoid arthritis (RA) is a autoimmune disease characterized by autoantibodies against post translational modifications (i.e. citrullination, acetylation and carbamylation), together these are called anti-modified protein antibodies (AMPA). What initially leads to the breach in tolerance is still unknown, but there are indications that this occurs in the intestines. Since antibody responses in the mucosa are dominated by IgA, we set out to characterize the AMPA IgA response. Sera from AMPA-positive RA (N=12), AMPA-negative RA (N=3) and healthy donors (N=3) was fractionated using size exclusion chromatography (SEC). Next, all fractions were tested by ELISA for AMPA IgA, and the total Ig profile. In addition, AMPA IgA was purified from serum to determine their size by western blot and to analyze the tailpiece by tandem mass spectrometry (MS/MS). Results show that AMPA IgA is mainly present in polymeric form, ~70%, while total IgA contains only ~20% , this was also confirmed by western blot. MS/MS data showed differences in the AMPA IgA tailpiece, in the dimeric fraction ~60% had the whole tailpiece, in the monomeric fraction this was only ~20%. This suggests that AMPA IgA responses are differently regulated since polymeric IgA only makes up a small proportion of serum IgA and is more frequently present in mucosal secretions, therefore they are potentially of mucosal origin.

AUTOANTIBODIES FROM SYSTEMIC SCLEROSIS PATIENTS AFFECT THE ANGIOTENSIN II TYPE 1 AND ENDOTHELIN-1 TYPE A RECEPTOR THEREBY INDUCING ENDOTHELIAL CELL ACTIVATION AND PRO-FIBROTIC RESPONSES

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Systemic sclerosis (SSc), a severe and heterogenous autoimmune disease, is hallmarked by dysregulated immunity, vasculopathy, and fibrosis. Autoantibodies (aAbs) directed against the angiotensin II type 1 receptor (AT1R) and endothelin-1 type A receptor (ETAR) might be associated with more severe disease complications. Therefore, the AT1R- and ETAR-mediated effects of patients or healthy controls (HCs) IgG on endothelial cells (EC) were assessed using ELISA and RT-qPCR followed by correlation to serum AT1R and ETAR aAb levels. SSc IgG induced upregulation of EC activation markers MCP-1, ICAM-1, and E-selectin compared to HC IgG which seemed to be in an AT1R- and ETAR-specific manner. Moreover, SSc IgG induced AT1R- and ETAR-mediated expression of IL-6, IL-8 and TGF-β whereas this induction was not observed for HC IgG. Although higher levels of both aAbs were associated with stronger EC responses, EC activation was not observed in all patients with AT1R and ETAR aAb. In conclusion, AT1R- and ETAR-targeting aAbs impact ECs resulted in EC activation, and pro-inflammatory and pro-fibrotic cytokine release.

MYELOID HIF-2A REGULATION OF TUMOUR PROGRESSION

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Macrophages are known to be a key element of the tumor microenvironment (TME) that modulate tumor progression, shaping anti-tumor immune responses and influencing therapeutic outcomes. However, macrophages are a highly heterogeneous population with either an embryonic origin (tissue resident macrophages, TRM) or a bone-marrow derived origin (BMDM). While TRM are highly specialised with distinctive organ-specific functions and self-renewing capacity, BMDM differentiate from blood-circulating monocytes under inflammatory circumstances. Both have shown to regulate tumour progression either promoting anti-tumor immunity or restraining it. In addition, other features of the TME, including limited oxygen availability (hypoxia) also modulate the anti-tumor capacity of macrophages. Transcriptional response to hypoxia is regulated by Hypoxia inducible factors (HIFs) and although the role Hif-1α is well documented, to date, the contribution of Hif-2α remains largely unexplored. Thus, to study the role of myeloid Hif-2α, in the regulation of anti-tumor immune responses in TRM and BMBM, we used a myeloid lineage specific knockout mice (LysM-cre x *Epas1*$^{fl/fl}$). We observed that mice with Hif-2α depleted macrophages showed increased tumour growth and altered immune infiltrating populations indicating the relevant role of Hif-2α in the regulation of anti-tumour immunity by myeloid cells.

A NOVEL FHR-2 HOMODIMER ASSAY SHOWS HIGHER ABUNDANCE IN BLOOD PLASMA

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Factor H-related protein 2 (FHR-2) is the smallest and most elusive member of the factor H (FH) protein family. Like other FHRs, FHR-2 is thought to function as de-regulator of the complement system by acting as an antagonist of complement regulator FH. It mainly seems to circulate as heterodimer with the more abundant FHR-1, with whom it shares a high similarity in sequence. FHR-2/2 homodimers are also present, but quantification is greatly hampered by the presence of FHR-1/2 heterodimers and the lack of sufficiently sensitive and specific reagents. Consequently, FHR-2/2 levels have thus far only been calculated based on FHR-1/1 and FHR-1/2, assuming a dimer equilibrium that solely depends on the total FHR-1 and FHR-2 levels, and unbiased formation through free exchange of dimers. However, with theoretical FHR-2 levels being associated with diseases such as age-related macular degeneration and non-small lung cancer, exact measurement of FHR-2/2 is needed. Here, we describe newly generated FHR-2 specific mouse anti-human antibodies and the development of a novel FHR-2/2 homodimer assay. Surprisingly, first results suggested a higher abundance of FHR-2/2 in blood plasma than expected. Use of this novel assay allows us to directly measure FHR-2, revisit the reported associations, and further elucidate the role of FHR-2 and its homo -and heterodimers within the complement system.

COMPARISON OF IMMUNE VARIABILITY BETWEEN HEALTHY AFRICAN AND EUROPEAN ADULTS REVEALS BROAD SIMILARITIES AND SPECIFIC DIFFERENCES

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Recent immuno-modulating therapies and their application to a range of pathologies is a major advance. Responses to these treatments vary between patients highlighting the need to better understand immune response variability. This is particularly striking regarding low resources countries, as most immunological studies have focused on western populations. To address this, we compared the MI cohort, consisting of 1000 healthy French donors and the HGGP cohort, consisting of 48 healthy Senegalese donors. These two populations are subjected to different environmental and pathogen exposures with different genetic backgrounds, lifestyles and diets. We characterized their baseline immune cells using flow cytometry, their functional immune responses using stimulated whole blood with stimulus covering bacterial, viral, and T cell immunity. 13 cytokines were measured in the supernatants, and cell pellet was assessed by Nanostring arrays. We also assessed fecal and nasal microbiome populations. Our results show comparable cellular population and cytokine levels in both baseline and stimulated phenotypes. Notable exceptions were observed for certain phenotypes, *ie* Senegalese donors had significantly lower naïve CD4 T cells, higher IL-8 in Null and LPS conditions. The microbiome composition in fecal and nasal samples was significantly different, and much diverse in the Senegalese donors.

DEVELOPMENT OF A VERSATILE CANCER VACCINE FORMAT WITH PROXIMITY-BASED SORTASE A MEDIATED LIGATION

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Immunotherapy response is correlated with the presence of activated immune cells in the tumor. Cancer vaccines can be utilized to increase tumor-specific immune responses in non-responding patients and should contain tumor-specific antigens. Our aim is to develop a versatile cancer vaccine format in which patient-specific tumor antigens can be site specifically conjugated by a proximity-based sortase A mediated ligation (PBSL) approach to antibodies (Abs) targeting antigen presenting cells to stimulate immune responses.

We used the CRISPR/HDR platform to produce mouse heavy chain IgG2a Abs with a sortase A recognition motif followed by a SpyTag. The engineered DEC205, CD169-specific Abs and isotype control IgG2a Ab were produced and the binding functionality was confirmed. In addition, we produced recombinant protein that consists of Sortase A linked to a SpyCatcher protein. PBSL was used to successfully ligate FITC label to the Abs. FITC ligated Abs efficiently stained DEC205-expressing dendritic cells and CD169-expressing macrophages *in vitro* and *in vivo*. In future studies we will ligate an ovalbumin peptide or melanoma antigen to the Abs and test the induction of T cell responses *in vivo*. We expect that the PBSL technic will enable fast and efficient production of patient-specific cancer vaccines.

TGF-ß1 ACTIVATING ANTIBODIES AS A TREATMENT FOR GRAFT-VERSUS-HOST DISEASE

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Graft-versus-host disease (GVHD) is a life-threatening complication of allogeneic hematopoietic cell transplantation which is a potent immunotherapy for hematological disorders. GVHD occurs when donor T cells trigger a strong alloreactive immune response against mismatched recipient cells. TGF-ß1 is a potent immunosuppressive cytokine produced by virtually all cell types in a latent, inactive form. Only a few cell types activate latent TGF-ß1 upon specific stimuli. Within the immune system, Tregs activate latent TGF-β1 through a mechanism involving transmembrane protein GARP. We derived monoclonal antibodies (mAbs) that activate human or mouse latent TGF-ß1. Here, we show that the administration of mAbs reduces severity and increases survival in mice with acute GVHD. The therapeutic effect correlates with increased Treg numbers and decreases Th1 responses in spleen and liver, while it does not impair donor cell engraftment. The increased number of Tregs might reflect the differentiation of naïve CD4+ cells into Tregs in the periphery. The beneficial effect of the mAbs was lost in absence of Tregs. We are currently studying the potential therapeutic effect of the mAbs on chronic GVHD and on graft-versus-leukemia effect.

FERROPTOSIS RELATED PROTECTIVE MECHANISMS ENSURE METABOLIC ADAPTION OF PATHOGENIC GROUP 2 INNATE LYMPHOID CELLS IN AIRWAY INFLAMMATION

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Innate lymphoid cells (ILC) serve the major task of protection and maintenance of the tissue barrier. However, chronic activation of ILC2s can promote inflammation and contribute to the development of asthma in the lung. This process is fueled by the acquisition of external lipids, which may render ILC2s susceptible to ferroptosis, a form of cell death caused by the accumulation of lipid peroxidation. We found that pathogenic ILC2s in allergic airway inflammation reside in a lipid rich environment and increase the incorporation of poly unsaturated fatty acids into their membrane thus increasing their susceptibility to lipid peroxidation. At the same time, we observed that pathogenic ILC2s show lower abundance of cellular reactive oxygen species (ROS) and lipid peroxidation, indicating that pathogenic ILC2s are protected from ferroptosis. Further investigation revealed that pathogenic ILC2s change their expression profile in response to allergen-induced airway inflammation. Anti-oxidant systems important for balancing excessive ROS as well as anti-ferroptotic systems that directly counteract lipid peroxidation are highly expressed in pathogenic ILC2s. Finally, we found that up-regulation of anti-oxidant and anti-ferroptotic systems is enabling pathogenic ILC2 to acquire high amounts of external lipids fueling the function of ILC2 and the progression of airway inflammation.

TRANSCRIPTIONAL REWIRING OF MACROPHAGES DURING MYCOBACTERIAL GRANULOMA FORMATION

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The mycobacterial granuloma is the defining pathological feature of pulmonary tuberculosis. Granulomas are highly organized host immune cell structures that encapsulate the invading pathogen, *Mycobacterium tuberculosis* at its core. Macrophages are pivotal in the formation of granuloma, making up the majority of the cell population. During this process, the typically motile macrophage undergoes a substantial behavioural reprogramming termed epithelioid differentiation. The resulting epithelioid macrophages upregulate adherence junction molecules including E-cadherin, allowing them to form interdigitations with neighbouring cells to become the central scaffold of the granuloma structure. This upregulation of E-cadherin was shown to be dependent on IL-4/ Stat6 signalling pathway and critical for granuloma formation and mycobacterial pathogenesis. The transcriptional reprogramming that underlies IL-4/Stat6 signalling in E-cadherin induction during granuloma organization is not known but represents a potential target for therapeutic intervention. Hence, we aim to understand this IL-4/Stat6/E-cadherin transcriptional regulation using *in vitro* conditionally immortalized Hoxb8 macrophages and *in vivo* zebrafish-*Mycobacterium marinum* model.

NOVEL IMMUNOTHERAPY APPROACH TO MYELOID MALIGNANCIES BY DUAL-TARGETING OF MYELOID CHECKPOINT RECEPTOR CLEVER-1

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The treatment options remain few for acute myeloid leukemia (AML), and for myelodysplastic syndrome (MDS), even fewer. The potential of additive immunotherapies in AML and MDS remains still under investigation. Clever-1 (also known as Stabilin-1) is a multifunctional scavenger and adhesion receptor expressed by monocytes and immunosuppressive macrophages. Bexmarilimab, a Clever-1 targeting antibody, demonstrates many immunomodulatory effects along with promising anti-tumor activity against solid tumors. In AML, high STAB1 (mRNA) levels associate with poor survival and resistance to therapy.

This project is the first comprehensive investigation of Clever-1 expression in AML and MDS patient BM blasts and monocytes. Our results confirmed Clever-1 protein expression in all AML types and bexmarilimab treatment induced metabolic and growth inhibition of KG1 and TF1 blasts (Clever-1^{high}). Ex vivo treatment of the primary AML BM cells with bexmarilimab, alone or in combination with azacytidine or venetoclax, indicate significantly enhanced antigen presentation capability and immunological activation. These results validate the therapeutic potential of bexmarilimab in myeloid malignancies. The safety, tolerability and preliminary efficacy of bexmarilimab is now further investigated in combination with venetoclax and/or azacytidine in a phase I/II clinical trial BEXMAB (NCT05428969).

[1]Viitala et al., Clin Cancer Res 2019;25:3289-303;[2]Bono et al., Annals of Oncology; 2021. p 32 (suppl_5): S1283-S346;[3]Lin et al., Mol Ther Nucleic Acids 2019;18:476-84

IDENTIFICATION OF DISTINCT IMMUNE LANDSCAPES OF INFILTRATING T CELLS IN COLON CANCER USING MULTIPLEX IMMUNOFLUORESCENCE STAINING

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The immune system recognizes foreign microorganisms as ”non-self” and reacts to destroy these disease-causing agents, playing a similar role in protecting the body from malignancies. The spatial distribution of T cell subsets in tumor tissues, like colon cancer, may provide information on the role of the immune system in tumor development.

We simultaneously assessed CD3, CD8, Foxp3, Ki67, Granzyme B and pan cytokeratin in ninety colon cancer cases using a tyramide signal amplification based mIF approach. Moreover, in a previous study[1], next-generation sequencing was performed on the same samples, resulting in consensus clustering based on the immunologic constant of rejection (ICR) genes, segregating colon cancer patients in three different groups: ICR low, medium and high.

All T cell subtypes were more prevalent in the stromal fraction than in the epithelial fraction, but the proportion of Ki-67+ or Granzyme B+ T cells was significantly higher in the tumor epithelium than in the tumor stroma. In both tumor epithelium and tumor stroma, T cell densities were significantly higher in those with high ICR than in those with low ICR. Meanwhile, the median distance between immune cells and epithelial cells was significantly smaller in ICR-high than in ICR-low. Interestingly, patients with an ICR high/Th cells high experienced improved overall survival ($p = 0.016$).

[1] Roelands J, Decock J, Boughorbel S, et al. A collection of annotated and harmonized human breast cancer transcriptome datasets, including immunologic classification. F1000Research. 2017; 6 :296.

DEIMMUNIZATION OF PEPTIDOGLYCAN HYDROLASES FOR THERAPEUTIC TREATMENT OF SYSTEMIC *S. AUREUS* INFECTIONS

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Staphylococcus aureus is an opportunistic pathogen colonizing roughly 30% of the human population, causing a wide range of diseases. Due to the emergence of resistant strains, novel antimicrobials are of high interest. Phage-derived peptidoglycan hydrolases (PGH) could be used as a treatment against drug-resistant bacterial strains. Fast lysis, high specificity and activity against drug-resistant bacteria are just a few advantages for the use of PGHs as protein therapeutics. A major drawback of protein therapeutics is the immunogenicity of foreign proteins. T cells play a key upstream role in the activation of the adaptive immune system and therefore, the immune reaction against protein therapeutics. Antigen presenting cells sample the environment and proteolytically process proteins, which are then presented as T cell epitopes on MHCII on the cell surface. This leads to the activation of CD4 T cells inducing the activation and differentiation of other T and B cells. Therefore, deimmunization approaches usually focus on T cell epitope prediction and deletion. This project applies computational tools to predict T cell epitopes and calculate epitope deleting mutations with low impact on protein activity and structure. Deimmunized variants undergo *in vitro* activity testing, *ex vivo* immunogenicity assays and *in vivo* immunogenicity and efficacy studies in humanized mice.

MECHANISM OF THE INDUCTION OF B CELL PROLIFERATION AND LYMPHOMAGENESIS BY THE EPSTEIN-BARR VIRUS ONCOGENE LMP1

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EBV is associated with B cell malignancies while usually kept in check by immune surveillance. During lifelong EBV latent infection, once immune surveillance fails, rare infected B cells can be activated and spread the virus to other B cells which, as a result, undergo proliferation and ultimately malignant transformation. Previous research of our group has shown that the expression of a single EBV oncogene LMP1 in B cells of immunodeficient mice is sufficient to induce rapid, fatal lymphoproliferation and lymphomagenesis. I investigate the early effect of LMP1 on primary B cell proliferation, activation, and gene expression, and reveal that LMP1 is able to induce the formation of liquid-liquid phase separation condensates of the transcriptional coactivators MED1 and BRD4. By *in situ* labeling the interacting proteins of MED1 and BRD4 with BioID2, transcription (co)factors in the condensates can be identified. Cap analysis of gene expression will be performed at different time points to describe the transcriptome transition after the presence of LMP1, and by identifying enhancer RNAs to indicate the active enhancers. This might lead to a mechanistic explanation that LMP1 orchestrates the activation of a gene expression program that massively promotes lymphoproliferation and lymphomagenesis by recruiting specific transcription (co)factors into condensates at certain genomic loci.

[1]Zhang B, Kracker S, Yasuda T, et al. Immune surveillance and therapy of lymphomas driven by Epstein-Barr virus protein LMP1 in a mouse model. Cell. 2012;148(4):739-751. doi:10.1016/j.cell.2011.12.031. [2] Sabari BR, Dall'Agnese A, Boija A, et al. Coactivator condensation at super-enhancers links phase separation and gene control. Science. 2018;361(6400):eaar3958. doi:10.1126/science.aar3958.

DC3S IN INFLAMMATORY ARTHRITIS

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Dendritic cells (DCs) are important players in the initiation and exacerbation of autoimmune diseases, such as inflammatory arthritis. DCs can be divided into different subsets with the two main subsets being plasmacytoid dendritic cells (pDCs), and conventional dendritic (cDC) subdivided into type I (cDC1s) and type II (cDC2s). A previous study has shown the presence of the newly identified DC3 subset in osteoarthritis, where they are involved in the formation of ectopic lymphoid-like structures that are associated with disease severity. In this study, we performed a first characterization of the DC3 subset in the synovial fluid of patients with inflammatory arthritis. Moreover, we show that the addition of synovial fluid induces the conversion of cDC2s into DC3s, indicated by the expression of CD14 and CD163. This work provides first insights into the characteristics of the DC3 subset in arthritis.