6th Annual Meeting of the Upper-Rhine Immunology (URI) Group

Karlsruhe, October 21, 2022

TALKS

T1: The multifaceted immunologic and molecular profiling of multivisceral human and murine colorectal cancer

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Colorectal cancer (CRC) is the second most prevalent cancer in females and the third most common in males, with about 0.8 million deaths worldwide per year. Whereas primary CRC (pCRC) in early stages is mostly curable by surgery, death rates from metastatic cancer remain still high and are the major cause of CRC-related death. Metastasized colorectal cancer is associated with poor prognosis and rapid disease progression. Beside hepatic metastasis, peritoneal carcinomatosis (PC) is the major cause of death in patients with stage IV pCRC. There are only few therapeutic options available for PC and these are considered palliative treatments. Therapeutic strategies include cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) or pressurized intraperitoneal aerosol chemotherapy (PIPAC). Overall, there is only limited profit for the prognosis of pCRC patients associated with severe side effects. Therefore, it is tremendously urgent to better understand the tumor microenvironments (TME) of pCRC multivisceral metastases, especially for the development of new immunotherapeutic approaches. Particularly the PC TME is poorly understood. Therefore, we analysed all microenvironments in a multi-omics approach with the focus on single cell and bulk RNA sequencing, 16S amplicon seq and spatial analyses. We used patient samples and set up a translational mouse model to mimic the clinical situation. With all the data, we are able to show a complete immune cell-extracellular matrix-microbiome crosstalk map. Herein, we figure out that different visceral pCRC metastases have distinct TMEs with distinct immune phenotypes and

functionalities. Especially the immune responses are completely altered in each metastasis location. Whereas we see B and T cell-mediated antitumoral responses in the liver metastasis, peritoneal carcinomatosis contains a myeloid-driven protumoral microenvironment. In conclusion, our data show different, metastasis-specific immunologic routes and phenotypes. Thus, the results highlight the need for an individual and adapted metastasis-specific immunotherapeutic approach in a personalized medicine manner.

T2: Bone marrow-derived cells impact the metastatic process in pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by early dissemination and associated with poor prognosis. The dynamic mutational landscape generates a heterogeneity that hampers tumor targeting. In order to successfully develop into metastases, disseminated cancer cells require appropriate niches at distant organs, that support their survival and immune evasion. Metastatic niches are formed, at least in part, by bone marrow derived cells (BMDCs). Tumor-secreted factors alter the extracellular matrix (ECM) in distant organs. In turn, the remodeled ECM plays a crucial role in the migration and adhesion of mobilized BMDCs to the distant organ, while BMDCs have been demonstrated to secrete components involved in ECM remodeling, such as fibronectin, collagen and matrix metalloproteinases. Prior the arrival of cancer cells, BMDCs and the remodeled ECM form pre-metastatic niches supporting adhesion, growth, survival and immune evasion of incoming cancer cells.

Our group has shown that inhibition of the cell adhesion molecule CD44, on cancer cells and on host cells, in several mouse and rat pancreatic cancer models, blocked the metastatic process. Additionally, prior to arrival of metastases in target organs, we have detected clusters of BMDCs, which express high levels of CD44. Thus, we hypothesize that the inhibition of CD44 might not only have prevented metastasis of cancer cells but also might have destabilized the metastatic ecosystem and niche-associated cells.

We are currently assessing the impact of a Cd44 knockout specifically in BMDCs using the $Cd44^{fl/fl}$; $VavCreER^{T2}$ inducible mouse model, before and during metastatic niche and metastasis formation. We have also setup an in vitro system of primed BMDCs, PDAC cells and other cells from the microenvironment. With both experimental settings, we will address the immunosuppressive function of BMDCs and the involvement of CD44.

T3: Specific targeting of dendritic cells using plant-derived Virus Like Particles: towards the development of an innovative anti-tumor vaccine

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In recent years, nanoparticles of various formulations have been developed as targeting cargos to improve efficacy and reduce side effects of conventional anti-tumoral therapies. Engineered nanoparticles that encapsulate a molecule of interest and display a targeting moiety on their surface allow both protection of the molecule from degradation and enhanced cell-specific delivery.

Here we used Virus-Like Particles derived from the capsid protein of the Grapevine Fanleaf Virus (VLPGFLV)[1] as adaptable platforms for targeted antigen delivery. These non-infectious self-assembling structures produced in planta were functionalized via nanobodies with anti-Clec9A antibodies in order to target ovalbumin (OVA, as a model antigen) to dendritic cells (DC). Clec9A is a C-type lectin expressed by a DC subset specialized in antigen cross-presentation[2]. We evaluated in vivo the ability of the functionalized VLPGFLV to induce Clec9A-mediated antigen delivery and assessed the subsequent OVA-specific CD4 and CD8 T lymphocyte activation. Our results demonstrate that the use of DC-targeted antigen-containing VLPGFLV is a promising strategy to generate specific and cytotoxic anti-tumoral immunity.

References:

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T4: COVID-19 booster vaccination elicits a transient effector CD8+ T cell response while conserving virus-specific memory T cells

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SARS-CoV-2 mRNA vaccines induce a robust spike-specific CD8+ T cell response followed by the establishment of a memory pool. However, waning of the humoral immune response led to worldwide booster vaccination campaigns and discussions about frequent booster immunization. Thus, there is an urgent need to improve our knowledge about the effects of booster immunization on the CD8+ T cells. To address this question, we collected blood of healthy donors without prior SARS-CoV-2 infection history throughout the 1st, 2nd and 3rd and 4th mRNA vaccine dose and of individuals with SARS-CoV-2 breakthrough infections after the 3rd mRNA vaccination. Subsequently, we performed phenotypic profiling of SARS-CoV-2-specific CD8+ T cells applying tetramer-based enrichment and high dimensional flow cytometry analysis. Functionality of the cells was assessed by peptide-specific 14-day in vitro expansion followed by intracellular cytokine staining upon peptide re-stimulation.We could show that the antigen contact by 3rd and 4th vaccine dose as well as in breakthrough infections induces a transient activation and expansion of virus-specific CD8+ T cells that lasts about 1 month with a subsequent contraction phase. These dynamics of the SARS-CoV-2-specific CD8+ T cell response are comparable to what we observed after the 1st and 2nd vaccine dose and are irrespective of the vaccine or infection trigger. Hence, the vaccine-induced SARS-CoV-2-specific CD8+ T cells are effectively reactivated during breakthrough infections. Importantly, we could not detect drastic changes in the phenotypic and functional characteristics of the SARS-CoV-2-specific CD8+ T cell memory pool at later time points (3-9 months) after the 3rd vaccine dose compared to corresponding time points after the 2nd dose. Additionally, the frequencies of SARS-CoV-2-specific CD8+ T cells expressing high levels of BCL-2 remained stable throughout all three vaccinations. Thus, the 3rd vaccine dose elicits a transient effector response while conserving the SARS-CoV-2-specific CD8+ T cell memory response.

T5: The humancytomegalovirus (HCMV) glycoprotein RL11/gp34 antagonizes C-reactive protein (CRP)-mediated activation of FcγRI/CD64

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We established a novel reporter-cell assay measuring C-reactive protein (CRP)-mediated FcγR activation and compared the responses with IgG-mediated triggering of FcγRs. The assay-platform is based on BW5147 cells stably expressing FcγR-CD3ζ-chain fusion chimeras leading to secretion of mIL2 upon FcγR-crosslinking (Corrales-Aguilar et al. 2013). In accordance with literature, we confirmed FcγRI/CD64, andFcγRIIa/CD32a (only allelic variant CD32aR131 but not CD32aH131) activation by immobilized and soluble CRP in a dose dependent manner, whereas inhibitory FcγRIIb/CD32B was only triggered by immobilized CRP. Generally, CRP-binding seems to be not sufficient for FcγR activation, as we observed binding to FcγRIIIa/CD16, but no FcγR triggering. Viral FcγRs, encoded by certain herpesviruses, bind IgG thereby interfering with host FcγRs to escape immune responses. Specifically, HCMV vFcγRs gp34/RL11 and gp68/UL119-118 antagonize IgG-mediated FcγR activation (Corrales-Aguilar et al. 2014). Since IgG and CRP (i) share homologous regions and (ii) activate FcγRs, we hypothesized that vFcγRs might also interfere with CRP-mediated FcγR-activation. Indeed, we could demonstrate for the first time a vFcγR to bind and interact with CRP in addition to IgG. gp34, but not gp68, directly binds to CRP and antagonize CRP-mediated FcγRI/CD64-activation. Mechanistically, we showed that gp34 pre-bound to CRP blocks subsequent FcγRI/CD64-binding, similarly as C1q, which is known to bind directly to CRP and displaces FcγRI.

In summary, we demonstrate robust gp34 binding to CRP, pointing to a potential entry or immune attenuating mechanism of HCMV

T6: Primitive macrophages: unseen string-pullers of the late immune system development

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The key role played by macrophages i organ development and homeostasis starts being appreciated and dysfunctional macrophages have been associated with inflammatory fetal and neonatal disorders. In mammals, primitive macrophages originate from the yolk sac in a hematopoietic stem cell (HSC)- independent fashion and seed the fetal niche wherein HSC expand and differentiate. Whether/how primitive macrophages modulate HSC biology is still not clear. et, niche defects are involved in myeloid malignancies, calling fora thorough study of the environmental cues regulating HSC and late immune system development. In vivo studies are still hampered by the complexity of the mammalian immune system and by the scarcity of specific tools. Using Drosophila as simple and evolutionary conserved animal model meets these needs. As in mammals, Drosophila macrophages derive from different hematopoietic waves. Primitive macrophages originate during embryogenesis. These migratory cells sense and react to internal/environmental stimuli, connect distant tissues and are ideal candidates to regulate development and homeostasis of several larval organs, including the lymph gland, which accounts for the late, HSC-dependent, hematopoiesis. Interestingly, we found that the loss of primitive macrophages severely impacts lymph gland growth and differentiation. This effect does not correlate with increased inflammation in the animal, suggesting that the observed phenotypes are caused by the lack of specific primitive macrophage functions. Notably, Drosophila macrophages produce and secrete several components of the extracellular matrix (ECM) and the downregulation of this function affects the lymph gland development. In sum, our findings unveiled a pivotal role of primitive macrophages in shaping the development of the late immune system through the ECM pathway, thus improving the current knowledge of the developmental function of these cells.

T7: Microbial triggering of myelin-specific immune cells in the gut drives central nervous system inflammation

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Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system (CNS) of unknown etiology. It is a prototypic complex disease in which genetic and environmental factors are thought to lead to dysregulated immune responses targeting myelin antigens. In that regard, changes in gut microbiota composition have recently been implicated in MS pathogenesis. How alterations in gut microbiota composition influence systemic immune responses in an antigenic specific manner and whether this interaction can trigger myelin-reactive immune responses in the gut through molecular mimicry with commensal bacteria in MS is unknown. Here, we studied antigen-specific triggering of gut immune cells and their encephalitogenic potential in experimental models of autoimmune neuroinflammation. We found that myelin peptide-expressing - but not bacteria expressing ovalbumin - were capable of triggering or exacerbating disease in different experimental murine models of MS. Our results provide novel insights into antigen-specific microbial triggers of MS with implications for the development of novel therapeutic strategies that aim at manipulating the MS gut microbiome.

T8: A particular HDAC: cytoplasmic Histone Deacetylase 6 regulates antimicrobial functions and immunometabolism in macrophages

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Macrophages are the first line of defence against invading pathogens. They detect infection via their Toll-like Receptors which trigger a rapid change in gene expression and the production of cytokines/chemokines and antimicrobial proteins. These inflammatory responses are also regulated by post-translational modifications, such as phosphorylation and acetylation. Histone deactetylases (HDAC), which deacetylate lysine residues, are important regulators of macrophage functions through their capacity to control signalling and gene expression. HDAC6 is uncommon in that it mainly deacetylates cytoplasmic targets. It is also unique from a structural perspective: HDAC6 is the only HDAC composed of two catalytic domains (CD1/CD2) and one C-terminal zinc finger ubiquitin binding domain (ZnF-UBP). The function of the different HDAC6 domains and their targets remains unclear. The GTPase Mitofusin1 is a compelling target of HDAC6 as it modulates the mitochondrial dynamic. After the deacetylation of its Lysine K222, Mitofusin1 promotes mitochondrial fusion. This oscillation between fused and fragmentated mitochondrial states drives the macrophage activation, and recently, we found that mitochondrial fission promotes inflammatory and antimicrobial response in macrophages. Thus, as anticipated, targeting HDAC6 via a genetic or pharmacological approach, blocks Mitofusin1 activation and increases mitochondrial fission in macrophages which, in turns, promotes bacterial clearance. In addition to mitochondria dynamics, HDAC6 interacts also with key metabolic enzymes from the glycolysis pathway and the tricarboxylic acid (TCA) cycle. We found that HDAC6 inhibition reduces the production of metabolites such as Lactate and Itaconate in macrophages. In addition, using Seahorse technology, we showed that HDAC6 inhibition reduces dramatically mitochondrial functions (Oxygen Consumption Rate - OCR) and glycolysis (extracellular acidification rate – ECAR). Collectively, our findings demonstrate a central role for HDAC6 in bacterial clearance and immunometabolism. Thus, targeting this enzyme could be one avenue for the treatment of acute and/or chronic inflammation and bacterial infections.

T9: Contact-dependent activation of TGF-β induces adaptation of macrophages to peripheral nerves

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Immune cells contribute towards peripheral nerve function under regular and pathologic conditions. In the dermis, a highly differentiated subset of tissue resident macrophages called sensory nerve-associated macrophages (sNAMs) interacts with sensory nerves and contributes to nerve maintenance and axon regeneration after nerve injury. However, the analysis of their development and interaction with nerves is hampered by the rarity and difficulties related to purification from the tissue. Hence, we have established murine as well as human in vitro models with myeloid cells and sensory neurons derived from primary cells or induced pluripotent stem cells. Using these models, we demonstrate that monocyte-derived macrophages upregulate specific signature genes such as Cx3cr1 in the presence of sensory nerves and mimic the phenotype and behaviour of sNAMs in vivo. Transcriptomic profiling of in vitro-generated sNAMs revealed that more than 700 of the differentially expressed genes were related to TGF-β signalling. Blocking of the TGF-β receptor and physical separation of the different cell types in transwells prevented immunophenotypic changes of macrophages in co-culture, while activation of latent TGF-β in cultures induced them. Moreover, activation of TGF-β was partly mediated by the integrin subunit β5, which was highly upregulated in macrophages in the context of sensory nerves in vitro and in vivo. In summary, our model uncovered a contact-dependent role of TGF-β in the phenotypic adaptation of macrophages to peripheral nerves. This allows to further unveil bidirectional heterocellular communication processes, which eventually may open new perspectives to improve the innervation of skin grafts and foster regenerative approaches for neurological disorders.

T10: HLA antibody affinity assessment: from HLA-specific monoclonal antibodies to donor-specific HLA antibodies (DSA) in patient sera

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Organs transplanted across donor-specific HLA antibodies (DSA) are associated with various clinical outcomes. However, neither the route of pre-sensitization nor readily available DSA-characteristics allow the discrimination between potentially harmless DSA and DSA with detrimental effect. DSA affinity has the potential to provide better information to predict the hazardous potential of circulating DSA. Several biophysical technologies allow the assessment of antibody binding strength, but prior knowledge of antibody concentration is required. The objective of the present study was therefore to develop an approach that combines DSA-affinity with DSA-concentration determination for patient sample evaluation. We first investigated several platforms including bio-layer interferometry (BLI), surface plasmon resonance (SPR), flow induced dispersion analysis (FIDA), and a Luminex Single Antigen Bead (SAB) titration assay, testing representative sets of HLA-specific monoclonal antibodies (mAbs) by incubating them with their cognate recombinant HLA molecules. Binding affinities were assessed either based on real time antibody on- (ka) and off-rate (kd) determinations (BLI, SPR) or by end-point measurements using the steady state equilibrium dissociation constant (steady state KD) (FIDA, SAB). FIDA is particularly suitable for measuring DSA-affinities in patient serum samples and simultaneously determines DSA-concentration. We investigated DSA of twenty pre-transplant patient samples, all with negative complement-dependent cytotoxicity (CDC)-crossmatch results and SAB signals ranging between 571 and 14899 MFI. DSA-concentration ranged between 11.2 – 1223nM (median 81.1nM) and their measured affinities were between 0.055 – 24.7nM (median 5.34nM; 449-fold difference). In 13/20 patients (65%), DSA accounted for more than 0.1% of total serum antibodies, and 4/20 sera (20%) revealed a proportion of DSA even higher than 1%. This study strengthens the presumption that pre-transplant patient DSA consists of various concentrations and different net affinities. Clearly, validation of these results in larger patient cohorts is the critical next step in assessing the clinical relevance of the two measures.

T11: H3K27me3 marks in thymic epithelial cells: Does dose matter for development and function?

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The thymus provides the physiological microenvironment for the development of the majority of T lymphocytes. Hence, its function is critical for the successful establishment and maintenance of the immune system's capacity to distinguish between vital self and injurious non-self. This competence is primarily instructed by thymic epithelial cells (TECs). However, the genetic and epigenetic mechanisms that control thymus formation, homeostatic maintenance and function are incompletely understood. Previous work by our group has shown the repressive epigenetic mark H3K27me3 to play an important role in TEC biology. However, such studies have relied on the complete ablation of these epigenetic marks by conditional knockout of the PRC2 complex, which is responsible for the deposition of H3K27me3 marks. For this study, we generated a murine model (namely TECK27M mice) in which a TEC-specific decrease in H3K27me3 marks is achieved by overexpression of the dominant negative histone H3 lysine 27-to-methionine (H3K27M) mutant, without altering the PCR2 machinery itself. This approach has allowed us to investigate how changes in the physiological epigenetic landscape impact TEC maturation and function in a dose-dependent manner. We have shown that thymi from TECK27M mice display altered thymocyte development, with a partial block at the positive selection stage, as well as a higher propensity to develop autoimmune reactions in the form of lymphocyte infiltrations in peripheral organs with age. These findings demonstrate the importance of normal histone modifications in TEC for the cell's maturation, metabolism and function and advance our incomplete understanding of their role in thymus biology.

T12: Optogenetics: Oscillatory TCR stimulation differently impacts on the different signalling pathways

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SARS-CoV-2 mRNA vaccines induce a robust spike-specific CD8+ T cell response followed by the establishment of a memory pool. However, waning of the humoral immune response led to worldwide booster vaccination campaigns and discussions about frequent booster immunization. Thus, there is an urgent need to improve our knowledge about the effects of booster immunization on the CD8+ T cells. To address this question, we collected blood of healthy donors without prior SARS-CoV-2 infection history throughout the 1st, 2nd and 3rd and 4th mRNA vaccine dose and of individuals with SARS-CoV-2 breakthrough infections after the 3rd mRNA vaccination. Subsequently, we performed phenotypic profiling of SARS-CoV-2-specific CD8+ T cells applying tetramer-based enrichment and high dimensional flow cytometry analysis. Functionality of the cells was assessed by peptide-specific 14-day in vitro expansion followed by intracellular cytokine staining upon peptide re-stimulation. We could show that the antigen contact by 3rd and 4th vaccine dose as well as in breakthrough infections induces a transient activation and expansion of virus-specific CD8+ T cells that lasts about 1 month with a subsequent contraction phase. These dynamics of the SARS-CoV-2-specific CD8+ T cell response are comparable to what we observed after the 1st and 2nd vaccine dose and are irrespective of the vaccine or infection trigger. Hence, the vaccine-induced SARS-CoV-2-specific CD8+ T cells are effectively reactivated during breakthrough infections. Importantly, we could not detect drastic changes in the phenotypic and functional characteristics of the SARS-CoV-2-specific CD8+ T cell memory pool at later time points (3-9 months) after the 3rd vaccine dose compared to corresponding time points after the 2nd dose. Additionally, the frequencies of SARS-CoV-2-specific CD8+ T cells expressing high levels of BCL-2 remained stable throughout all three vaccinations. Thus, the 3rd vaccine dose elicits a transient effector response while conserving the SARS-CoV-2-specific CD8+ T cell memory response.

T13: Study of TSLP-TSLPR signaling through dendritic cells in driving skin T cell responses

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Background: Atopic dermatitis (AD) is a skin chronic inflammatory disease that affects up to 20% of the children and 3% of the adults worldwide. It is an immune-mediated disease characterized by pruritic eczematous skin lesions, which is often associated with other atopic diseases including asthma and food allergies. AD typically presents an aberrant type 2 immune response, shown by skin infiltration of T helper 2 (Th2) cells, eosinophils and basophils, accompanied by an elevation of blood immunoglobulin E levels. The thymic stromal lymphopoietin (TSLP) is a keratinocyte-derived cytokine that has been recognized to play a key role in AD pathogenesis, but whether and how TSLP signals through its receptor (TSLPR) expressed on dendritic cells (DCs) to drive the Th2 response remain to be investigated.
Objective: Study TSLP-TSLPR signaling through mouse DCs in driving skin T cell responses.
Methods: We used a TSLP overexpression-induced AD model originally established in the lab. We employed DC-selective knock-out mouse lines and gene reporter mouse tools, combined with flow cytometry and scRNAseq analyses, to dissect the TSLP-TSLPR signaling axis.
Results: We showed that TSLP-TSLPR signaling in DCs induces not only Th2 but also regulatory T (Treg) cells. We found that TSLP-activated DCs upregulated their expression of the costimulatory molecule OX40L, but surprisingly, OX40L in DCs was not required for the induction of Th2; instead, it was crucial for the induction of Treg. By taking use of our newly generated OX40L reporter mice, we characterized the OX40L-expressing DCs for their surface marker expression and transcriptomic profiles. Thus, our results provide interesting insights on how skin TSLP signals through DCs to drive different T cell responses.

T14: CNS infection wakens a dormant autoimmune B response

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The exact pathogenetic role of B cells in MS is still unclear. We developed a model that links B cell biology to some of the known risk factors for MS, namely previous brain infection and EBV. Our hypothesis predicts that (1) autoimmune B cells are part of the normal human B cell repertoire; (2) a viral brain infection provokes infiltration of autoimmune B cells into brain parenchyma; (3) these B cells can be triggered to produce autoantibodies by a CD40L signal as it is provided by latent EBV infection. Using a membrane capturing assay we could identify myelin oligodendrocyte glycoprotein (MOG) specific B cells in the repertoire of 3/3 healthy human donors. We established an animal model using genetically modified virus injection into the striata of transgenic mice with B cell receptors specific for viral antigens or MOG. B cells infiltrate perivascular spaces of the brain from day 2 post injection independent of their specificity. From day 5 on they migrate into brain parenchyma. Immunofluorescent microscopy and intravital two-photon imaging of infected tissues showed intimate contact between the B cells and antigen-expressing cells in the infected area. ELIspot analyses demonstrated that only brain-infiltrated B cells present their specific antigen to T cells indicating capture of antigen within brain parenchyma. When CD40L signal is provided, in the brain, significant demyelination ($P<0.0001$) was observed in B cell infiltrating areas in IgH MOG transgenic mouse brains compared to the wildtype controls, obtained by immunofluorescence analysis. These results show that B cells are able to acquire their target antigen directly in infected tissue. Upon CD40-CD40L which can be provided by latent EBV infection they can evade activation induced cell death and thereby circumvent an important immune check point. Our study provides new insights into possible mechanisms of B cell mediated pathogenesis in MS.

T15: Tissue-engineered human skin as a new model to study arboviral cutaneous infection

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Arboviral infections occur through the bite of virus-infected arthropods, including mosquitoes, while blood-feeding on the skin. These infections represent an increasing challenge for public health and economies worldwide, exposing millions of people to death or long-lasting diseases. Despite advances in vector surveillance and public health awareness, little medical progresses have been made notably due to the lack of adequate research models recapitulating human skin biology while taking into account the role of the virus-delivering arthropod in the infection. Organoids, referring here to three-dimensional (3D) cell cultures, have already proven useful to study human diseases, such as cancer and infections, with foreseeable applications in drug testing and regenerative medicine. To investigate whether such approach could be used to study arboviral cutaneous infections, we developed and characterized a tissue-engineered 3D human skin model integrating self-organizing primary human keratinocyte-based epidermis over a collagen/chitosan matrix populated with dermal fibroblasts, monocyte-derived macrophages and iPSC-derived sensory neurons. These 3D skin models were exposed to ZIKA and Sindbis-infected Aedes mosquitoes which were found to actively probe the tissues. Using quantitative PCR, immunofluorescence staining and viral titration, we showed that the engineered skins were readily infected by these two arboviruses. Taken together, we established a novel pluridisciplinary experimental workflow allowing to monitor infection within a tissue-engineered skin model using infected insect vectors. This system opens unprecedented venues to explore immune response to arbovirus and human cutaneous pathophysiological mechanism.

T16: Distinct changes in endosomal composition promote NLRP3 inflammasome activation

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Inflammasome complexes are pivotal in the innate immune response to pathogens and other danger signals. The NLRP3 inflammasome is activated in response to a broad variety of cellular stressors. Most of the stimuli act in a potassium efflux-dependent manner but a primary and converging sensing mechanism by the NLRP3 receptor initiating inflammasome assembly remains ill-defined. A recent study reported binding of NLRP3 to phosphatidylinositol 4-phosphate (PI4P) as a prerequisite of NLRP3 inflammasome activation. Here we demonstrate that NLRP3 inflammasome activators primarily converge on disruption of ER-endosome membrane contact sites (EECS). This defect causes endosomal accumulation of PI4P and a consequent impairment of endosome-to-TGN trafficking (ETT), necessary steps for binding of NLRP3 to endosomes and subsequent inflammasome activation. Lowering endosomal PI4P levels prevents endosomal recruitment of NLRP3 and inhibits inflammasome activation. Disruption of EECS or ETT is sufficient to enhance endosomal PI4P levels, to recruit NLRP3 to endosomes and to potentiate NLRP3 inflammasome activation. Mice with defects in ETT in the myeloid compartment are more susceptible to LPS-induced sepsis in a NLRP3-dependent manner. Our study thus identifies a distinct cellular mechanism leading to endosomal NLRP3 recruitment and inflammasome activation.

POSTERS

1: Nucleobase adduct-containing metabolites are MR1 ligands that stimulate self-reactive MR1T cells

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MR1T lymphocytes are a recently identified population of T cells that recognize unknown self-antigens presented by the non-polymorphic MHC-I-related molecule, MR1. MR1T cells are heterogeneous, recognize and kill tumor cells and can also modulate the functions of other immune cells. Due to their functional capacities and target specificities, MR1T cells have promising potential in therapeutic applications. Despite several microbial metabolites or drugs were characterized by their ability to bind MR1, the nature of self-antigens presented by MR1 on tumor cells and recognized by MR1Ts still remains unknown.

By integrating genetic, pharmacological and biochemical approaches we identified distinct metabolic pathways that promote recognition of tumor cells by MR1Ts. Among these pathways, we focused our attention on carbonyl stress, in which reactive carbonyl species are generated, and nucleobase metabolism, that regulates the intracellular availability of purines and pyrimidines. We observed that when different pathways were perturbed together, tumor cells stimulated MR1Ts better than when pathways were modulated individually. Therefore, we hypothesized that nucleobases modified by reactive carbonyls could be accounted for increased antigenicity of tumor cells. We chemically synthesized different nucleobase adducts and tested their antigenicity. Our results show that purine- and pyrimidine-adducts bind MR1 and stimulate different MR1T cell clones, confirming that nucleobase adducts are a group of self-antigens recognized by MR1T cells. We will present data suggesting that MR1T cells are surveyor of cellular metabolic alterations occurring in conditions of metabolic stress, such as cancer, and lay the groundwork for the development of novel HLA-unrestricted T cell-based therapies.

P2: A novel immune evasive effect on B cells is mediated by the multi- leukocyte manipulator E3-49K of human adenoviruses

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Introduction: The early transcription unit 3 (E3) of human adenoviruses (HAdVs) encodes for immunomodulatory functions. The E3 of species D HAdVs contains an open reading frame called conserved region 1-beta (CR1-beta). Its protein product (E3-49K) is expressed at the cell surface as transmembrane protein, where it is cleaved by cellular metalloproteases and shed. Soluble E3-49K of HAdV-D64 binds to CD45, thereby affecting CD4 T cell and NK cell functions.

Objectives: To date, a CD45 modulatory activity is only described for the highly pathogenic HAdV-D64, but all species D HAdVs express E3-49K orthologues. To address whether the E3-49K type has any disease association, we designed a comparative analysis to investigate if the highly diverse E3-49K molecules from disease-causing and non-disease-causing HAdV-D types have distinct biochemical or physiological properties. Additionally, we extended the target spectrum of E3- 49K-mediated CD45 modulation.

Materials & methods: We expressed N-terminally HA-tagged codon-optimized E3-49K orthologues in stable transfected A549 cells. Quantitative analysis of production and secretion was established and functionality of the different species D orthologues was compared in cell-based assays. With usage of a soluble CD45 protein, the expression of CD45–binding activity of other HAdVs was explored.

Results: We could show that like untagged E3-49K, all tested HA-tagged orthologues share a similar functional activity. They are all secreted and bind to CD45, thereby inhibiting T cell and B cell activation. In HAdV infection, only the species D infected cells showed a CD45-binding activity.

Conclusions: Comparative studies between the different E3-49K orthologues demonstrated a similar function independent to the pathological association of the corresponding viruses. Modulation by binding to CD45 seems to be a unique property of the species D among HAdVs. As key result of this research, we have been able to show the manipulation of B cell function by HAdVs for the first time.

P3: Flt1 determines neuronal regeneration by titrating Vegf levels and innate immune response at the neuro-vascular interface

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Zebrafish have the unique capacity to regenerate injured organs including the heart, skeletal muscle, and the nervous system. In the spinal cord of zebrafish embryos, neural stem cells are able to re-activate the molecular programs required for nervous system regeneration. The micro-environment that surrounds the neural stem cell niche, constituted by blood vessels, connective tissue and pro- and anti-inflammatory cytokines, produced by neutrophils and macrophages play a crucial role herein. In the spinal cord radial glia and neurons determine the onset and extent of spinal cord vascularization by producing Vascular Endothelial Growth Factor (Vegf), and soluble Vegf receptor-1 (sFlt1), acting as a Vegf trap. Loss of (neuronal) sFlt1 causes spinal hypervascularization. We hypothesized that stimulating spinal cord vascularization by genetically deleting *flt1*, and thereby improving blood and nutrient flow, may accelerate the regeneration process. Surprisingly, although having increased angiogenic capacity, *flt1*$^{-/-}$ mutant embryos failed to regenerate their spinal cord upon injury.
Mechanistically we found that spinal cord repair required soluble Flt1 (sFlt1) acting as a Vegf trap, independent of mFlt1 signaling. Accordingly reducing Vegfaa or neuron specific overexpression of sFlt1 rescued spinal cord regeneration defects in *flt1*$^{-/-}$. Macrophages and neutrophils are critical for spinal cord repair. Flt1 mutants showed aberrant kinetics in both neutrophil and macrophage recruitment toward the lesion site. Macrophages furthermore showed a shift in M1-M2 polarization. In a parabiosis setup combining *flt1* mutant embryos with WT embryos restored macrophage behavior and rescued spinal cord regeneration in *flt1*$^{-/-}$ upon injury. We conclude that *flt1* is essential for Spinal Cord regeneration through control of the innate immune responses at the level of the neuro-vascular stem cell niche. This may open novel insights into understanding the etiology of neurodegenerative diseases and potential therapeutic strategies.

P4: Deciphering the implication of lymphoid organ stromal cells in autoimmune responses during Systemic Lupus Erythematosus

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Secondary Lymphoid Organs (SLO) such as Lymph Nodes (LN) harbor a very complex but well-organized microarchitecture, based on an intricate network of stromal endothelial and mesenchymal cells that surround and support immune cells. Lymph Node Stromal Cells (LNSC) provide scaffolds allowing lymphocyte migration and recruitment through cytokine and chemokine secretion. Thus, a crosstalk between LNSC and the hematopoietic compartment is involved in the initiation and regulation of immune responses and in the maintenance of tolerance through peripheral tissue antigen presentation[1]. More recently, a few studies have highlighted the plasticity of the stromal compartment in pathological situations such as a viral infection[2] or cancer metastasis[3]. Here, we study stromal cells in the context of systemic lupus, an autoimmune syndrome that involves tolerance breakdown, autoantibody production and kidney disease. Of interest, the absolute numbers, and relative frequencies of the main LNSC subsets are specifically altered in the kidney-draining LN of lupus NZB/W mice. Moreover, we performed the first RNA-Sequencing analysis of these sorted cells, and we were able to evidence modifications of the stromal transcriptomic signature specifically linked to disease development, suggesting alterations of cellular (auto)immune processes that are currently under investigation.

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3 *Riedel, A., Shorthouse, D., Haas, L., Hall, B. A. & Shields, J. Tumor-induced stromal reprogramming drives lymph node transformation. Nat. Immunol.* ***17****, 1118–1127 (2016).*

P5: Identification of rheumatoid arthritis-specific extracellular vesicles

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Extracellular vesicles (EVs) are membrane enclosed particles produced by all cell types and were previously shown to contribute to the pathogenesis of different diseases like rheumatoid arthritis (RA) – a chronic inflammatory autoimmune disease. Preliminary data showed microRNA (miR)- 221-3p as a driver of the proinflammatory M1-phenotype *in vitro*, displaying increased expression in synovial fluid (SF) of RA patients, especially in the EV-fraction. Further, the SF of RA patients contain EVs with macrophage- (CD68) specific surface markers. Comparison of RA- and osteoarthritis (OA)- SF exhibited a higher amount of CD68-positive EVs in RA samples, indicating the enrichment of specific EV populations in disease conditions. However, the role of EV subpopulations, their specific cargoes and the molecular mechanisms, which contribute to pathogenesis of RA are still unknown.
Here, we identified FAPa and CD90 on the surface of synovial fibroblast derived EVs, two cell markers of fibroblast subpopulations found in RA synovium. For that, EVs derived from either conditioned medium of synovial fibroblast cell culture (*in vitro*), SF or plasma were isolated via size exclusion chromatography and compared between RA and OA or healthy donor samples. Western blot analysis detected CD90 on the surface of synovial fibroblast derived EVs *in vitro*, while FAPa was identified on EVs derived from SF, plasma and *in vitro*.
The identification of RA specific EV markers will allow the separation of distinct synovial EV subpopulations and subsequent investigation of their functional cargo via proteomics and miRomics.

P6: Characterizing the role of exhausted T cells in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is a major contributor to the worldwide cancer burden and has high mortality. The role of the immune response in HCC remains unclear. T cells can mediate protection against tumor cells but are frequently dysfunctional and exhausted in cancer. Exhausted CD8+ T cells (TEX) play an important role in chronic infections and cancer and are known as immunotherapy targets. T cell exhaustion is described as a type of T cell dysfunction characterized by reduced effector function and high co-expression of immunoregulatory molecules. It has been demonstrated that regulation through immune checkpoints (e.g. PD-1, CTLA-4, LAG-3, TIM-3) is an important driver of exhaustion. However, an extensive assessment of TEX diversity in human disease is lacking. This is particularly relevant for HCC, in which only 30% of patients respond to immunotherapies.

We are interested to study the cellular mechanisms of the immune system in response to chronic inflammation and to be able to understand the therapeutic effects of cancer immunotherapy. Therefore, we plan to determine the composition of the immune microenvironment in HCC by using imaging mass cytometry (IMC) for a fine-profiling of the tumor microenvironment in HCC resections and biopsies.

Furthermore, we want to identify differences in immune cell composition based on different tumor etiology and genomics and to understand the spatial interaction and the role of metabolic niches in the HCC microenvironment. We will use spatial IMC analysis and spatial transcriptomics combined together on formalin fixed paraffin embedded (FFPE) tissue sections. Moreover, we will compare peripheral blood and intrahepatic exhaustion profiles and will perform peripheral and intrahepatic single-cell assessment of exhausted T cell populations using exhaustion-directed deep immune profiling. We would like to contribute to the understanding of the interplay between etiology, cancer genomics and immunotype of HCC to advance personalized treatments for patients in the future.

P7: Impact of the removal of CD44 in pancreatic cancer-associated fibroblasts on tumor growth and metastasis

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Pancreatic cancer is the deadliest type of cancer due to its invasive and fast metastasizing nature. In various pancreatic cancer mouse and rat models, our group could show that blocking of CD44v6, a member of the CD44 family of transmembrane proteins with species-specific peptides, not only on human cancer cells (L3.6pl) but also on endogenous murine (host) cells, led to reduction of tumor volume and metastatic burden. My PhD focuses on the role of CD44 on cancer-associated fibroblasts that likely influence tumor progression by interactions with other stromal cells and cancer cells. Results in our group showed that the knockout of *Cd44* in pancreatic stellate cells (PSC), a unique fibroblastic cell type of the pancreas led to a reduction of their activation consequently to decreased fibrosis. The PSCs belong to the cancer-associated fibroblasts (CAF) that also comprise other cell types, amongst which inflammatory CAFs, playing roles in immunosuppression and fibrosis and which are crucial for tumor progression. To investigate the role of CD44 in the whole CAF population, we have removed *Cd44* by means of $Cd44^{fl/fl}$; $PDGFR\beta CreER^{T2}$ mice and are examining the consequences of the absence of all CD44 isoforms in CAFs on tumor growth, tumor composition and metastasis.

P8: CD169+ macrophages in lymph node and spleen critically depend on dual RANK and LTβR signaling

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$CD169^+$ macrophages, which play an important role in the immune defense against pathogens, are strategically localized at the lymphatic sinuses of lymph nodes (LNs) and the marginal zone of the spleen where they capture lymph- and blood-borne antigens, respectively (Gordon et al, 2014). As resident macrophages they are responsive to environmental cues to shape their tissue-specific identity and function. We have previously shown that LN $CD169^+$ macrophages require RANKL for formation of their niche and their differentiation (Camara et al, 2019). Here we demonstrate that they are also dependent on direct lymphotoxin beta (LTβ) receptor (R) signaling.

In the context of partially overlapping functions, we scrutinized the implication of the RANK-RANKL and the LTβR-LTαβ axes in the differentiation of LN and splenic $CD169^+$ macrophages. Using *Cd169*-directed conditional deficiency of RANK or LTβR, we report by cytometry and immunofluorescent microscopy that direct RANK and LTβR signalling is required for their differentiation in the LN and the spleen. In the absence of the receptors, LN $CD169^+$ macrophages were replaced by myeloid cells phenotypically similar to the $SIGN\text{-}R1^+$ medullary sinus macrophages. Combined haploinsufficiencies of RANK and LTβR revealed that both receptors contribute equally to LN and splenic $CD169^+$ macrophage differentiation. Altered macrophage differentiation had a negative impact on lymph-borne antigen transport to B cells and in the spleen compromised viral capture and the formation of the virus-specific $CD8^+$ T cell response. By the use of a novel RANKL-reporter mouse together with RT-qPCR of sorted splenic stromal subsets, we identified $CCL19^+$ splenic MRCs as a source of RANKL and demonstrated in *Ccl19*-cre RANKL-deficient mice that stromal RANKL participates in MMM differentiation.

Taken together, the data provide evidence that $CD169^+$ macrophage differentiation in LN and spleen is dependent on the dual signals emanating from LTβR and RANK with implications for the immune response to lymph and blood borne pathogen.

P9: Involvement of LC3-conjugation to single membrane in B-cell antigen receptor trafficking

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Antigen presentation by B cells is central in the humoral immune response. B cells present antigens to helper T cells which stimulate their differentiation into plasma cells or memory cells. Autophagy-related (ATG) proteins participate in antigen presentation, but their precise contribution is still unclear. Previous work in the laboratory demonstrated the preponderant role of the ATG5 protein. We recently showed that ATG proteins are involved in immobilized antigen processing after engagement by the B cell receptor (BCR) through polarized endocytosis. We aim now at precising how ATG proteins participate in BCR trafficking. We generated human B cell lines expressing tagged ATG16L1, ATG5 or MAP1LC3B. Using mass spectrometry approaches, we identified novel ATG partners in the context of BCR crosslinking. Significant candidate proteins are associated with lysosome exocytosis and fusion with endosomes. We particularly focused on SNAP23, involved in lysosome exocytosis and LC3-associated phagocytosis (LAP). SNAP23 colocalization with BCR after crosslinking is impaired upon ATG5 or ATG16L1 depletion. We are now generating SNAP23-deficient B cell lines to validate its involvement in BCR trafficking. We also intended to define if LC3-conjugation to single membranes is involved in BCR trafficking and antigen acquisition. We therefore studied the impact of RUBCN depletion, essential LC3 associated phagocytosis/endocytosis protein. We stimulated primary B cells isolated from a *Rubcn*-/- mice, with microbeads-tethered antigens. Upon RUBCN depletion, super-resolution imaging show polarization defects of internalized BCR-containing vesicles. This suggests the involvement of RUBCN-dependent processes in BCR endocytosis. We are now investigating whether RUBCN participates in antigen presentation by B cells after BCR engagement.

Altogether we showed that ATG proteins polarize BCR trafficking and identify new partners in this RUBCN-dependent process. We are now validating involvement of identified partners, and generating mouse models to address the question of the *in vivo* role of this pathway.

P10: Vaccine-elicited CD4 T cells prevent the deletion of antiviral B cells in chronic infection

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Chronic viral infections subvert protective B-cell immunity. An early type I interferon- (IFN-I-) driven bias to short-lived plasmablast differentiation leads to clonal deletion, so-called “decimation”, of antiviral memory B-cells. Therefore, prophylactic countermeasures against decimation remain an unmet need.

We show that vaccination-induced CD4 T cells prevented the decimation of naïve and memory B-cells in chronically LCMV-infected mice. Although these B-cell responses were largely T-independent when IFN-I was blocked, pre-existing T help assured their sustainability under conditions of IFN-I-driven inflammation by instructing a germinal center B-cell transcriptional program. Prevention of decimation depended on T cell-intrinsic Bcl6 and Tfh progeny formation. Antigen presentation by B-cells, interactions with antigen-specific T helper cells and costimulation by CD40 and ICOS were also required. Importantly, B-cell-mediated virus control averted Th1-driven immunopathology in LCMV-challenged animals with pre-existing CD4 T cell immunity.

Our findings show that vaccination-induced Tfh cells represent a cornerstone of effective B-cell immunity to chronic virus challenge, pointing the way towards more effective B-cell-based vaccination against persistent viral diseases.

P11: Niche-specific metabolic plasma cell heterogeneity in lupus-prone NZB/W F1 lupus mice

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While plasma cells (PCs) chiefly contribute to protective antibody titers, in autoimmune diseases, they can drive disease pathology. Hence, understanding commonalities and differences between conventional and pathogenic PCs could help to target autoantibody-secreting PCs selectively, sparing protective humoral immunity. PCs persist in specified niches and continuously secrete high amounts of immunoglobulins along with strong energy demands. So far, it is rather unexplored if PC differentiation, survival and function are linked with specific metabolic needs. Furthermore, the impact of niche composition and inflammation on these aspects of PC biology remain unresolved.
To decipher niche-specific metabolic characteristics of protective vs. autoreactive PCs, transcriptional profiling of PCs from different sites (spleen, bone marrow (BM) and kidney) of lupus-prone NZB/WF1 versus C57BL/6 mice was performed. Metabolic requirements of PCs and PC subsets from these different sites and strains were assessed by different ex vivo and in vitro strategies.
RNA sequencing revealed a generally high PC heterogeneity and differential expression of genes involved in major metabolic pathways, most predominantly in PCs from inflamed kidneys. Short- term culture under nutrient starving conditions further showed that BM and splenic PCs rely on both glucose and glutamine for optimal survival, while PCs from inflamed kidneys show higher glucose dependence and greater susceptibility to inhibition of glycolysis. Furthermore, treatment with metabolic inhibitors revealed variable sensitivity in different PC populations, which will be followed-up. For instance, we found that putative auto-reactive IgG-expressing PCs, are more sensitive to metabolic inhibitors and differ in ROS production, autophagy and glucose uptake.

In summary, our preliminary data may indicate metabolic differences between niche-specific PC subpopulations, which need to be validate by further experimental approaches and in vivo. In a next step, we will compare homeostatic and inflammatory/autoimmune conditions, to reveal potential vulnerabilities specific for autoreactive PCs, which might be employed for therapeutic targeting.

P12: CD44 as a regulator of colorectal cancer cell plasticity

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The incidence of colorectal cancer (CRC), the third most frequently diagnosed cancer worldwide, is still rising. Early-stage CRC is often curable, while effective therapies for later and already metastatic stages are lacking. Besides this, tumor relapse after therapy complicates a successful treatment. New concepts in treatment of CRC are therefore urgently needed. One finding that revolutionized our understanding of molecular aspects of CRC is a phenomenon called plasticity, a process which enables cancer cells to perform a dynamic switch from a differentiated to an undifferentiated (cancer stem cell (CSC)) stage. One prominent marker of CSCs is CD44, a family of proteins which is also highly expressed in intestinal stem cells as well as colorectal CSCs. Interestingly, our lab could already show a reduced stem cell functionality in the absence of *Cd44* in mouse intestinal organoids. In order to investigate the role of CD44 in cancer cell plasticity *ex vivo,* mouse CRC organoids in which CSCs are marked by the expression of eGFP, were used. To observe the plastic process, eGFP negative cancer cells were sorted, and the reappearance of eGFP, reflecting the reappearance of CSCs, was followed. We could demonstrate that blocking the specific CD44v6 isoform resulted in an inhibition of the dedifferentiation of cancer cells in CSCs, suggesting a role of CD44 in the plastic process. Given that dynamic switches govern the progression of CRC, blocking CD44 is a therapeutic avenue that should be investigated in depth.

P13: Cross-talk between the immune system and the nervous system in Drosophila melanogaster

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The interaction between the nervous and the immune systems, which both allow us to sense, respond and adapt to internal/external environments, make the object of intense investigation, due to the potential implication in medical research. In major nervous system diseases, one specific type of immune cell, the macrophage, presents a bipolar role either by enhancing inflammation and accelerating the onsets of the diseases or by secreting anti-inflammatory factors and reducing the damages. Although the mechanisms underlying this bipolar role are still under debate, the macrophages clearly appear as strong therapeutic targets.
It has been recently recognized that the role of macrophages goes well beyond that of immune cells that engulf and eliminate dying cells and pathogens. The mobility and ability of these scavenger cells to connect tissues and organs make them ideal sensors of the internal state in physiological and challenged conditions. This implies that the peripheral tissues, including the nervous system, have an impact on the state of macrophages.
Our current project is using *Drosophila* to characterize the impact of the nervous system on the development and function of the macrophages. *Drosophila melanogaster* is devoid of adaptive immune system and elicit exclusively an innate immune response. The main cellular immune response is carried out by plasmatocytes, macrophage-like cells that constitute 95% of the immune cells of the organism. In the larvae, the majority of the plasmatocytes resides in compartments between the cuticle and the muscles, where they are in close range to the peripheral nervous system. Our recent data suggest a direct communication channel between the macrophages and the nervous system necessary for the normal development of the plasmatocytes.

P14: Effect of therapeutic peptide P140 in a mouse model of gout arthritis

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Gout is painful form of inflammatory arthritis characterized by the deposition of monosodium urate (MSU) crystals in the joints. Gouty arthritis is a disease that affects 1- 2% of the adult population worldwide. The prevalence of gout has risen sharply within the last decades, rendering this disease an important health care issue. The current therapies are not effective in all patients, especially those with longstanding hyperuricemia. Novel therapeutic approaches are therefore eagerly awaited to improve the existing strategies or to provide additional possibilities to treat gout patients. In this context, we evaluated the possible effect of the therapeutic phosphopeptide P140, a regulator of autophagy, which was found to display potent protecting effects in several murine models of autoimmunity and inflammation, and in clinical trials including patients with systemic lupus erythematosus. Here, we used a classical mouse model in which gout was induced by injecting MSU crystals into the knee joint. Post-induction, treating mice with P140 provoked a decreased neutrophil influx and a weaker production of pro-inflammatory cytokines. Furthermore, P140 induced a significantly reduced hyper-nociception in treated animals. *In vitro*, P140 modulated the activity of neutrophils by increasing apoptosis pathways through augmented caspase 3 activity; it reduced NFκB phosphorylation. Moreover, P140 increased the production of the pro-resolving mediator annexin A1 and decreased expression of autophagy-related ATG5-ATG12 complex and HSPA8 chaperone protein. Overall, these findings suggest that P140 exerts a significant beneficial effect in a neutrophilic inflammatory model of gout that can be of special interest in the design of new therapeutic strategies that are in development to treat gout arthritis.

P15: Identification of cell-intrinsic regulators of T cell population homeostasis

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Recent work from our laboratory suggests the existence of a mechanism regulating T cell population homeostasis that is coordinated by coronin proteins to balance pro-survival pathways with homotypic kin-to-kin apoptotic signaling in a cell density dependent manner. Upon cell population density increase, the expression of the main coronin expressed in T cells, coronin 1, is upregulated to maintain pro-survival signaling until threshold densities are reached, at which point increased cell-to-cell interactions induce population growth arrest and apoptosis to adjust the population to its appropriate size. The mechanisms via which coronin 1-dependent pathway allows cell density sensing is not known. In my project, I aim to uncover cell-intrinsic regulators of T cell population homeostasis through the identification of the coronin 1 interactome. My results suggest that coronin 1 co-opts multiple pathways via which the control of the appropriate size of the T cell population size is being coordinated.

P16: NOD-1 Agonist Synergize with CD40L to Induce Proliferation and Plasma Cell Differentiation of Mouse B Cells especially in CD23^{high} B cells

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Since a few years, it has been demonstrated that, like innate cells, B cells expressed also Pathogen Recognition Receptors (PRRs) to danger signal such as tissue damage or intrusion of a pathogen. Thus, production of specific antibody by plasma cells results from activation and differentiation of B cells following 3 signals (i) antigen recognized by B Cell Receptors, (ii) T-cell help and (iii) recognition of danger. However, a question arises: is T-cell help dispensable for B cell activation and differentiation? Among PRRs, Toll Like Receptors (TLRs) agonists alone have been shown by our team (Boeglin et al. 2011) and others to induce B cell proliferation and differentiation, even if T cell signal such as CD40L or BCR stimulation enhanced the process. Few studies investigated the role of cytosolic PRRs such as NOD1 on B cell differentiation. In the present study, we showed that B cell expressed NOD1 and that treatment of B cells with C12-iE-DAP NOD1 agonist induce activation, proliferation and differentiation in plasma cells even in absence of T-dependent signal. As splenic B cells from marginal zone (CD23low) have different properties than those from follicular zone (CD23high), we investigated NOD1 pathway stimulation on each subpopulation. Surprisingly, CD23high B cells were more sensitive than CD23low B cells. Taken together, ours results suggest that NLR pathway could induce antibody development during infections and be exploited for development of more effective vaccination.

Boeglin, E., C. R. Smulski, S. Brun, S. Milosevic, P. Schneider & S. Fournel (2011) Toll-like receptor agonists synergize with CD40L to induce either proliferation or plasma cell differentiation of mouse B cells. PLoS One, 6, e25542.

P17: T Cell Exhaustion in STING Gain-of-Function Mice

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Stimulator of Interferon Genes (STING) belongs to cytosolic DNA-sensing pathways in innate immunity and has emerged as a central player in antiviral immunity, autoinflammation and cancer. When activated by cyclic dinucleotides produced by cGAS, STING translocates from endoplasmic reticulum to ERGIC/Golgi where it activates TBK1, which then phosphorylates IRF3 and induces the expression type I interferons (IFNs). Type I IFNs trigger then inflammation through the expression of a set of interferon-stimulated genes, consecutively to the activation of the type I IFNs receptor (IFNAR). Gain-of-function (GOF) heterozygous mutations in STING- coding gene is leading to constitutive activation of the protein and have been described in patients with an autoinflammatory phenotype designated STING Associated Vasculopathy with onset in Infancy (SAVI). While studying the role of STING in the pathophysiology of the disease by using a STING GOF mouse model (heterozygous V154M mutation, corresponding to the human V155M mutation), our team highlighted an unexpected role of the protein in adaptative immunity that is not explained by the action of type I IFNs as these mice develop T, B and NK lymphopenia in the presence or absence of the IFNAR receptor. Moreover, the remaining lymphocytes present functional, especially proliferation defects. Interestingly, transcriptomic analysis of both sorted CD4+ or CD8+ T cells revealed an exhausted phenotype that can be confirmed at the protein level with the overexpression of characteristics immune checkpoints. Trying to understand the underlying mechanisms, we showed that this phenotype is acquired only in the periphery since thymic double-negative T cells do not present exhaustion markers, but very early as evidenced by the mature T cells of 2 weeks old mice that are already exhausted. As IFNAR KO didn't allow any rescue, T cell exhaustion must involve type I IFN independent mechanisms in STING GOF mice, like the Ca^{2+}/NFAT pathway for example.

P18: Allogenic HER2-CAR T cells overcome intrinsic trastuzumab resistance *in vitro* and *in vivo* in a preclinical model of breast cancer

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The advent of anti-HER2 therapy has prominently prolonged the time of disease progression and survival for metastatic breast cancer patients, where a decent proportion is suffering from a HER2+ tumor. Beside classical approaches via antibodies against HER2, the use of Chimeric Antigen Receptor T (CAR-T) cells also in a solid cancer context is getting more and more attention. In our study, we evaluated the efficacy of Trastuzumab versus HER2 targeting CAR-T cells in a panel of human cancer cell lines (SK-OV3, Hs578T and JIMT-1) with different HER2 expression levels in vitro in 2D as well as 3D and in vivo in immunocompromised mice. In vitro, the tumor growth and invasion of the CAR-T cells was measured via fluorescence-based live cell imaging. On the last experiment day, a metabolic read-out (CellTiter-Glo, CTG assay) was performed. In vivo, we measured tumor growth inhibition (TGI) via caliper measurement and tumor tissue and hematopoietic organs were analyzed by flow cytometry (FC), Immunohistochemistry (IHC) and multiplex cytokine analysis. The HER2 targeting CAR-T cells eradicated the 3D spheroids of the HER2+ SK-OV3 as well as JIMT-1 in a dose-dependent manner. The untransduced control T cells did not influence the tumor growth in vitro. Trastuzumab displayed efficacy in 2D and 3D in SKOV3. As expected, JIMT-1 cells were resistant to trastuzumab treatment despite their positive HER2 status. The HER2- cell line Hs578T served as a negative control and proved to be resistant to any treatment in this study. The HER2-targeting CAR-T cells were applied in vivo in two different doses to JIMT-1 tumor bearing NSG mice (n=8/group) and tumor volume was measured over time. Again, the CAR-T cells were able to induce a complete remission. In contrast, the tumors displayed progressive disease under therapy with the untransduced T cells or Trastuzumab. Interestingly, the CAR-T cells induced a tumor swelling between eight- and twelve-days post injection very similar to the situation in the patient. Four weeks post injection the human T cells could be detected in spleen, peripheral blood, liver and bone marrow of the JIMT- bearing mice. The implication of this finding for possible side effects of the HER2 CAR´s remains to be elucidated. A PK/PD study is currently ongoing for this purpose. Taken together the 3D live cell imaging platform

proved to be a feasible tool for efficacy testing of biologics as well as cellular therapies. Our in house developed HER2 CAR-T cells proved to be specific and effective in eradicating the targeted cancer cells in vitro and in vivo. The mechanism behind the modulated sensitivity of the HER2+ JIMT-1 cells against HER2-targeted treatment will help to shed some light in possible resistance mechanism and hopefully have some translational value for patients suffering from this disease.

P19: Impact of Low Dietary Vitamin D Intake on Immunoregulation and Disease Pathology in Lupus-prone Mice

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Vitamin D (VD) deficiency is a highly prevalent worldwide phenomenon and extensively discussed as an environmental risk factor for the development of systemic lupus erythematosus (SLE) and other immune-mediated diseases. In addition, it is now appreciated that VD exerts a host of immunomodulatory effects, alongside its established role in the regulation of calcium and phosphate homeostasis. Here, we aimed to explore the impact of low dietary VD intake on lupus pathology in lupus-prone NZB/W F1 mice and to identify immunological effects that might contribute to disease progression. We show that low VD intake mildly accelerates lupus progression, reflected in reduced overall survival, more rapid proteinuria onset, as well as earlier and more pronounced autoantibody production. This unfavorable effect gained statistical significance with additional low maternal VD intake during the prenatal period, suggesting a role of maternal VD status in autoimmune development in offspring. Among the examined immunological effects, we found that low VD intake hampered the adoption of a regulatory phenotype in lymphocytes, significantly reducing both IL-10-expressing and regulatory $CD4^+$ T cells (Tregs). This goes along with a mildly decreased frequency of IL-10-expressing B cells. We did not observe consistent effects on phenotype and function of innate immune cells, including cytokine production and co-stimulatory molecule expression. Phagocytosis of apoptotic cells, the impairment of which is commonly observed in macrophages from SLE patients, was similarly unaffected by low VD intake. Hence, our data reveal that low VD intake

mildly promotes lupus pathology, potentially via the deviation of adaptive immunity. This study suggests that the correction of VD deficiency may serve as an important module in prophylaxis and as an add-on in the treatment of SLE and possibly other immune-mediated diseases.

P20: Targeting B and T lymphocyte attenuator (BTLA) regulates lupus disease development in NZB/W mice

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Systemic lupus erythematosus (SLE) is an auto-immune disease characterized by an excessive activation of the immune system leading to the production of auto-antibodies (auto-Ab). These auto-Ab, through immune complex formation and their subsequent deposit in targeted organs, can cause tissue damages. B cell differentiation into plasma cells requires communication between T and B cells, which is regulated by costimulatory or co-inhibitory molecules. B and T Lymphocyte Attenuator (BTLA) is an inhibitory receptor, expressed by most lymphoid and myeloid populations, whose protective role in SLE has been suggested in mice. In our laboratory, it was previously demonstrated in lupus patients i) an altered BTLA expression by a subset of memory B cells and by activated regulatory T cells and ii) an altered capacity of BTLA to inhibit $CD4^+$ T cell activation. These data led us to consider BTLA as a potential therapeutic target in the treatment of lupus.
We showed that the administration of an Ab targeting BTLA to 22-week-old lupus mice had a beneficial effect on the development of the lupus disease. Indeed, anti-BTLA treated NZB/W mice displayed a delayed onset of proteinuria, limited kidney damages and an increased survival rate. This beneficial effect was associated with a decrease in circulating B cell frequencies compared to mice administered with the isotype and required continuous exposure to anti-BTLA Ab. From a mechanistic point of view, BTLA administration leads to partial B cell depletion, reduces BTLA expression by all lymphoid cells which is not due to BTLA internalization, but doesn't prevent its binding to its ligand HVEM.

Our results are really encouraging and we now wish to define the mechanism(s) of action by which the administration of the antibody delays the onset of symptoms and to evaluate whether the administration to diseased mice can reverse the symptoms.

P21: Distinct humoral response in Africans and Caucasians: consequence on vaccine response?

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An efficient vaccine is desperately needed to fight against the worldwide HIV/AIDS epidemic. One recurrent question is whether the vaccine needs to be adapted to HIV clade, geographic and/or ethnic background. Recent attempts to reproduce and enhance the Thai RV144 vaccine efficacy in South Africa failed. The reasons for this misleading outcome may be numerous. We examined the influence of ethnicity, host genetic, and geographic background on HIV-specific antibody response in a vaccine trial performed concomitantly in USA and South-Africa

Volunteers were vaccinated with three multivalent DNA-HIV prime immunizations followed by a rAd5- HIV boost. Total and HIV-specific IgG and IgA responses were analyzed in pre-immune sera and 4 weeks after the final boost by ELISA. Antibody functions, neutralizing and antibody-dependent cellular cytotoxicity (ADCC), were determined by conventional TZM-bl and ADCC-LUC assay respectively. The Fc-receptor polymorphisms were genotyped by custom Taqman assays.

Compared to Caucasians, Africans had significantly higher total IgG and IgA. We observed a significantly higher ratio (post/pre-immune) of antibodies binding to gp140 ConS and gp160 MN-LAI in Africans. IgG recognition pattern of linear Envelope epitopes was overall comparable between the groups and was dominated by recognition of V3 and an epitope in gp41. Africans had significantly lower HIV- specific immune response background, lower neutralization ability against tier 1 virus, and lower frequency of FcR polymorphism rs10800309. Multivariate analysis showed the total IgA was correlated with a FcRIIa polymorphism independently of other parameters like sex, age, race, or location. ADCC induced following vaccination was low in both ethnic groups, therefore preventing any conclusions on the role of race, sex, and FcR polymorphism on ADCC function.

Overall, Africans induced distinct HIV-specific antibody responses after vaccination compared to Caucasians. Immunological differences between ethnic groups and regions should be studied more in detail to better understand their potential impact on HIV vaccine efficiency.

P22: Targeting the coronin 1 signaling pathway for the prevention of autoimmunity

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Autoimmune disorders and transplant rejection affect over 5% of the entire global population. Currently, these patients are treated with “non-selective” immunosuppression, aimed at targeting T cells that are the main drivers of both autoimmune disorders as well as graft rejection. However, T cell responses are equally important for the control of pathogenic microbes and cancers. Therefore, a major complication of currently used immunosuppressants includes opportunistic infections and cancers. Previous work from our laboratory has uncovered that genetic disruption of the coronin 1, a protein important for the homeostasis of T cells, allows tolerance towards allografts and prevents the development of autoimmune diseases while preserving an anti microbial pathogen immune response intact. In this work, we are developing an approach to deplete coronin 1 pharmacologically. We are currently analyzing the possible activity of these compounds in their ability to attenuate autoimmune disorders using a variety of model systems. Our preliminary data suggest the potential for targeting the coronin 1 pathway to develop novel and safe immunosuppressants.

P23: 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) targeting CTLA-4 and PD-(L)1 demonstrated potent and durable anti- tumor efficacy. Still, the majority of patients do not benefit from ICIs, while many others, who initially respond, later develop disease progression. Therefore, it is of the utmost importance to understand the mechanisms underlying therapy resistance.

The aim of our project is to understand the mechanisms of resistance to anti-PD-1/CTLA-4 combination therapy. To this end, 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. Moreover, the introduction of a second contra-lateral tumor allows us to study the tumor microenvironment (TME) at an early timepoint of response or resistance and follow the fate of the second tumor in the opposite flank of the mice. By doing this, we will obtain a longitudinal study of the tumor 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 5'UTR VDJ sequencing on intratumoral CD45+ cells, combined with multiplexed imaging.

P24: Macrophage origin during mycobacterial granuloma formation in the liver

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Mycobacterial infections pose a major threat to humanity. Best known is the disease tuberculosis caused by *Mycobacterium tuberculosis* (Mtb), which is estimated to have caused around 1.3 million deaths in 2020. The hallmark of mycobacterial infections is the formation of granulomas - organized aggregations of immune cells with macrophages as the main cell type. We sought to assess the ill-defined impact of resident macrophages in contrast to monocyte-derived macrophages during the granuloma formation.
By using intravenous infections of mice with the live attenuated *Mycobacterium bovis* BCG (Bacille Calmette-Guerin) - the only approved vaccine against tuberculosis - granulomas were forming within liver and spleen. The *Clec4f*$^{Cre\text{-}td\text{-}Tomato}$:R26-yfp reporter mouse specifically labeled resident liver macrophages, so called Kupffer cells (KCs), and enabled us to identify that granuloma macrophages passed through a KC stage and downregulated *Clec4f* afterwards. By specifically sorting granuloma core macrophages and performing RNA sequencing, we were able to identify *Mmp12* as one of the most differentially expressed genes. As MMP12 can reduce CCL2 and CCR2 expression, which are needed for monocyte recruitment, we wanted to assess the contribution of monocytes to the granuloma core. To this end, we performed irradiation experiments with bone-marrow transplantations. We observed that resident macrophages were present in the core, while monocyte-derived macrophages had a minor impact.

Furthermore, we also infected CCR2$^{-/-}$ mice, which lack Ly6C^{high} monocytes. We noticed a delay in granuloma formation compared to wildtype mice, as well as smaller granulomas. Altogether, the results indicate a necessity of monocytes to induce granulomas, while tissue resident macrophages are forming the granuloma core. Further work is required to understand this differential distribution and if the increased *Mmp12* levels in the core might be a reason for the lack of monocytes within it.

P25: Function of the Ikaros transcription factors in hematopoietic stem cells

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Hematopoietic stem cells (HSCs) sit atop the hematopoietic hierarchy which generates all blood and immune cells throughout life. This process is guaranteed by both self-renewal and differentiation, two essential features of stem cells. How these processes are regulated is still unclear, and transcription factors (TFs) have been implicated. Among them, our main interest concerns the Ikaros family. Ikaros and Helios, two of its members, are highly expressed in HSCs, and they are implicated in controlling stemness, multipotency and lineage commitment. We investigated how Ikaros and Helios control these processes using inducible murine models, which allow the deletion of one or both TFs in adult animals. We showed that both Ikaros and Helios are implicated in the proper maintenance of bone marrow hematopoiesis, to which they contribute at different levels and through specific and redundant functions. In the stem cell compartment, Ikaros positively regulates the maintenance of the HSC pool, while it appears to negatively regulate myeloid differentiation from multipotent progenitors. Helios, on the other hand, is specifically required to repress the megakaryocyte priming in HSCs. Both are required to promote the priming towards the lymphoid lineage. Our study provides new insight into the understanding of HSC regulation by Ikaros and Helios, and suggests that these factors control the self-renewal and lineage commitment of adult HSCs through specific and common mechanisms.

P26: Pulmonary macrophage heterogeneity and mycobacteria infection

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Mycobacterium tuberculosis infection is a leading cause of death worldwide. The only available vaccine, BCG (Bacillus Calmette-Guérin), is typically administered intradermally and has variable efficiency in preventing tuberculosis disease. More recently, however, alternative routes of BCG vaccination (intravenous and intrapulmonary) were shown to elicit near sterilizing immunity following Mtb challenge in non-human primates. Studies to investigate the mechanistic basis of this protection have mostly focused on epigenetic and metabolic rewiring of innate cell precursors in the bone marrow, while less is known about how BCG affects innate cells in the lung. To address this, we established a BCG immunization model in mice to examine the phenotype, function and spatial distribution of lung localized macrophages. Our preliminary data reveal that depending on the route of administration, BCG distinctly impacts the production of inflammatory cytokines and distribution of macrophage subsets in the lung. Future work will focus on assessing the longevity of these effects, their molecular underpinnings and the protective capacity of BCG-remodeled macrophages.

P27: The influence of Pseudomonas aeruginosa lectin LecB on dendritic cell migration and T cell activation in draining lymph nodes

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The interactions between T cells and dendritic cells (DCs) in the draining lymph nodes (LNs) are vital for initiating cell-mediated adaptive immune responses. LNs are strategically situated throughout the body at junctions of blood vascular and lymphatic systems to direct immune responses against antigens in peripheral tissues. When LecB, a lectin secreted by *Pseudomonas aeruginosa* (*P. aeruginosa*), is injected into mice, it may influence the host immune responses. We previously discovered that LecB binds to endothelial cells *in vitro* and *in vivo*, and disrupts the junctional VE-cadherin and the cytoskeleton leading to impaired leukocyte transmigration. However, the physiological impact was not known. Here, we show that LecB blocks the migration of DCs from the tissue via the lymphatics to the T cell zone and inhibits $CD4^+$ T cell activation in the LNs. However, a synthetic fucose-glycomimic restores DC migratory and T cell priming activity. These findings demonstrate that LecB suppresses the immune response by blocking DC migration into the T cell zone of LNs and that a synthetic LecB inhibitor could be employed to boost the anti-pathogen immune response.

P28: Tenascin-C orchestrates an immune suppressive tumor microenvironment harboring novel targeting opportunities

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The extracellular matrix (ECM) molecule tenascin-C (TNC) that is highly abundant in cancer promotes tumor progression by multiple mechanisms. Recently, we found TNC to corrupt tumor immunity. Here, mechanisms how TNC orchestrates an immune suppressive tumor microenvironment (TME) and novel tools to restore anti-tumor immunity by targeting the matrix will be presented.

In a carcinogen induced tongue tumor model TNC was found to induce CCL21 that bound TNC thereby generating an adhesive substratum for CD11c+ cells. This caused an inversion of the CCL21 gradient between lymph nodes, Treg infiltration of the tumor cell nests and retention of CD11c+ dendritic in the stroma. Inhibition of CCR7 caused release of CD11c+ and other immune subtypes from the stroma, reduced tumor growth and lymph node metastasis. This phenotype mimicked the TNCKO tumors. In the absence of TNC the matrisome and immune checkpoints were strongly downregulated revealing TNC as an orchestrator of the immune suppressive TME[1]. In the NT193 breast cancer model TNC induced CXCL12 that also bound to TNC thereby causing a sticky substratum for macrophages and CD8+ TIL, retaining these cells in the stroma thus blocking killing of the tumor cells. Inhibition of CXCR4 resulted in release of the CD8+ TIL from the matrix, infiltration into the tumor nests, tumor cell killing and reduced tumor growth and subsequent metastasis. CXCR4 inhibition phenocopied TNCKO tumors where the immune suppressive properties were largely reduced. Thus, we propose targeting "TIL-Matrix-Retention" as a novel approach to fight cancer[2]. We had recently generated TNC specific nanobodies and MAREMO peptides that blocked TIL chemoretention in vitro and may be useful to reactivate anti-tumor immune surveillance in vivo[3, 4].

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[2]Murdamoothoo et al., 2021, EMBO Mol Med 13(6):e13270

[3]Dhaouadi et al., 2021, Front Immunol 12:635166

[4]Loustau et al., 2022, Mat Bio 108, 20

P29: Contribution of CD44 expressed on tumor-associated macrophages to progression of pancreatic cancer

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The CD44 transmembrane glycoprotein family members, particularly the CD44v6 isoforms, have been shown to promote tumor growth and metastasis in different types of cancer including pancreatic ductal adenocarcinoma (PDAC). However, CD44 isoforms are not only expressed on cancer cells but also in the stroma, which makes up to 90% of the PDAC tumor volume. PDAC stromal cells include cancer-associated fibroblasts (CAF), pancreatic stellate cells (PSC), and immune cells including tumor-associated macrophages (TAMs). It has long been established that TAMs are one of the most abundant immune cell types in the tumor microenvironment (TME), and that they exhibit different phenotypes ranging from the classically activated M1 phenotype which favors inflammation to the alternatively activated phenotype which is anti-inflammatory and pro-tumoral. Since CD44 isoforms are involved in the progression of PDACs and are expressed on macrophages, the aim of this project is to investigate its potential role in the polarization of macrophages from the anti-tumor M1 phenotype to the pro-tumor M2 phenotype using the *Cd44* knockout (KO) mouse models: *Cd44*$^{fl/fl}$*; Csf1r-Cre* and/or *Cd44*$^{fl/fl}$*; Csfr1CreER*^{T2}. Using the same mouse models, we also aim at exploring the consequences of a *Cd44* KO on macrophages on tumor growth and metastasis. Finally, an interaction between CD44-expressing TAMs and other components of the TME such as the extracellular matrix (ECM) will be investigated. Such potential role of CD44 on TAMs can reveal a new mechanism by which the TAMs promote PDAC tumor growth and hence will lead to the possible development of targeted therapies against PDAC.

P30: From NSCLC tissues to tumoroids with its TME

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Despite the advances in the understanding of lung cancer pathophysiology and the development of novel therapeutic classes, treatment failures and acquired resistance are still reported by drugs' agencies and pharmaceutical industries[1]. For this purpose, tumoroids are currently being explored as they represent better physiological systems. In the implementation of such models, it is important to consider not only tumoral cells but also cells from the lung tumor microenvironment (TME) that participate to cancer progression or immunosupression[2]. We will therefore describe new optimized tumoroid model that recapitulates TME for the purpose of relevant drug screening.

NSCLC biopsies were dissociated to generate a heterogeneous suspension of tumor cells. Then, heterologous microvessels (MV) derived from adipose tissue were added to this suspension of tumor cells to generate our scaffold-free tumoroid model[3]. Once tumoroids were formed, the immunohistochemistry analysis revealed a depletion in CD45 + cells in comparison with the match patient tissue. Hence, patient autologous immune cells were added exogenously to emulate accurately the original tumor and its TME.

We show that our lung tumoroids closely recapitulate the histological characteristics of the original tumor although the addition of heterologous MV. Notably, following the addition of exogenous immune cells, we were able to generate a model with immune cells infiltrated inside the tumoroid, leading to the 1st step of an immune-oncology model. Consequently, we studied the potential effect of drugs such as chemotherapy and virotherapy on immune cell infiltration. A diminution of immune cells infiltration in tumoroids was observed with both treatments. Additional studies are in progress to assess and understand the mechanism of chemotherapy and virotherapy in this new tumoroid model. We have shown here that we reconstructed a comprehensive model that considers all the characteristics of lung cancer (tumor, immune cells and vascularization) and that accurately recapitulate patient histological features. In perspective, this model of tumoroids will be generated with exclusively autologous cells to increase the relevance of drug screening and fit the objectives of personalized medicine.

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P31: Investigating the function of coronin proteins in immune cells: Redundancy or Cooperation?

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T lymphocytes constitute an important part of the immune system: therefore, it is crucial to maintain homeostatic numbers of T cells to guarantee a continuous response to pathogenic triggers. The mammalian protein coronin 1 has been described to play a central role in T cell homeostasis. Deletion or mutation of coronin 1 in mice and humans results in profound peripheral T cell deficiency. Coronin 1 is a member of a family of 7 mammalian proteins, evolutionarily conserved from yeast to human. We have analyzed the possible contribution of other members of the coronin protein family, and results will be presented dissecting the role of such coronin protein family members in T cell homeostasis.

P32: Local antigen acquisition by B cells in brain and its potential to cause an autoimmune response

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B lymphocytes, target of recent anti-cd20 treatments, are known to excessively infiltrate the MS diseased brain and impacting the gadolinium enhanced lesion formation. To trigger B cell infiltration in the brain parenchyma of BCR transgenic mice with B cells specific for viral antigen or MOG, we injected genetically modified viruses in the striatum of the brain or the skin of the ear, to investigate whether B cells can acquire antigen locally. Using immunofluorescence and intravital two-photo imaging, we characterized antigen specificity independent B cells to infiltrate the brain 2 days p.i. in the perivascular space and from 5 days p.i. on, in the parenchyma. Additionally, intimate contact between infiltrated B cells and antigen, including MOG antigen acquisition and presentation from IgH MOG B cells to MOG specific 2D2 T cells have been examined using ELISpot. Activation-induced cell death of IgH MOG B cells was demonstrated using scTranscriptomics, as the number of infiltrated B cells in IgH MOG mouse brains decreased over time, proved by flow cytometry and immunofluorescence. If CD40L was provided to B cells in the brain, B cells could have been rescued and significant demyelination was observed, when comparing to the wildtype control mice, obtained by immunofluorescence analysis. Our results show that viral brain infection facilitates the infiltration of B cells including the autoimmune B cells, which can acquire their antigen locally in the tissue suggesting that the antigen acquisition is not limited to lymph nodes. Upon CD40-CD40L interaction which can be provided by latent EBV infection they can evade AICD and thereby circumvent an important immune check point. Our study provides new insights to understand the possible mechanisms of B cell pathogenesis in MS.

P33: Function of the RANK-RANKL axis in B cell associated stroma and LN formation

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LNs are structured into B cell follicles and T cell zones required for optimal immune response and regulation. B cell follicle formation and function depend on the CXCL13 chemokine produced by mesenchymal stromal cells such as Follicular Dendritic Cells (FDCs) and Marginal Reticular Cells (MRCs). However, the underlying molecular and cellular mechanisms for $CXCL13^{+}$ B cell stroma formation are incompletely understood. Here, we demonstrated its dependency on the RANK (Receptor Activator of NF-kB)-RANKL (Receptor Activator of NF-kB Ligand) axis. In mice lacking RANK in Lymphatic Endothelial Cells (LECs), B cell stroma was severely impacted, based on the reduced expression of B cell stroma markers CXCL13 and CD35. The loss of B cell stroma was associated with disorganization and a reduction in B cell numbers. LEC activation may rely on RANKL produced by Lymphoid Tissue Organizer cells (LTOs) and MRCs. However, mice lacking RANKL in LTO/MRCs only present an incomplete phenotype suggesting other cellular sources of the ligand. It was recently shown that Subcapsular Sinus Macrophages (SSM) are also dependent on RANK-RANKL signaling. In light of the requirement for the RANK-activated LECs to instruct a niche for SSM, we are currently exploring the possibility that lymphoid tissue inducer cells and B cells are similarly responding to niche signals. The local clustering of all three hematopoietic cell types may be required for MRC and FDC formation. LNs are very important for the formation of an efficient humoral response and understanding the mechanism of their formation and organization might open new therapeutical targets for autoimmune diseases or cancer.

P34: Epidermal maintenance of Langerhans cells relies on autophagy-regulated lipid metabolism

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Autophagy is a cellular mechanism that prevents the accumulation of harmful cytoplasmic components and mobilizes energetic reserves. Thus, it is especially important in the homeostasis of long-lived, self-renewing cells. Autophagy deficiency affects antigen presentation in conventional dendritic cells without impairing their survival. To date, no study has addressed the role of autophagy in Langerhans cells (LCs), the epidermal dendritic cells, which are maintained due to their proliferative capacities and extended lifespan. We generated $Atg5^{DCd207}$ and $Atg7^{DCd207}$ mouse models, where $CD207^{+}$ LCs become autophagy-deficient through the deletion of Autophagy-related gene 5 (Atg5) or Atg7. Autophagy-deficient LCs were gradually depleted from the epidermis and showed deregulated lipid metabolic pathways. Atg5-deficient LCs accumulated neutral lipid droplets, which eventually led to the accumulation of toxic lipidic reactive oxygen species and ferroptosis. LC ablation resulted in the upregulation of pro- inflammatory transcripts and decreased the innervation of the epidermis. Therefore, we reveal that autophagy represents a critical regulator for lipid metabolism in LCs and is necessary for their maintenance and for the epidermal homeostasis.

P35: Tenascin-C regulates TRAIL function in the NT193 model and breast cancer

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The death ligand TRAIL was promised to eradicate cancer, however clinical trials have not yet success. In MMTV-NeuNT and syngeneic tumor grafting breast cancer model we describe a novel function of the extracellular matrix molecule tenascin-C (TNC) in immune-suppression of cancer by counteracting TRAIL. Although cultured tumor cells expressed TRAIL and its receptor DR5, and were sensitive to killing by recombinant TRAIL, cells expanded and were metastatic. To understand TRAIL actions, we grafted tumor cells with a knockdown of TRAIL and observed bigger tumors and more lung metastasis which correlated with reduced infiltration of myeloid cells as determined by flow cytometry. No difference in tumor growth was seen upon grafting of cells with lowered DR5, supporting that TRAIL could regulates anti-tumor immunity. By using conditioned medium from tumor cells with lowered TRAIL we observed reduced myeloid cell invasion into tumor spheroids and the migration is CXCR4 dependent. TNC downregulating TRAIL expression via integrin α9β1, and physically confining TRAIL in the stroma as shown by surface plasmon resonance spectroscopy and negative electron microscopy. Moreover, through epithelial-to-mesenchymal transition, TNC conferred cancer cells resistance to TRAIL killing by lowering DR5 expression. Kaplan Meier analysis demonstrating combined low TNC with high TRAIL correlate with longer human breast cancer patient survival. Altogether our study demonstrated an important function of TNC in counteracting TRAIL which harbors therapeutic potential through cancer cell killing and myeloid cells recruitment.

P36: The role of Sialoglycans and Sia-binding immunoglobulin-like lectins (Siglecs) in modulation of human myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment

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Evasion of immune destruction and the generation of a tumor-promoting environment are important hallmarks of cancer and can be promoted by overexpression of sialic acid on glycans of tumor cells. Those sialoglycans can bind to inhibitory Siglec receptors on immune cells – like T cells – and thereby inhibit their immunoregulatory function.

Recently, it was shown that Siglecs are also expressed on the surface of MDSCs of glioma patients. Interestingly, elevated numbers of MDSCs are found in many cancer patients and can favor disease progression by limiting anti-tumor immunity for example through T cell inhibition.

Of note, we found high expression of Siglec-5, Siglec-7, Siglec-9 and Siglec-10 on MDSCs from lung cancer patients and healthy donors. To evaluate the functional role of Siglecs on the surface of MDSCs, we established an assay to generate highly immunosuppressive MDSC-like cells in vitro. Interestingly, decreasing the levels of Sialoglycans on MDSCs using neuraminidase resulted in less suppressive MDSCs.

Functional analysis of Siglec-E knockout, the murine paralog of Siglec-9, in the myeloid compartment in vivo showed survival benefits compared to their litter mates with functional Siglec-E on MDSCs. Of note, MDSCs lacking Siglec-E resulted in less CD8 T cells in the tumor in vivo and showed less immunosuppressive capacity in vitro.

In summary, our results provide first insights into the importance of Siglecs and Sialoglycans in mice and humans to modulate MDSCs in the TME. Further studies are needed to reveal the underlying mechanisms.

P37: Targeted deletion of Cd44 from pancreatic stellate cells impacts tumor growth

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The CD44 family of cell adhesion molecules is highly involved in tumor progression and metastasis. Most of the studies on CD44 however, relate to its function on tumor cells and very little is known on the contribution of CD44 proteins expressed on cells of the tumor microenvironment. The interplay between stromal components and cancer cells has a tremendous influence not only on tumor growth but also on metastasis formation. Interestingly, there are several evidence suggesting that CD44 isoforms are playing a paramount role in this tumor-stroma interaction. For example, a tumor stroma rich in the extracellular matrix component hyaluronan, the main ligand of CD44, induces the activation of stromal cells thereby enhancing tumorigenicity. Moreover, matrix metalloproteinase-9 expressed by activated stromal cells, is shown to be a processing enzyme for CD44 cleavage stimulating cell motility and migration. Our group has already demonstrated that CD44 expressed on endothelial cells plays a role in cancer progression, suggesting a tumor promoting role for CD44 expressed in the surrounding stroma. Using $Cd44^{fl/fl}$; *GFAPCre* mice, in which *Cd44* is specifically removed from pancreatic stellate cells, we could demonstrate that the loss of *Cd44* decreases the size of orthotopic primary tumors and influences the tumor tissue structure. Transcriptomic analysis of the tumors showed an impact on collagen expression as well as the activation of T cells both contributing to a desmoplastic, highly fibrotic and immunosuppressive environment. We identified a role of CD44 in the TGFβ/SMAD signaling-dependent activation of pancreatic stellate cells inducing the secretion of the extracellular matrix protein Col1a1 as well as their migratory and invasive ability. Moreover, CD44 is involved in the induction of an epithelial-mesenchymal transition-like process during the activation of stellate cells, all steps that are crucial in facilitating local and distant invasion of tumors.

P38: A coronin 1-dependent kin-to-kin density-sensing pathway defines T cell population size

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The maintenance of appropriate cell population size is fundamental to the proper functioning of multicellular organisms, yet the underlying mechanisms remain largely undefined. For T cells, the factors required for their sustained survival in the peripheral lymphoid tissues are well described, but it is unclear how the homeostatic population size is defined. Here, we describe a cell-intrinsic kin-to-kin density-sensing pathway that allows T cells to define their appropriate population size. Cell density-dependent expression of coronin 1 protein coordinated pro-survival signaling with inhibition of cell death until the cell population reached threshold densities. At or above threshold densities, coronin 1 expression leveled off allowing for the initiation of apoptosis through kin-to- kin adhesin signaling to return the cell population to homeostatic cell size. Our data suggest the existence of a coronin 1-regulated homeostatic mechanism by which cells are informed of and coordinate their population size.