TRANSLATIONAL IMMUNOLOGY –
FROM TARGET TO THERAPY IX

TWINSIGHT

TRANSLATIONAL TWINNING IN SYSTEMS IMMUNOLOGY
AND HIGH-THROUGHPUT TECHNOLOGY

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POSTER ABSTRACTS

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TWIN SIGHT

TWINSIGHT Clinician Scientist Project – The ERK5/KLF pathway as potential universal target for interference with therapy resistance in melanoma

01

Authors

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State-of-the-art systemic therapies using immune checkpoint blockade (ICB) and targeted MAPK inhibition (MAPKi) have drastically improved the prognosis of metastatic malignant melanoma, but are often limited by the development of resistance.

MAPKi leads to compensatory activation of the ERK5/KLF signalling pathway, which contributes to proliferation and survival under MAPKi and thus to resistance. However, ERK5 has also recently been reported to promote formation of tumor-associated M2 macrophages and suppress the TH1 polarisation of T cells in some tumours by secreting interleukin 6 (IL-6) thereby contributing to tumor-induced immune suppression.

Therefore, the question arises whether the ERK5/KLF signalling pathway is also involved in ICB resistance in malignant melanoma, e.g. by regulating IL-6 secretion by tumour cells and subsequently suppressing the T cell response.

This will be evaluated (A) by investigating the influence of ERK5 on IL-6 secretion and the influence of melanoma cells on immune cell responses and (B) by characterising melanoma cells and their microenvironment in resistant ICB patients using single-cell RNA sequencing. In the future, the use of ERK5 inhibition as a therapeutic option for ICB resistance will be investigated..

Gut Commensal Lactobacillus murinus Ameliorates Hypertensive Organ Damage

02

Background

Gut microbiota play an important role in the development of hypertension. We aimed to identify disease modulating factors in the microbiome using narrow-spectrum antibiotic modulation of the gut microbiome. Thus, using antibiotics lacking enteral absorption, we specifically aimed to deplete gram-negative or -positive bacteria in double transgenic rats (dTGR, transgenic for human renin and angiotensinogen) and analyzed microbiome composition, inflammation and hypertensive target organ damage.

Methods

Four-week-old dTGR (transgenic for human renin and angiotensinogen) were treated with oral Vancomycin (Vanco), Polymyxin B (Poly) or Vehicle (Veh) for 3 weeks. Seven-week-old SD rats were included as healthy controls. Microbiome, clinical and immune phenotype were analyzed by shotgun metagenomic sequencing, echocardiography, telemetric blood pressure (BP) measurement, clinical chemistry, bulk RNAseq and flow cytometry. For further investigations in another mammalian species, hypertensive wild-type NMRI mice received Lactobacillus murinus or control per daily gavage, as well as i.p. injections of the aryl hydrocarbon receptor (AhR) inhibitor CH-223191. To induce hypertension, mice were infused with angiotensin II (1.44 mg·kg−1·d−1 subcutaneous) for 14 days. Hypertensive organ damage was assessed using clinical chemistry, qPCR, histology, echocardiography, and flow cytometry.

Results

Independent of BP levels, hypertensive organ damage of rats was ameliorated by Vanco treatment as assessed by albuminuria and cardiac hypertrophy indices. Poly treatment did not affect organ damage. BulkRNA sequencing revealed altered AhR-related gene expression in the heart and kidneys after Vanco treatment. Vanco treatment led to a bloom of fecal Lactobacillus murinus alongside fewer pro-inflammatory immune cells (e.g., Th17) in the kidneys and the gut. Targeted oral administration of Lactobacillus murinus to hypertensive mice mirrored these benefits, decreasing kidney damage markers (Lcn2 expression, proteinuria) and inflammatory immune responses.

Conclusion

Our findings highlight the gut microbiome's role in modulating hypertensive organ damage. We identified gut commensal Lactobacillus murinus as the key microbe responsible for these beneficial effects. We are currently investigating the mechanism involved to identify potential targeted therapies for hypertensive organ damage.

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Aging remodels the epidermal T-cell network through interleukin 17A/F

03

Authors

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Aging is one of the major causes of skin pathology as it leads to skin susceptibility to inflammation, infection, and delayed wound healing. The skin, the body's largest barrier, is exposed to environmental threats and relies on immune cells for protection and maintenance of immune homeostasis. In the epidermis, dendritic epidermal T cells (DETC) and Langerhans cells (LC) maintain homeostasis and immune surveillance. How aging affects the epidermal immune network has not yet been investigated. While mouse ear and back models are commonly used, their tissue structure differs from the human epidermis, which has undulating microstructures called rete ridges. Recent studies revealed that the microstructures of the human skin can be recapitulated in the mouse tail epidermidis. Interestingly, the distribution of DETC and LC follows this microarchitecture. In contrast to the uniform immune network in the ear epidermis, DETC and LC in the tail are located exclusively in the mouse equivalent of the inter-ridge region, creating immune-privileged areas linked to melanoma development and susceptibility to inflammation and infection.

We investigate how aging affects the immune network, DETC and LC, in mouse tail. After embryonic seeding and perinatal expansion in the epidermis, DETC and LC remained constant until adulthood. From six months of age, the number of DETC decreased dramatically and they were virtually absent by 18 months. To compensate for the loss of DETC, dermal T cells infiltrate the epidermis in addition to a slight expansion of the LC. To gain insight into the underlying mechanisms, we first investigated the influence of the microbiota on the age-mediated shift of the epidermal T cell network using germ-free mice. In the absence of microbiota, DETC loss was delayed and contained, suggesting an indirect effect of the microbiota. We next investigated the contribution of the interleukin (IL-) 17 as a key player in host-microbial interactions at the tissue barrier. In the absence of IL-17A/F or the inducer cytokine receptor, IL-23 receptor, the epidermal DETC were maintained in the epidermis. Our data showed that aging remodels the epidermal immune network through IL-17, suggesting a degeneration of the epidermal immune network. Further investigations will address the origin of the newly arrived dermal T cells in the aged skin and understand the functional consequences.

Analysis of the immune system under anti-IL17 therapy and correlation with treatment response in patients with hidradenitis suppurativa

04

Authors

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Hidradenitis suppurativa (HS) is a chronic, underdiagnosed inflammatory skin dissease characterized by painful, draining lesions with unpleasant odors, disfiguring scars, and significant psychosocial burden. It affects approximately 0.4% of the population in Germany.

HS is characterized by abnormal differentiation of keratinocytes in the hair follicle-sebaceous gland unit and persistent inflammation [(1)]. Interleukin (IL)-17 and tumor necrosis factor-alpha (TNF-α) have been identified as key players in the inflammatory process. However, many patients do not respond adequately to therapy with IL-17 or TNF-α inhibitors.

Accordingly, TH1 and TH17 signatures were detectable in both lesional and peri-lesional skin of HS patients [(2)]. The development of TH17 cells is driven by IL-23, IL-1β, and IL-6, produced by innate immune cells such as dendritic cells and macrophages [(3)]. IL-17, produced by TH17 cells, further promotes the production of chemokines, recruiting neutrophils and innate lymphoid cells (ILCs) in advanced stages of HS. Furthermore, increased numbers of IL-17-producing CD4+ T-cells have been observed in HS lesions. CD163+ tissue macrophages infiltration was lower in HS- compared to healthy skin [(4)]. Because CD163 is a typical marker of anti-inflammatory M2 macrophages, we hypothesize that an increased recruitment of this macrophages subset in HS lesions under anti-IL-17 therapy in the tissue at initiation or during therapy of this therapy correlates with treatment response [(5, 6)].

This could explain why IL-17 inhibitors such as secukinumab and bimekizumab show notably lower response rates in HS (45%) than in psoriasis (>85%). Different origins of inflammation may account for this gap in response between HS and psoriasis. In HS, the hair follicle, which is the source of inflammation, could represent a privileged niche.

The primary focus of the research is to analyze changes in the immune system (macrophages, dendritic cells, neutrophils, B cells, T cells, fibroblasts) under IL-17 inhibition. Specifically, the study will examine whether the presence of anti-inflammatory M2 macrophages in HS lesions prior to therapy or their increase during treatment correlates with clinical response.

Therefore, we will collect tissue and blood samples from 39 HS patients with inguinal lesions before the initiation of treatment with an IL-17 antagonist. After 6 months, patients will be categorized as responders (HiSCR 75) and non-responders (HiSCR 50). Tissue from 4 responders and 4 non-responders will be collected before and after treatment and subsequently analyzed alongside blood samples.

The interaction of innate immune cells with regulatory T-cells and TH1, TH2, and TH17 cells, B-cells and the non immune compartment will be assessed using spatial transcriptomics, scRNASeq FACS analysis, immunohistochemistry, immunofluorescence and functional *in vitro* assays.

In addition, an AI-based mobile application will be used to track disease activity and treatment response. Patients will document their condition weekly using the app, which includes skin images, questionnaires on pain (VAS), quality of life (DLQI). This data will be correlated with clinical and immunological findings to identify predictive factors for treatment outcomes.

The results aim to provide insights into the immune mechanisms that contribute to varying responses to IL-17 inhibitors in HS, ultimately guiding personalized treatment strategies.

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The potential role of long-chain acyl-CoA synthetases (ACSLs) in tumor-adipocyte interaction at the invasive front of Colorectal Carcinoma (CRC)

05

Introduction

Recent findings suggest that adipocytes in the CRC tumor microenvironment (TME) contribute significantly to the lipid metabolic reprogramming of colorectal cancer (CRC) cells, which promotes CRC progression. Recently, a new concept (histopathological pattern) of tumor-adipocyte interaction has been introduced, called SARIFA (Stroma AReactive Invasive Front Areas). SARIFA was reported to be associated with poor clinical outcomes in gastrointestinal cancers. We examined the contribution of ACSLs to CRC lipid metabolic reprogramming initiated by direct contact between tumor cells and adipocytes at the CRC invasive front.

Materials and Methods

Direct contact between tumor cells and adipocytes (SARIFA status) was assessed using H&E-stained slide analysis of 130 primary CRC samples. Immu-

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nohistochemistry and multiplex immunofluorescence were used to analyze the expression and spatial distribution of ACSLs (ACSL1, ACSL3, ACSL4, ACSL5, and ACSL6).

Results

We found that SARIFA was significantly associated with the clinicopathological data of CRC patients, including tumor differentiation, lymph node metastasis, and vascular and lymphatic invasion. Additionally, we found that SARIFA-positive cases have significantly poorer survival rates than SARIFA-negative cases. Primary CRCs exhibiting direct tumor cell-adipocyte interaction sites at the invasive front (SARIFA-positive tumors) were significantly associated with high ACSL3 expression ($p = 0.002$). Multiplex immunofluorescence revealed high ACSL3/ACSL5 expression, particularly in tumor cells in direct contact with adipocytes. Interestingly, ACSL3 expression was increased in both tumor cells and adipocytes, suggesting its role in tumor-adipocyte crosstalk. High ACSL3 expression in primary CRCs was significantly associated with low CD8+ T cell infiltration.

Conclusion

Our data suggest that ACSL3/ACSL5 may play a role in lipid metabolic reprogramming induced by direct tumor-adipocyte interaction at the CRC invasive front. Further research is required, mainly focusing on the role of ACSL3 in tumor-adipocyte crosstalk using co-culture models of CRC cancer cells with adipocytes.

Cell type specific effects on Vγ9Vδ2 T cell activation and Vγ9Vδ2 TCR binding for cell surface expressed Butyrophilin (BTN) 2A1 proteins and immobilized BTN-Fc constructs

06

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Vγ9Vδ2 T cells are the major subset of γδ T cells, comprising 1-5 % of human peripheral blood T lymphocytes. These cells detect cancerous cells and cells infected by pathogens. They respond to phosphoantigens (PAg), phosphorylated intermediates of the isoprenoid synthesis of microbial or host origin via butyrophilins (BTN) expressed by target cells. The binding of PAg to the B30.2 domain of BTN3A1 functions as a molecular glue initiating the intracellular interaction between the B30.2 domains of BTN2A1 and BTN3A1. This complex of BTN2A1 homodimers and BTN3A heterodimers alters the BTN topology, resulting in the interaction of the V domains of BTN2A1 and BTN3A2 with the Vγ9Vδ2 T cell receptor (TCR). In this particular instance, the T cell receptor undergoes a conformational change, thereby facilitating the binding of BTN2A1 to the Vγ9 chain and BTN3A2 to the Vδ2 chain.

As BTN2A1 is a cognate ligand for Vγ9Vδ2 TCR and is mandatory for Vγ9Vδ2 T cell activation by Pag. The effects of altered BTN2A1 expression on Vγ9Vδ2 T cell activation were tested using PAg-dependent activation of 53/4 γδ TCR MOP hybridoma cells by different cell lines as a readout. Surprisingly, we observed that BTN2A1 overexpression in 293T (stable clone derivative of

HEK293) as stimulatory cells exhibited a reduced PAg-response. However, this immunosuppressive fashion was not exhibited by the human RAJI B cell lymphoma. Furthermore, the suppression was found to be PAg-specific, as it was not observed in the activation of NY-ESO-1 tumor peptide-specific TCR-transduced Jurkat cells.

The generation of soluble BTN2A1 molecules is of considerable interest for tumor therapy, owing to the pivotal role of BTN2A1 as a ligand for the Vγ9Vδ2 T cell receptor (TCR). These molecules enable the investigation of BTN2A1-mediated immunomodulatory functions. While all γδ TCR MOP tetramers bound to cell surface expressed BTN2A1, the binding efficiency of immobilized BTN2A1-Fc fusion proteins differed depending on the cellular origin of their production. The experiment involved the coating of human Fcγ receptor 1A (CD64A)-transduced cells or cell-sized protein A/G beads with BTN2A1-Fc constructs or CD80-Fc constructs (negative control). Subsequently, the cells were stained with Vγ9Vδ2 TCR tetramers. While BTN2A1-Fc constructs produced by ExpiCHO (high-expressing CHO cell line) showed clear Vγ9Vδ2 TCR tetramer binding, constructs produced by HEK293 did not demonstrate this binding characteristic. This finding suggests that TCR-binding is modulated through post-translational modifications that are unique to specific cell types.

In summary, the initial finding indicates that BTN2A1 overexpression in specific cell types may function as a suppressor of the PAg-induced response against certain tumors and cell types. Therefore, it is of interest for the development of Vγ9Vδ2 TCR or BTN-targeting tumor therapies. Secondly, cell-specific post-translational modifications of BTN2A1-Fc constructs likely modulate Vγ9Vδ2 TCR binding. These effects on ligand binding are of wider interest, as BTN(L)-Fc constructs produced by HEK 293 cells are used to analyze BTN(L) molecule-mediated ligand binding and immune modulation.

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GLUT3-mediated Redox Balance in CD8+ T Cells enhances antimicrobial response against *Staphylococcus Aureus*

07

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Staphylococcus aureus (S. aureus) is a common opportunistic pathogen and a leading cause of healthcare-associated infections. The innate immune system primarily uses neutrophils as its first line of defense against S. aureus, while Th17 cells also play a key role by attracting neutrophils to the infection site. However, the role of CD8+ T cells in S. aureus infections is less well understood.

CD8+ T cells are crucial components of the adaptive immune system and contribute to immune responses against both intracellular and some extracellular pathogens, including S. aureus. For a robust antibacterial response, CD8+ T cells need to activate and differentiate into cytotoxic effector T cells (Teff), which have antimicrobial functions. This activation process requires metabolic reprogramming, where naive CD8+ T cells switch from oxidative phosphorylation to aerobic glycolysis, providing the energy and resources for rapid expansion and effector function. A critical part of this shift is the upregulation of glucose transporters to boost glucose uptake.

In this study, we identify a novel function of glucose transporter 3 (GLUT3) in supporting redox balance in activated CD8+ T cells. Effector T cells lacking GLUT3 maintain normal glycolysis and also mitochondrial respiration but fail to reduce intracellular ROS levels, leading to increased cell death and accelerated terminal differentiation. This impairs their ability to mount effective antimicrobial responses. Notably, this function of GLUT3 is largely conserved in human CD8+ T cells, as shown through CRISPR/Cas9 genome editing. These findings highlight potential translational opportunities for enhancing CD8+ T cell responses in the treatment of bacterial infections, particularly those involving antibiotic-resistant S. aureus.

Anti-inflammatory effect of TNFR2-mediated Treg activation is diminished in PD

08

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Background

Parkinson's disease (PD) is an incurable neurodegenerative disorder that significantly deteriorates patients' quality of life. It is characterized by the loss of dopaminergic neurons in the substantia nigra (SN) and accumulation of aggregated alpha-synuclein (aSyn) in the brain. Overexpression of this protein in animal models results in PD-like characteristics, such as neuronal loss and motor dysfunction. Both PD patient autopsies and animal models also demonstrate T-cell infiltration to the brain and microglia activation as a result of the aSyn accumulation in the SN. CD4+ T cells from PD subjects are alpha-Synuclein specific and are neurotoxic to PD-derived neurons *in vitro*. Importantly, lack of total CD4+ T-cells eliminated the neuronal loss in an aSyn mouse model of PD, pointing towards T-cells driven neuroinflammation as a crucial part of PD pathology. Thus, modulating T-cells towards an anti-inflammatory response to aSyn may be a promising tool to halt neurodegeneration in PD.

The tumor necrosis factor receptor 2 (TNFR2) agonist, NewSTAR2, can effectively increase anti-inflammatory regulatory T-cells (Treg) levels and suppressive function *in vivo* in wt animals. Hence, we utilized NewSTAR2 to activate Tregs aiming to reduce the inflammatory response and neurodegeneration in a PD mouse model.

Methods

To model the neuroinflammation, neurodegeneration and motor dysfunction, we injected an adeno-associated virus (AAV) encoding the mutated form of human alpha-synuclein (haSyn) or an empty vector (EV) into the SN. Animals were treated intraperitoneally with the NewSTAR2 or phosphate-buffer saline (PBS) once per week. Ten weeks after the AAV injection, Tregs functioning was evaluated via the conventional T-cells (Tconv) suppression assay. The effect of NewSTAR2 on PD pathology was assessed via immunohistochemistry (IHC) in the brains. Total splenocytes response to the NewSTAR2 stimulation was studied in culture and assessed by Flow cytometry (FC).

Results

TNFR2 activation *in vivo* led to increased Treg suppressive function in both EV and haSyn mice. However, haSyn-induced neurodegeneration was unaffected by the restored Treg functionality. *In vitro*, Tregs of PD animals could be successfully activated and expanded with NewSTAR2 when cultured in isolation. However, in the presence of other cell types, haSyn-derived Tregs do not expand in response to NewSTAR2. Moreover, pro-inflammatory TNFR2+ Tconv show increased activation upon TNFR2 stimulation in cells from haSyn, but not EV condition.

Conclusions

NewSTAR2 increased suppressive function *in vivo* and expanded Treg *in vitro* in PD and control conditions. Nevertheless, activation of these anti-inflammatory T-cells failed to provide neuroprotection. We revealed a type of pro-inflammatory cells that change upon TNFR2 activation in PD mice, which might decrease the effectiveness of immunosuppressive Treg function. These findings suggest the need for specific targeting of Tregs in order to provide successful therapy in this incurable disease.

Long-lasting reprogramming of immune-cell-stroma circuits in the lung following repeated pathogen exposure

09

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Life-long pathogen exposure to pathogens alters immune responses and affects gene expression across various organs, raising concerns about the translational relevance of “clean” specific pathogen-free (SPF) laboratory mice for modeling human tissue immunity. SPF mice lack differentiated and mucosally distributed memory T cells, in contrast to pathogen-experienced (“dirty”) mice, which display immune cell populations and gene signatures more closely resembling those of adult humans.

To address this gap, we established a sequential infection model (SIM) to generate specific pathogen-experienced (SPE) mice under BSL2 conditions, using sequential exposure to murine γ-Herpes virus (MHV-68), mouse Cytomegalovirus (MCMV), and Influenza strain WSN—murine analogs of prevalent human pathogens EBV, CMV, and IAV.

Flow cytometric analyses of SPE mouse lungs revealed significant increases and alterations in both circulating and tissue-resident immune cell populations. Single-cell mRNA sequencing of CD45+ and CD45- lung cells from SPF and SPE mice, both at resting memory and following respiratory viral challenge, demonstrated long-lasting changes in immune-related gene expression patterns in both, hematopoietic and non-hematopoietic compartment.

Thus, our SIM approach enables the study of immune responses in a potentially more physiologically relevant context and provides a platform for investigating the mechanisms by which life-long pathogen exposure shapes immune function.

Single-cell study reveals clonally expanded CD8+ non-CAR T cells in cerebrospinal fluid in a case of parkinsonism after treatment with ciltacabtagen-autoleucel

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Introduction

The use of ciltacabtagen autoleucel (cilta-cel) in RRMM patients within the CARTITUDE-1 study has exposed previously unknown late onset neurotoxicities, referred to as movement, neurocognitive treatment emergent events (MNT)[(1)]. We report the case of MNT in a patient who developed a clinical syndrome of parkinsonism 30 days post CAR T infusion. By day 57 parkinsonism worsened, reflected by an elevated MDS -UPDRS score. Multimodal analysis, including flow cytometry, and simultaneous scRNA- and scTCR-seq, was performed on peripheral blood (PB) and cerebrospinal fluid (CSF) longitudinally to the course of illness.

Results

CAR T cell expansion in PB peaked in the first month post infusion, while peak infiltration of the CSF occurred at day 57 coinciding with parkinsonism deterioration. We performed scRNA-seq and scTCR-seq on 8 longitudinally collected samples (day 20- day 204 post CAR-T) of the patient's CSF and 6 matched PB samples. CAR T as well as non-CAR T cells were present in the CSF with CAR-T proportions decreasing over time. CD8+ as well as CD4+ CAR T cells showed marked expression of cytotoxicity associated genes (PRF1, granzymes, GNLY). While CAR T cells did not clonally expand, scTCR-seq revealed clonally expanded CD8+ non-CAR T cells in the CSF concurring with the deterioration of parkinsonism. Major CD8+ non-CAR T clones were detectable from day 30 post CAR T, albeit at low numbers, and were also detectable in the PB. Phenotypically, these clones were marked by the expression of cytotoxicity genes. Furthermore, we observed an increased interferon response in the CSF that preceded the deterioration of the patient, which was absent in PB.

Conclusion

Our case study provides for the first time longitudinal single-cell CSF data of a patient with late neurotoxicity and uniquely highlights expanded CD8+ non-CAR-T cells potentially driving parkinsonism deterioration. This aligns with recent findings that implicate a role of CD8+ T cells in neurodegeneration[(2,3)].

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Optimizing CAR-T Cell Therapy: Dual Targeting and Metabolic Reprogramming to Improve Efficacy and Persistence

11

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Introduction

Chimeric antigen receptor-(CAR-) T cell therapies have shown promising responses in many patients with hematologic malignancies. However, two major challenges often lead to disease relapse: the loss of tumor-associated surface antigens and the limited persistence and effector function of CAR-T cells after reinfusion. These issues reduce long-term treatment success and highlight the need for improved CAR-T cell design and functionality.

Methods and further approach

To address antigen loss, we explored a dual-targeting strategy using CAR constructs directed against two distinct tumor antigens – BCMA and SLAMF7 – and tested for their efficacy in multiple myeloma (MM) cell line models.

In parallel, we aim to enhance CAR-T cell persistence through metabolic engineering. Specifically, we are targeting ATP-citrate lyase (ACLY), a key enzyme in de novo lipogenesis that may also influence the effector differentiation of T cells via epigenetic regulation. By inhibiting ACLY pharmacologically and genetically via 2-Hydroxycitrate (2-HC) and CRISPR/Cas9 genome editing, respectively, we seek to maintain the stemness and polyfunctionality during the manufacturing of CAR-T cells. This approach is expected to favor the generation of long-lived memory-like T cells with improved persistence and therapeutic potential.

Intermediate results and outlook

The dual-antigen targeting approach showed selective cytotoxicity and good killing efficacy in MM cell lines. Notably, this strategy also enhanced the efficacy of CAR-T cells in an antigen escape model, in which tumor cells expressed only one of the targeted surface markers.

Furthermore, we have found that suppression of ACLY favors memory-like features in murine T cells during both acute and chronic viral infections. Building on these findings, we aim to test the hypothesis that ACLY inhibition similarly enhances the longevity, persistence and functional capacity of human CAR-T cells. In our preliminary experiments, treatment of human T cells with the ACLY inhibitor 2-HC resulted in reduced secretion of inflammatory cytokines upon restimulation, indicating a shift toward a less differentiated, memory-associated phenotype. Furthermore, phenotypic analysis revealed that BCMA CAR-T cells treated with 2-HC for six days maintained a more naïve-like phenotype compared to untreated controls.

Conclusion

Combining dual-targeting CAR constructs with metabolic engineering represents a promising strategy to overcome the limitations of current CAR-T cell therapies. This approach may improve both efficacy and durability of treatment in MM and potentially other malignancies.

Identifying the immune cells mediating neuropathology in Parkinson's disease

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Authors

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Introduction

Parkinson's disease (PD) is characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra (SN), with increasing evidence suggesting that immune dysregulation and neuroinflammation play significant roles in disease progression. Recent studies have focused on the immune cell interactions as key contributors to the pathophysiology of PD. However, it is crucial to understand the immune cell types infiltrating the brain and their interplay with neurons. In this study, we use a combination of cell culture models and advanced spatial transcriptomics in a mouse model of PD to identify the specific immune cell subtypes contributing to PD progression.

Objective

To investigate the specific immune cell populations infiltrating the brain in a PD model through spatial transcriptomics and elucidate the role of CD8+ T cells in PD-associated neurodegeneration *in vitro*.

Methods

The study used a PD model where wildtype mice are stereotactically injected with an Adeno-associated Virus serotype 1/2 (AAV1/2), that is empty (control) or expressing the human mutant form of α-Synuclein (hαSyn) into the SN. The brains of these mice were harvested for spatial transcriptomics and the splenic CD8+ T cells for co-culture with either control of hαSyn-expressing MN9D neurons. Apoptotic activity was measured through immunocytochemistry for caspase3, while cytokine levels in the co-culture media were quantified using IsoLight.

Results

From our *in vitro* co-culture experiments, we observe a PD-specific increase in caspase3 signaling, indicating an alpha-synuclein specific CD8+ T cell mediated neurodegeneration observed as enhanced apoptotic activity and neuronal loss in PD models. Furthermore, cytokine analysis from the hαSyn CD8+T cell with hαSyn MN9D neurons co-culture showed a significant increase in pro-inflammatory cytokines, supporting the notion that CD8+ T cell mediated neuroinflammation is a critical driver of PD progression. Spatial transcriptomics displayed a marked elevation in astrocyte containing spots in hαSyn PD mouse model brains.

Conclusions

Our study suggests the role of dysregulated immune cells in PD progression. We show that CD8+ T cells can exacerbate neurodegeneration in an α-Synuclein-specific manner. In addition we also observe an upregulation of astrocytes in PD brains compared to EV. These findings contribute to a deeper understanding of PD pathology and provide new insights that can help to develop therapeutics targeting these specific cell types.

Distinct preferences of CD8+ and CD4+ CAR-T cells for transcription factor enhancement

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Authors

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Background

Transcription factor (TF) engineering is an emerging strategy to enhance attributes of CAR T-cells. Previous data emphasize the potential of AP-1 family members - comprising the Jun, Fos, ATF and Maf subfamilies – to enhance the function, persistence, resistance to exhaustion, and “stemness” of CAR-T cells.

Methods

Here we conducted a systematic analysis to determine the effect of specific AP-1 family members – BATF, BATF2, BATF3 and cJUN - on distinct CD8+ and CD4+ CAR T cell functions. We designed constructs encoding the second-generation CAR, different TFs and a truncated surface marker. We used co-culture assays, mouse xenograft models, flow cytometry, metabolic flux assays and mRNA analysis to characterize the cell products on functional, phenotypic, metabolic and transcriptional level, respectively.

Results

We identify BATF2 as a lead candidate for CD8+ CAR T cell modification providing significantly improved specific proliferation, reduced activation-induced cell death, diminished exhaustion-marker expression, and a memory-like phenotype. BATF2 attenuated IL-2 secretory capacity compared to conventional CAR T cells, specifically with a 4-1BB but not with a CD28 CAR. Conversely, CARcJUN T cells consistently exhibited the highest levels of IL-2 and IFN-γ secretion accompanied with the best Aglow tumor control. Importantly, none of the tested TFs permitted antigen-independent proliferation. Transcriptional analysis of CD8+ CARBATF2 T cells revealed a signature of lower activation, reduced exhaustion, and a unique chemokine receptor profile compared to conventional CAR T cells. The characteristics induced by BATF2 significantly minimized lung sequestration compared to conventional CAR T cells due to increased LFA-1 recycling via βII-spectrin *in vivo*.

BATF2-modification proved detrimental to the potential of CD4+ CAR T cells to elicit effector functions, highlighting distinct preferences of T cell subsets for TF-modification. However, CD8+ CARBATF2 T cell proliferation was synergistically enhanced in the presence of CD4+ CARcJUN T cells both *in vitro* and *in vivo*, resulting in improved engraftment of CD8+ CARBATF2 T cells compared to a CD4+/CD8+ CARcJUN T cell product.

CD8+ CARBATF2 T cells had lower antigen sensitivity, conferred less cytolytic activity and IL-2 secretion mediated by lower levels of LCK. This deficiency was rescued by a CAR-LCK (CAR-L) fusion while further augmenting the proliferative capacity of CD4+ and CD8+ T cells *in vitro*. A blend of CD4+ CARcJUN and CD8+ CAR-LBATF2 T cells proved superior in a solid tumor xenograft model *in vivo*, providing augmented tumor control compared to conventional CAR T cells.

Conclusions

Overexpression of BATF2 endows CD8+ CAR T cells with favourable attributes facilitating engraftment, persistence, homing and exhaustion resistance.

Our data pinpoint a cell product blend of CD4+ CARcUN and CD8+ CAR(L)BATF2 T cells to augment the intrinsic properties of CAR-T cells conferring superior engraftment and maximum antitumor efficacy *in vivo*. Our data show that CD8+ and CD4+ CAR T cells have distinct preferences and define an optimal blend for TF-enhancement.

Disclosure of interest

FF, VG and MH are inventors of patent applications related to CAR T technologies filed by the University Hospital Würzburg.

Targeted Plasma Proteomics Links Inflammation with Chronic Kidney Disease Progression in a Multi-National Pediatric Cohort

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Authors

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Aims

Preventing progression of chronic kidney disease (CKD) is of outstanding importance, especially in children where treatments such as SGLT2 inhibitors are not yet available. Inflammation has emerged as a key player in the pathogenesis and progression of CKD, as persistent low-grade inflammation contributes to kidney damage by promoting fibrosis, oxidative stress, and vascular injury. We aim to identify inflammation-related proteome signatures as potential therapeutic targets and biomarkers to predict progression of CKD in children.

Methods

We analyzed clinical data longitudinally from the prospective, multi-center Cardiovascular Comorbidity in Children with CKD (4C) Study. At study entry, inflammation-related serum proteins were assessed using the OLINK Target 96 Inflammation assay. After standard QC, we retained 77 plasma proteins for further analysis. We used linear models, LASSO regression, confounder-aware analysis (MetadeconfoundR), random forest machine learning and cox regression to identify associations with kidney function, patient demographics and a composite kidney endpoint (eGFR loss >50% or start renal replacement therapy).

Results

We included 683 children to this analysis (mean age 12.1 ± 3.3 years) with a mean eGFR of 28.8 ± 11.4 ml/min*1,73m2. Step-wise LASSO regression analysis indicated that CKD-related data (eGFR, albuminuria, CKD stage) explained the highest amount of variance for the majority of proteins. This was similarly reflected in the principal component analysis. Using linear modeling, we demonstrate that 46 out of 77 proteins associate negatively with eGFR, of which 44 remained significant after adjustment for age, sex, BMI, country of origin and diagnosis. To investigate whether inflammatory profile at baseline visit associate with kidney disease outcome, we performed a confounder-aware analysis and a random forest classifier with an 80/20 training/test data-split and k-cross validation. A subset of 5 proteins (CD40, PD-L1, CD137, CX3CL1

and IL15RA) was positively associated with the composite kidney endpoint in both methods, without being confounded by other parameters. We defined an inflammation score, where each protein was considered elevated if its residual level exceeded the 80th percentile after correcting for age, sex, eGFR, and proteinuria. In a Cox proportional hazard model, the inflammation score was associated with an increased risk of reaching the endpoint, with a hazard ratio of 1.15 (CI 1.05-1.27). Notably, this risk score remained significant, even after adjusting for additional risk factors in a more comprehensive Cox model.

Conclusions

Our data shows an inflammatory signature depending on CKD stage in a representative pediatric cohort. We describe a subset of serum proteins related to kidney outcomes, independently of known risk factors, highlighting use of these proteins as predictive biomarkers.

Glucose metabolism controls neutrophil immunity to C. albicans and S. aureus

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Authors

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Neutrophils play a central role in eliminating bacterial and fungal pathogens. In their function as a first-line immune defense against invading microorganisms, neutrophils require a rapid supply of nutrients and energy to cope with the pathogens.

Thus, neutrophils rely primarily on aerobic glycolysis, making glucose metabolism indispensable to meet their metabolic demand. Peripheral granulocytes express the two main hexose transporters GLUT1 and GLUT3 to utilize glucose, but the specific function of the two transporters in neutrophils remains incompletely understood.

Here we show that neutrophilic immune responses to Candida albicans and Staphylococcus aureus are dependent on environmental glucose uptake and aerobic glycolysis. The expression of GLUT1 in neutrophils was increased, while GLUT3 expression was downregulated, upon stimulation with live pathogens. Genetic ablation of both GLUT1 and GLUT3 almost completely abolished glucose uptake and lactate secretion of bone marrow-derived neutrophils, demonstrating that GLUT1 and GLUT3 are the main glucose transporters in neutrophils. Surprisingly, the loss of GLUT1 and GLUT3 did not affect homeostatic granulopoiesis, suggesting that neutrophil development does not require glucose consumption per se. However, the glycolytic flux into the pentose phosphate pathway (PPP), which is essential for neutrophil ROS production, was in the absence of GLUT1/3 redirected in increased utilization of metabolites for purine biosynthesis. This underlines the severe defects in the effector function of GLUT1/3-deficient neutrophils after a microbial challenge by affecting the synthesis of immunomodulatory lipids and reactive oxygen species (ROS) production.

To investigate the roles of GLUT1/3 in human neutrophil responses and the involvement of GLUT1/3 in aerobic glycolysis we used an pharmacological in-

hibitor to modulate the granulocyte function and the results revealed a comparable dependency on GLUT1/3 as observed in murine neutrophils. This suggests that the role of GLUT1/3 in antifungal and antibacterial immune responses is largely conserved between humans and mice. These findings also shed light on the development of glycolytic intervention strategies to treat T cell-mediated autoimmune diseases. However, in this context, the anti-inflammatory effects of GLUT1/3 inhibitors must be carefully balanced against the risk of impairing protective antifungal immune responses in neutrophils.

Inflammatory remodeling of the spatial bone marrow niche drives cytopenias after CAR-T cell therapy of multiple myeloma

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CAR-T cell therapy is an effective treatment for relapsed or refractory multiple myeloma. Nevertheless, a number of potentially fatal adverse events with unknown biological underpinnings have been linked to this treatment, including a failure of bone marrow regeneration leading to dysfunctional hematopoiesis. To address this issue, we analyzed bone marrow (BM) core biopsies of CAR-T cell treated patients by combining single-cell RNA-sequencing with single-cell resolution spatial transcriptomics. Focusing on patients with dysfunctional hematopoiesis, our analysis revealed hematopoietic stem cell (HSC) niche alterations towards a cellular network around inflamed mesenchymal cells that relay IFN-γ signaling from T cells to monocytes. Monocytes in turn provide pro-inflammatory feedback to mesenchymal cells through IL1B signaling. HSCs from cytopenic patients exposed to this niche up-regulate a senescence signature, indicating a cause of dysfunctional hematopoiesis. Our study deciphers a BM niche interaction network that maintains prolonged inflammation and impairs bone marrow regeneration by inducing HSC senescence.

Authors

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GDF-15 neutralization enhances T cell infiltration in solid tumor models

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Authors

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Background

Growth Differentiation Factor 15 (GDF-15) has emerged as a critical immunosuppressive factor in the tumor microenvironment, shown to inhibit endothelial adhesion of leukocytes and limiting T cell infiltration into tumors[1,2]. Both chimeric antigen receptor (CAR) T cell therapies and bispecific T-cell engagers have shown remarkable success in hematological malignancies; however, their efficacy in solid tumors remains limited[3,4]. Overcoming factors that restrict T cell infiltration and function in solid tumors is crucial for expanding T cell-based therapy applications.

Materials and Methods

T cell infiltration was examined in two tumor models: a pancreatic ductal adenocarcinoma model in C57BL/6 mice treated with EpCAM-specific CAR-T cells with or without visugromab, a monoclonal antibody targeting GDF-15; and a melanoma model in NOG mice reconstituted with human peripheral blood mononuclear cells and treated with tebentafusp, a bispecific T-cell engager, in combination with visugromab or isotype control. Flow cytometry was used to evaluate T cell infiltration and antigen-presenting cell activation, with serum GDF-15 levels measured via ELISA.

Results

GDF-15 neutralization enhanced T cell infiltration into tumor tissue across both models. Mice with splenic CAR-T cells subjected to GDF-15 neutralization exhibited a larger fraction of above-threshold CAR-T infiltration at the tumor site. Tebentafusp-treated mice receiving GDF-15 neutralization also demonstrated improved T cell infiltration into tumor tissue. Additionally, GDF-15 neutralization in the tebentafusp model was associated with increased infiltration of activated antigen-presenting cells, as evidenced by elevated numbers of CD80 and CD86-expressing dendritic cells and macrophages.

Conclusions

Our findings identify GDF-15 as a potential barrier to effective T cell infiltration in solid tumors and suggest that its neutralization may enhance T cell-based therapies by promoting T cell extravasation and facilitating the recruitment and activation of antigen-presenting cells in the tumor microenvironment, potentially expanding the application of these therapeutic modalities beyond their current limitations.

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Visualization of cardiac inflammation using 19F MRI at 7T in large animal model after Acute Myocardial infarction

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Authors

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Introduction

Heart injury triggers a rapid immune response from leukocyte sources [Epelman S]. Various methods exist to assess and monitor immune response, and 19F MRI serves as an option for tracking inflammation after acute myocardial infarction (AMI) due to its uptake by circulating immune cells, allowing for detection without background noise in combination with 1H MRI. In this study, we established imaging of myeloid cells using 19F MRI at ultra-high field (UHF) in a large animal model of AMI. The approach introduces the first 19F MR inflammation imaging at 7T MRI following AMI in large animals allowing for monitoring egress of fluorinated immune cells from their sources and their infiltration into the infarcted heart over time.

Methods

For this, pigs were subjected to an art tool of high-resolution 1H/19F MRI protocol at baseline and between days 4-15 after AMI for determination of MI and edema. For direct monitoring of myeloid cells by 19F in pigs, an intravenous PFCE-NE tracer was injected into pigs after a body weight-adjusted volume (5mL/kg) at day 2-3 of AMI, followed by non-invasive *in vivo* & *ex vivo* 19F MRI visualization of immune cells and monitoring their egress into infarcted heart.

Results and Discussion

In the first step, cardiac function was assessed before and after MI. As expected, the induction of MI resulted in a severe deterioration of the pigs' cardiac functions, with increases in left ventricular end-systolic volume after MI, as well as end-diastolic volume, and a severely impaired ejection fraction. Fluorine results demonstrate the feasibility of cardiac inflammation *in vivo* (Figure 1) and *ex vivo* (Figure 2) in the infarcted heart with matching patterns of LGE-positive myocardium. The sharp decline in 19F signal at 4, 7, and 15 days after infarction revealed differential immune cell patterns post-MI and indicated a drop of myeloid cell levels over time (Figure 3). Overall, our results introduce the first *in vivo* and *ex vivo* approach for 19F MRI at UHF with strong and reliable acquisition time. Furthermore, the 19F specific-intensity differences between 4-15 days after AMI may allow us to monitor the changes in the myeloid cell content during the inflammatory course and indeed help us to specify the timing of PFCEs-NE intravenous administration.

Conclusion

The results of this work characterize for the first time noninvasively 19F MRI imaging of cardiac inflammation following infarction in a large animal model at 7T MRI. Translation to the clinic is challenging but appears reasonable with the right tools and people in place.

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Maternal microbial metabolites drive embryonic fibroblast fate for neonatal wound healing

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Authors

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Background

The skin is the first line of interaction and defence of our body. When compromised, defective wound healing can lead to susceptibility to infection, particularly in preterm infants. The microenvironmental cues that programme the fate decision and commitment of skin fibroblast progenitors during the embryonic development for efficient wound healing early in life remain unknown. We have previously shown that maternal microbial metabolites reach embryonic tissues and promote neonatal cutaneous and intestinal barrier function. As superior wound healing is assured by fetal fibroblasts, we investigate the role of maternal microbiota in fibroblasts cell fate to facilitate the differentiation of lineages and subsets involved in wound healing and the expression of receptors and signal integration adaptors required for fibroblast to coordinate immune cells, keratinocytes, and adipocytes for the skin reconstruction.

Methods and Results

For a control exposure to maternal microbial metabolites, germ-free pregnant mice were gestational colonized with an auxotrophic E. coli HA107. Analysis of the embryonic and early post-natal fibroblast compartment revealed that maternal microbial metabolites promote the expansion of fibroblast lineages associated with hair-follicle formation (Bmp3 positive subset) and extracellular matrix remodelling (Tnfaip6 high positive subset). In the absence of the microbial metabolites, the progenitors of adipocytes (Lpl and Fabp4 high positive subset), associated with skin atrophy and fibrosis, expanded embryonically and mature adipocytes populate the perinatal skin. In this line, postnatal wound healing was superior in the skin from the offspring of gestational colonized dams, illustrated by a rapid clot formation and re-epithelization in addition to an enhanced tissue remodelling. Notably, this wound was able to novogenerate hair follicles. A detailed cellular analysis showed the accumulation of papillary fibroblasts. In contrast, the unhealed wounds from germ-free offspring accumulated En1 profibrotic fibroblasts and inflammatory myeloid cells. Furthermore, endothelial cells and myeloid cells were the major cell population that received signals from pro-wounding fibroblasts, including collagen and chemokines signalling pathways, both associated with the wound repair.

Conclusions

Our results indicates that maternal microbiota plays a critical role in shaping the fate of the embryonic fibroblast compartment, thereby promoting postnatal wound healing.

Targeting TAMs via the glucocorticoid receptor sensitizes melanoma to immunotherapy

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Authors

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Melanoma is among the five most prevalent malignancies on a global scale. Immune checkpoint inhibitors (ICIs) have been shown to be an effective treatment for metastatic melanoma, with a consequent improvement in patient survival. However, the occurrence of massive immune-related side effects, in addition to the development of resistance, limits the efficacy of this treatment in half of the patients.

Regulatory T cells, myeloid-derived suppressor cells, and tumor-associated macrophages (TAMs) are components of the immunosuppressive tumor microenvironment (TME), which contributes to therapeutic resistance to ICIs. TAMs can be categorized into an immunoreactive M1-like phenotype and a protumoral, immunosuppressive M2-like phenotype. In many tumors, a high percentage of TAMs is associated with a poor prognosis.

Targeting of M2-like TAMs is currently being investigated as a potential therapeutic approach to overcome ICI resistance, with some groups using lipid nanoparticles to target M2-like macrophages via CD163.

Our approach aims to change the polarization state from M2-like TAMs to anti-tumorigenic M1-like TAMs. *In vitro*, the loss of the glucocorticoid receptor (GR) has been shown to alter the polarization state of macrophages leading to an M1-like state. This, in turn, results in an increase in antigen uptake and processing. *In vivo*, the loss of the GR in the myeloid compartment has been shown to overcome resistance to ICIs. Furthermore, a CITE-seq analysis revealed a strong increase in Cadm1^{hi} TAMs upon anti-PD-1 treatment which was subsequently confirmed on protein level by immunofluorescence staining. This Cadm1^{hi} macrophage subset has been shown to exhibit an upregulated interferon-γ dependent gene signature that is critical for an effective response to ICIs. The results of this study suggest that GR inhibition is a promising target to sensitize melanoma to ICIs.

Postnatal antibiotic therapy prevents normal b-cell maturation in preterm infants

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Authors

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Background

The high risk of infection and complications in preterm infants leads to an increased use of antibiotics. Exposure to antibiotics is known to affect the gut microbiome. The gut microbiome is thought to be an important partner for the development and maturation of B-cells. Therefore, antibiotic exposure in the early infancy might affect the development of the B-cell compartment.

Objective

Our aim is to elucidate potential influences of the gut microbiome and antibiotic therapy on the development of adaptive immunity in preterm infants.

Methods

Peripheral blood from premature infants was sampled in the first and fourth week of life as part of a longitudinal clinical study (IRoN: Immuno-Regulation of the Newborn). Using 6 different staining panels, immune cell subpopulations were analyzed by flow cytometry. The data pertaining to the gut microbiome were obtained from stool samples. The bacterial DNA was extracted, amplified using 16S-PCR, and analyzed with metagenomic sequencing. Clinical data were obtained from questionnaires and physicians' letters. T-test and multiple linear regression analyses considering gestational age, birth weight, presence of sepsis and administration of antibiotic treatment, were used for statistical analysis. Follow-up examinations (after one year of life) are currently being carried out.

Results

The analysis included 68 premature infants. The median gestational age was 29 weeks. 48 infants were treated postnatally with antibiotics, among them 11 patients with an episode of blood-culture positive sepsis. Total B-cell counts, in particular naive and non-switched B-cell counts, were lower in the antibiotic-treated group compared to the non-treated group at four weeks of age. In the multiple linear regression analysis, antibiotic therapy was identified as the factor most significantly associated with a low B-cell count after four weeks of life, suggesting that the differences are not only explained by the infection itself or the gestational age at birth. This antibiotics-mediated effect was found selectively for B cells, while T cell, T helper, cytotoxic T and natural killer cell counts remained unaffected.

Conclusion and Outlook

In our study we found a significantly impaired development of B-cells in preterm infants treated with antibiotics. The fact that the differences in B-cells are not present in the first week of life samples supports our hypothesis that that the influence of antibiotics could be mediated by a disruption of the microbiome during the course of treatment, and therefore lead to impaired B-cell development. The upcoming microbiome analyses using metagenomic sequencing will investigate the influence of gut microbiome dysbiosis in more detail. A more profound comprehension of these relationships could not only engender a more restrained utilization of antibiotics, but also facilitate the implementation of probiotic therapy in premature infants. The objective of this endeavor would be to foster natural immune development in the most optimal manner in the future.

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Modulating the Acetyl-CoA Metabolism to Enhance the Stemness and Longevity of (CAR) T cells

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Authors
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Cellular metabolism regulates the differentiation and function of lymphocytes by controlling their bioenergetics, signaling pathways and gene expression. Post- translational histone modifications by intermediary metabolites, such as acetylation and methylation, controls the structure of the chromatin, thus regulating the gene expression. Because posttranslational histone acetylation depends on the availability of nuclear acetyl-CoA as substrate, subcellular acetyl-CoA levels are tightly regulated. One important source of acetyl-CoA is the conversion of mitochondrially-derived citrate into acetyl-CoA by the nuclear-cytosolic enzyme ATP-citrate lyase (ACLY). Thus, ACLY connects mitochondrial me-

tabolism to epigenetic programming and signal transduction though posttranslational protein modification. Recent studies showed that ACLY controls the effector differentiation and functional exhaustion of CD8 T cells. However, the role of ACLY in T cell fate decision early during lymphocyte activation remains unexplored. In this study, we found that ablation of ACLY in virus-specific CD8 T cells impaired the differentiation into effector T cell subsets while promoting their polarization into memory lymphocytes. Epigenetic, transcriptional and metabolomic analyses revealed that ACLY controls the early glycolytic reprogramming of T cells, shaping their subsequent proliferation, effector differentiation and tissue homing. Collectively, our findings demonstrate that ACLY-mediated acetyl-CoA production serves as a critical metabolic checkpoint that dictates CD8 T cell fate decision early during activation. Our findings also have important implications for (CAR) T cell-mediated cancer immunotherapy. Modulating acetyl-CoA metabolism during the manufacturing process of T cells products represent a promising strategy to enhance their persistence and functionality in cancer treatment.

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Glucose transporters in leukocytes as novel therapeutic targets after myocardial infarction

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Authors

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Myocardial infarction (MI) triggers a cascade of immune responses, playing a pivotal role in tissue repair and remodeling. It is known that the pro-inflammatory phase of this immune response, while essential for clearing debris and initiating repair, can exacerbate tissue damage if dysregulated. Targeting these proinflammatory responses could be an intriguing therapeutic target to improve the outcome post MI.

Upon activation leukocytes undergo a variety of metabolic changes, an increased glucose uptake facilitated by glucose transporters (GLUTs) being a hallmark for in particularly proinflammatory leukocytes. Therefore, in our study we established tools to determine the current metabolic state of leukocytes within the infarcted mouse heart and subsequently investigate glucose transporters as potential therapeutic targets.

We were able to show an upregulation of glycolysis in infiltrating leukocytes, especially in neutrophils and macrophages during early days post MI and an upregulation of GLUT1 predominately in pro-inflammatory leukocytes *in vivo*. *In vitro* studies revealed a specific dependency on GLUT1 to form pro-inflammatory macrophages, whereas targeting GLUT3 shows impairment of anti-inflammatory macrophages and no effect on pro-inflammatory macrophages. Using transgenic mouse models in which macrophages specifically lack GLUT1 and GLUT3 we were able to show significant improvement of heart function and increased survival post MI. We furthermore explore the therapeutic inhibition of GLUTs, for which we target GLUT1, GLUT1 and GLUT3 or glycolysis by treating mice post MI with GLUT1 inhibitor (BAY-876), GLUT1+3 inhibitor or 2-DG, experiments that are currently ongoing but preliminary data shows tendencies for an improved survival after inhibitor treatment.

By inhibiting glycolysis in a targeted manner, the aim is to modulate the pro-inflammatory immune response, striking a delicate balance between necessary inflammation for healing and preventing unwarranted tissue damage.

CAR T cells capable of multi-antigen targeting in lymphoma via co-expression of an adapter CAR

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Authors

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Therapy with gene-modified T cells expressing a chimeric antigen receptor (CAR T cells) is effective in many patients suffering from B cell malignancies. However, tumor antigen escape is a common mechanism of resistance to CAR T cell therapy, accounting for up to a third of all relapses. Here, we generate conventional and adapter dual (ConvAD) CAR T cells by co-expressing a lymphoma-specific CAR and the modular P329G adapter CAR that uses P329G Fc-mutated antibodies as adapter molecules. Instead of binding directly to the tumor antigen via the extracellular binding domain, adapter CAR T cells are specific for an adapter molecule, which in turn binds to the tumor antigen, allowing for flexible and controlled antigen targeting. Our cell product is the first to synergistically combine conventional and adapter CARs, which are typically viewed as competing technologies. We demonstrate bifunctionality of ConvAD CAR T cells acting through direct engagement of the conventional CAR as well as through binding of adapter, resulting in enhanced anti-lymphoma dual targeting compared to P329G CAR T cells alone. By introducing modularity into anti-lymphoma CAR T cells, ConvAD CAR T cells are able to prevent tumor outgrowth in models of antigen escape and effectively eradicate lymphoma with heterogeneous antigen expression *in vitro* and *in vivo*. The ConvAD CAR platform thus provides a potent tool to counter lymphoma antigen escape in a personalized manner.

T-cells as a therapeutic target in an acute myocardial infarction pig model

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Authors

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Problem

Following myocardial infarction (MI), both wound healing and long-term survival are frequently impaired. Studies in mice have shown that shifting the balance between Foxp3+ CD25+ regulatory T cells (Treg) and conventional T cells (Tconv) towards the Treg can enhance healing and improve survival outcomes. However, currently it is unclear whether this can also be achieved in a large animal model under human-like conditions.

Aim

The aim of this project is to evaluate the efficacy of manipulating the Treg/Tconv ratio in a pig model of MI as a key intermediate step before translating this approach from mice to humans. To increase the Treg/Tconv ratio we either preferentially activated Treg over Tconv by injection of a superagonistic anti-CD28 monoclonal antibody (mAb), abbreviated as CD28-SA, or by preferentially inhibiting Tconv versus Treg with an anti-CD28 mAb that blocks ligand binding to CD28 (ligand-blocking anti-CD28 mAb – LBA-CD28). Both approaches had been efficacious in mouse models of MI.

Methods

To induce an MI, the left anterior descending artery (LAD) was assessed using a C-bow with contrast agent via a femoral arterial approach and occluded with a balloon for 90 minutes mimicking a common ischemia-reperfusion scenario

in humans. Echocardiography of the heart (lateral position) was performed on days 0, 3 and 7 measuring stroke volume (SV) through PW Doppler and the diameter of the left ventricular outflow tract below the aortic valve. Therapeutic or control antibodies were applied on day 3 post MI through i.v. infusion by surgeons blinded to the chosen substance. On day 7, pigs were euthanized and samples were taken from spleen, thymus, mediastinal lymph nodes and the heart for flow cytometric analyses of myeloid cells and lymphocytes.

Results

8 out of 33 animals could not be resuscitated after developing ventricular fibrillation during the MI procedure. The remaining 25 animals were assigned to 5 treatment groups: NaCl infusion (n=2), control mAb 1 mg/kg (n=4), control mAb 10 µg/kg (n=6), CD28-SA (10 µg/kg, n=6), and LBA-CD28 (1 mg/kg, n=7) and remained in the experiment until day 7. Echocardiographic analyses showed no significant differences between the treatment and control groups. Immunologically, CD28-SA infusion increased the proportion of CD25+ Foxp3+ Treg among heart-infiltrating CD4+ T cells (13.8% vs. 6.6% in controls; $p<0.05$, One-Way-ANOVA und Tukey's post-hoc Test), but not among CD4+ T cells of mediastinal lymph nodes, spleen or CD4-single positive thymocytes. LBA-CD28 treatment had no effect on Treg frequencies among CD4+ T cells in any of the tissues studied (8.7% in the heart). The increase in the proportion of Tregs among CD4+ T cells induced by CD28-SA infusion was accompanied by a reduction in the proportion of TNF-secreting cells among heart-infiltrating myeloid cells.

Conclusions

CD28-SA treatment induced a marked increase in the Treg population, while blocking CD28 did not appear to have a significant effect. To further validate these findings, we are currently establishing histological stainings for Foxp3 and CD4 to enable direct detection of Tregs in the infarcted heart. Additionally, a long-term study has been initiated in which animals will be examined and euthanized 8 weeks after MI to assess the impact of manipulating the Treg/Tconv ratio on wound healing and cardiac function in pigs after MI.

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In vitro generation of monocytic MDSC from the conditionally immortalized HoxB8 cells

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Authors

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Myeloid-derived suppressor cells (MDSC) play a pivotal role in modulating immune responses by inhibiting various inflammatory and immunogenic cells, like T cells and dendritic cells (DC). These cells appear as granulocytic or monocytic subtypes (G-MDSC, M-MDSC) and have evolved to regulate immune reactions and accumulate during chronic infections and cancer. Myelopoiesis of MDSC is driven by GM-CSF, which is produced by various cell types, including activated T cells. Due to its central function, GM-CSF has been employed to produce MDSC from murine bone marrow (BM) cells *in vitro*.

MDSC suppress nearby immune cells through different mechanisms, like expression of the inducible nitric oxide synthase (iNOS) and arginase 1 (Arg1), both of which impact the metabolism of L-arginine, which is crucial for T-cell

function. Another potential suppression mechanism involves the expression of programmed death-ligand 1 (PD-L1).

In our study, we present a novel protocol to generate murine M-MDSC from conditionally immortalized multipotent progenitor (MPP) cells, the HoxB8 cells. HoxB8 cells are generated by fusing the estrogen-binding domain of the estrogen receptor to the HoxB8 gene and transducing this construct into murine BM via retrovirus. In the presence of estrogen together with growth factors, HoxB8 cells grow as conditionally immortalized cells of the MPP stage. Upon estrogen removal and addition of different colony-stimulating factors, HoxB8 cells differentiate into various myeloid cell types.

By following our previous protocol established for *in vitro* generation of BM-MDSC, HoxB8 cells were employed to generate MDSC through two successive signaling events involving 3 days of GM-CSF culture and subsequent overnight activation with LPS/IFN-γ ± IL-4 to up-regulate iNOS and Arg1. These HoxB8-MDSC mimic BM-generated MDSC in phenotype and function, offering an innovative method for (i) overcoming the mouse inherent limited life span, (ii) allowing precise CRISPR/Cas9 gene editing and (iii) comprehensively understanding MDSC biology.

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Microbial metabolites steer key regulatory pathways in CAR T cells to achieve enhanced anti-tumor immunity

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Background

Several studies underscored the crucial role of the intestinal microbiome in cancer immunotherapy[1,2]. Our research has shown that pentanoate, a short chain fatty acid (SCFA) produced by the commensal bacterium Megasphaera massiliensis in the gut, significantly enhances the efficacy of chimeric antigen receptor (CAR) T cell immunotherapy[3]. Preliminary findings point towards epigenetic as well as metabolic changes as key factors, sparking increased interest into its mechanism of actions. Gaining insight into pentanoate's effects on lymphoyte functionality could pave the way for advancements in T cell-based therapies.

Aims

Here, we seek to implement microbial metabolites in the CAR T cell manufacturing process. In the following, we aimed to perform in-depth characterization of pentanoate-induced changes of the T cell phenotype *in vitro* and *in vivo*.

Methods

In this study, we developed a protocol for the generation of CAR T cells implementing pentanoate's stimulatory benefits during the engineering process. To dissect its mode of action, we utilized pharmacological agents with histone deacetylase (HDAC) or metabolic modulatory properties to replicate the pentanaote-induced phenotype. Additionally, we employed isotope-labeled substrates to conduct a detailed molecular characterization. In a final step, we assessed the functionality of generated CAR T cells *in vivo* in two immunocompetent solid tumor models.

Authors

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Results

Compared to conventionally engineered CAR T cells, CARPenta T cells exhibited significantly higher transduction efficiency and enhanced functional activity in terms of cytotoxicity and effector cytokine secretion. While the clinically available drugs mocetinostat and dichloroacetate, functioning as HDAC class I inhibitor and metabolic modifiers, respectively, improved CAR T cell function upon individual treatment, only the dual application of both was able to induce a comparable pentanoate-like phenotype *in vitro*. Metabolic tracing of 13C-glucose and -glutamine revealed uncoupling of the TCA cycle from glycolysis independently of glutamine anaplerosis as a unique feature of pentanoate. This effect was not recapitulated by the clinically relevant drugs. Pentanoate-engineered CAR T cells demonstrated superior anti-tumor control and enhanced cell persistence *in vivo*. Furthermore, scRNA sequencing of *ex vivo* tumor-infiltrating lymphocytes revealed a unique naïve-like T cell state in addition to reduced T cell exhaustion

Conclusion

The integration of the microbial metabolite pentanoate into the CAR T cell manufacturing process led to distinct shift in the CAR T cell phenotype granting superior functionality *in vitro* and *in vivo*. Beyond pentanoate's HDAC-inhibitory effects and indirect metabolic modulation, we demonstrated its capacity to reprogram the TCA cycle, in contrast to clinically available drugs, making it a unique modulator of T cell biology[4]. This study introduces a novel perspective, emphasizing the critical connection between microbiome-derived metabolites and cancer immunotherapy that can be utilized to improve engineered T cells.

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Heart-to-lung miscommunication: Role of phosphodiesterase 2A in pulmonary endothelial inflammation after myocardial infarction.

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Pulmonary congestion and oedema are major complications of acute and chronic left heart diseases. Together with increased "backward" pressure, endothelial barrier dysfunction contributes to lung inflammation. Here we studied whether induction of the cGMP/cAMP-degrading phosphodiesterase (PDE) 2A participates in a proinflammatory heart-to-lung crosstalk after acute myocardial infarction (AMI).

Authors

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In mice, two days after AMI, the expression of PDE2A in microvascular lung endothelial cells (MLEC) was significantly increased. This was associated with signs of pulmonary inflammation, such as extravasation of albumin, augmented expression of VCAM-1, ICAM-1, CXCL-1 and Interleukin-6, and enhanced neutrophil infiltration. In cultured murine lung endothelial cells (MLEC), Interleukin (IL)-1β, a cytokine released during cardiac ischemia, induced PDE2A expression. The functional relevance was studied using the PDE2A inhibitor BAY-60-7550. In MLEC, BAY enhanced ANP-induced cGMP as well as Adenosine- or Isoprenaline-induced cAMP formation. Moreover, BAY attenuated Thrombin-induced barrier dysfunction and IL-1β-evoked increases of VCAM-1 and ICAM-1 expression. To dissect the pathophysiological implications, we studied a novel genetic mouse model with conditional, endothelial (EC)-restricted PDE2 deletion (KO). PDE2 deletion enhanced MLEC cGMP and cAMP levels and prevented the inhibitory effect of IL-1β. Furthermore, in such PDE2A-deficient MLEC, the baseline and TNF-α and IL-1β-induced VCAM-1 expression was reduced. Most importantly, after experimental AMI, the EC PDE2A KO mice had diminished pulmonary infiltration by immune cells (as compared to control littermates).

Our observations indicate that increased endothelial expression and activity of PDE2A contributes to pulmonary congestion and inflammation after cardiac ischemia. PDE2A might be a target for therapies aiming to prevent or reduce such clinically relevant complications.

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Keywords
PDE2A, endothelial barrier, pulmonary inflammation

γδ T Cells as Gatekeepers of Skin Autoimmunity: Uncovering Their Non-redundant Role in Blistering Diseases

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Bullous pemphigoid (BP) is a chronic autoimmune skin disease characterized by autoantibodies targeting hemidesmosomal proteins, which impair dermal-epidermal adhesion and trigger subepidermal blister formation. These autoantibodies activate the complement system and promote the recruitment of granulocytes, resulting in inflammation and tissue damage. Relapsing-remitting BP is clinically treated with topical or systemic corticosteroids, but the underlying immunopathology is poorly understood, particularly the role of the adaptive immune system. In this study, we uncovered a crucial and non-redundant role for dermal γδ T cells in the effector phase of BP. Using an established animal model of BP induced by passive transfer of autoantibodies targeting hemidesmosomes, we found that Rag1−/− mice, which lack all lymphocytes, were completely protected from the disease. However, the absence of either B cells or conventional αβ T cells did not prevent BP, indicating the involvement of unconventional lymphocytes. Notably, the ablation of γδ T cells was sufficient to protect mice from BP-induced skin inflammation. Using both constitutive and inducible γδ T cell-deficient mice, we demonstrated that the absence of γδ T cells reduced neutrophil recruitment and prevented tissue damage in the effector phase of BP. Mechanistically, we identified dermal γδ T cells as the predominant source of IL-17A and IL-17F production in the skin following autoantibody-mediated tissue injury. Ablation of IL-17A/F completely protected mice

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from BP, preventing neutrophil recruitment to the skin. These findings highlight a novel, non-redundant role of dermal γδ T cells in initiating and amplifying autoantibody-mediated skin inflammation. Future work will investigate longitudinal changes in dermal T cell clonality and composition, alongside metabolomic profiling, to further elucidate BP disease progression. A better understanding of how γδ T cells promote autoimmune blistering diseases will pave the way for novel therapeutic strategies that selectively target autoimmune pathology while preserving protective immune responses.

Preterm birth impairs the long term development of mucosal B cell immunity

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Objectives

Preterm infants have an increased risk of hospitalization due to infectious diseases that persists into adulthood. Additionally, they exhibit lower vaccine-specific antibody titers compared to term-born children during early childhood. This suggests a persistent impairment in the development of a protective adaptive immune memory in preterm infants. Through systems immunology-based analysis of adaptive immune trajectories in term and preterm neonates, we aim to identify which components of the adaptive immune response are most affected. In the long term, the goal is to identify potential targets for interventions that promote the development of a protective immune memory in preterm infants.

Materials and Methods

We perform longitudinal analyses of clinical data, immune phenotyping (flow cytometry, single-cell RNA sequencing), and the microbiome in healthy term neonates (MIAI cohort, n = 230) and preterm neonates (IRoN cohort, birth weight <1500 g, n = 115) during the first year of life.

Results/Summary

At birth, significant differences in the cellular composition of the adaptive immune system are observed between preterm and term neonates. These differences show distinct, gestational age-dependent trajectories for T, B, and NK cells during the first 50 days of life. These trajectories are further modulated by perinatal inflammation or typical complications of prematurity.