

RTG 2408 | Maladaptive processes across
physiological barriers in chronic diseases



Annual
RETREAT

SEPTEMBER
24-26 **2025**

USEFUL INFORMATION

VENUE & ACCOMMODATION

Schloss Beichlingen

Schloßberg 1

99625 Beichlingen

TIMES

Check-in: Day 1 from 14:00

Check-out: Day 3 by 10:00

Breakfast 7:00–9:00

ROOMS

Breakfast, lunch, dinner: Restaurant, 1st floor

Talks, meetings, evening events: Conference room (Parketsaal)

Coffee breaks: Goldener Salon

BUS TRANSPORT

Meeting point: Sep 24, 2025, 9:00-9:10. Parking lot behind House 33

<https://maps.app.goo.gl/iPrmg7GFmp8q9s4v5>

PROGRAM

Day 1 | September 24, 2025

11:45–13:00 **Arrival & Welcome Lunch**

13:00–13:15 **Welcome**

Session 1

Chairs: Vladyslava Dovhan, Nora Siegelt

13:15–13:50 *USP48-dependent regulation of NF- κ B in the *H. pylori* infected gastric mucosa*
P1-2 | Lorena Ferino

13:50–14:25 *Characterization of the specific functional relevance of perivascular mast cells in skin inflammation*
P4-2 | Aaron Hoffmann

14:25–14:40 **Coffee Break**

Session 2

Chairs: Mahsa Abedi, Arun Kanthasamy

14:40–15:15 *Impact of T-Cell Oxidative Stress on Prognosis in Acute Myeloid Leukemia*
CS6 | Tobias Ronny Haage

15:15–15:50 *Th2 cell-dependent effects on the airway epithelial barrier during chronic asthma*
P12-2 | Anna Krone

15:50–16:05 **Coffee Break**

Session 3

Chairs: Somayeh Alinaghi Arjas, Kyrill Herwartz

16:05–16:40 *Analyzing the impact of mesenchymal stromal cells as tolerance promoting barrier in the context of CAR T cell therapies*
P13-3 | Lea Reemts

16:40–17:15 *The role of cold shock proteins in mitochondrial homeostasis and for tubular cell phenotype determination during cell stress*
P8-2 | Sohail Ahmad

17:15–17:30 **Coffee Break**

Session 4

Chairs: Sandra Freier, Shivani Singh

17:30–18:05 *Local and systemic effects of intestinal inflammation on secondary challenges*
P2-3 | Nina Lindemann

18:05–18:40 *Exploitation of epithelial/endothelial microenvironment crosstalk*
P15-2 | Sandro Gogia

19:00–20:00 **Dinner**

20:00 **Pub Quiz**

PROGRAM

Day 2 | September 25, 2025

Session 5	Chairs: Nina Lindemann, Lea Reemts
9:00–9:35	<i>Optimized models for studying intra- and intercellular crosstalk in biliary diseases</i> P11-3 Shivani Singh
9:35–10:10	<i>Mechanisms of induction of classical NF-κB by <i>H. pylori</i></i> P15-3 Arun Kanthasamy
10:10–10:25	Coffee Break
Session 6	Chairs: Sohail Ahmad, Aaron Hoffmann
10:25–11:00	<i>Balancing Immunity and Pathology: Lipid Metabolism and Siglec Signaling in Th2 and Autoimmune Responses</i> P12-3 Sandra Freier
11:00–11:35	<i>Characterization of the genotype underlying hereditary forms of intrahepatic cholestasis</i> AP11 Somayeh Alinaghi Arjas
11:35–11:50	Coffee Break
Session 7	Chairs: Tobias Ronny Haage, Lorena Ferino
11:50–12:25	<i>Interleukin-7 dependent infiltration of acute lymphoblastic leukemia across the testicular endothelial barrier</i> P14-2 Vladyslava Dovhan
12:25–13:00	<i>Investigating the role of mast cells in central and peripheral trained immunity upon skin inflammation</i> MD12 Kyrill Herwartz
13:00–14:00	Lunch Break
14:00–15:45	Case Discussion with PIs (including Coffee Break)
16:00–18:30	Team Event
19:00–20:00	Dinner
20:00	General Assembly

PROGRAM

Day 3 | September 26, 2025

9:00–9:30 **Students/PI-Meeting**

Session 8

Chairs: Sandro Gogia, Anna Krone

9:35–10:10 *Bidirectional cross-talk between mast cells and endothelial cells in homeostasis and inflammation*

P4-3 | Mahsa Abedi

10:10–10:45 *Screening for systemic factors in biliary diseases*

P16-3 | Nora Siegelt

10:45–11:00 **Coffee Break**

11:00–12:30 **Graphical Abstracts + Voting**

12:30–12:45 **Wrap up**

12:45–13:30 **Farewell Lunch**

USP48-dependent regulation of NF-κB in the *H. pylori* infected gastric mucosa

Background

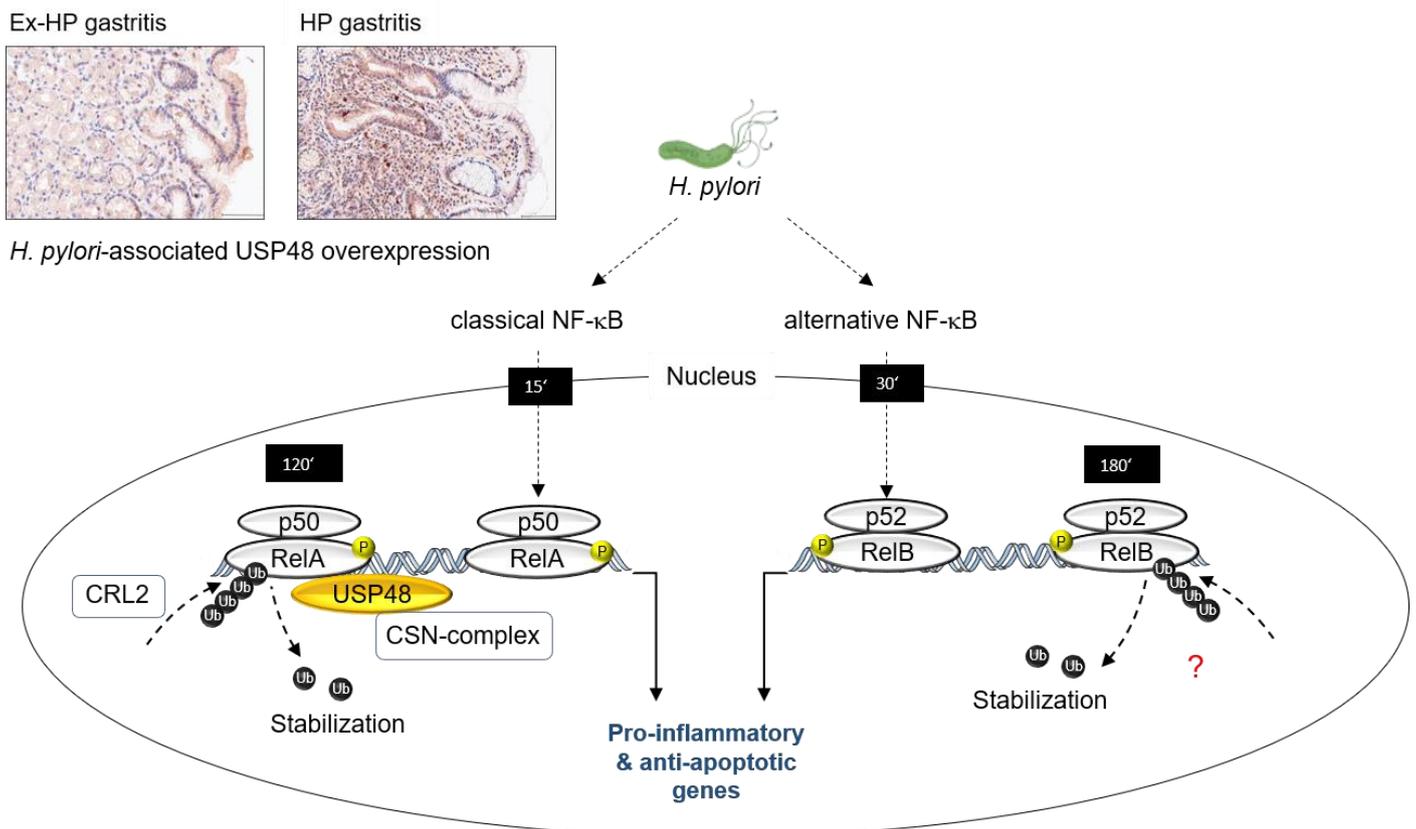
H. pylori induces classical NF-κB in the gastric epithelium and CSN-associated USP48 stabilizes nuclear RelA via deubiquitinylation upon infection. We aim to investigate the role of USP48 in alternative NF-κB and the inflammatory response.

Results

The NF-κB regulator USP48, a deubiquitinating enzyme (DUB), is upregulated in *H. pylori* gastritis and can serve as a prognostic disease marker. *H. pylori*-induced inflammation is closely connected to the activity of classical NF-κB. Classical NF-κB controls alternative NF-κB via RelA-dependent transcription and subsequent nuclear translocation of RelB. As a prerequisite for NF-κB clear translocation, the inhibitors IκBα and p100 become degraded by cullin-RING-E3 ligases. Neddylation of cullins promotes the ubiquitinylation of the inhibitors. Further, the multi-protein complex COP-signalosome (CSN) counteracts the neddylation and the CSN-associated USP48 stabilizes nuclear RelA. Knockdown of either USP48 or CSN2 caused a diminishment of nuclear RelA as well as RelB and a decline in NF-κB target gene expression. Interestingly, an inducible interaction between USP48, CSN and RelB was detected in co-IP upon infection. In addition, we observed nuclear RelB ubiquitinylation by an yet unknown E3 ligase.

Conclusions

CSN-associated USP48 interacts with RelB and knockdown of either leads to a diminishment of nuclear RelB. We hypothesize that CSN-associated USP48 serves as DUB for nuclear RelB, which remains to be validated by DUB assays.



Characterization of the specific functional relevance of perivascular mast cells in skin inflammation

Background

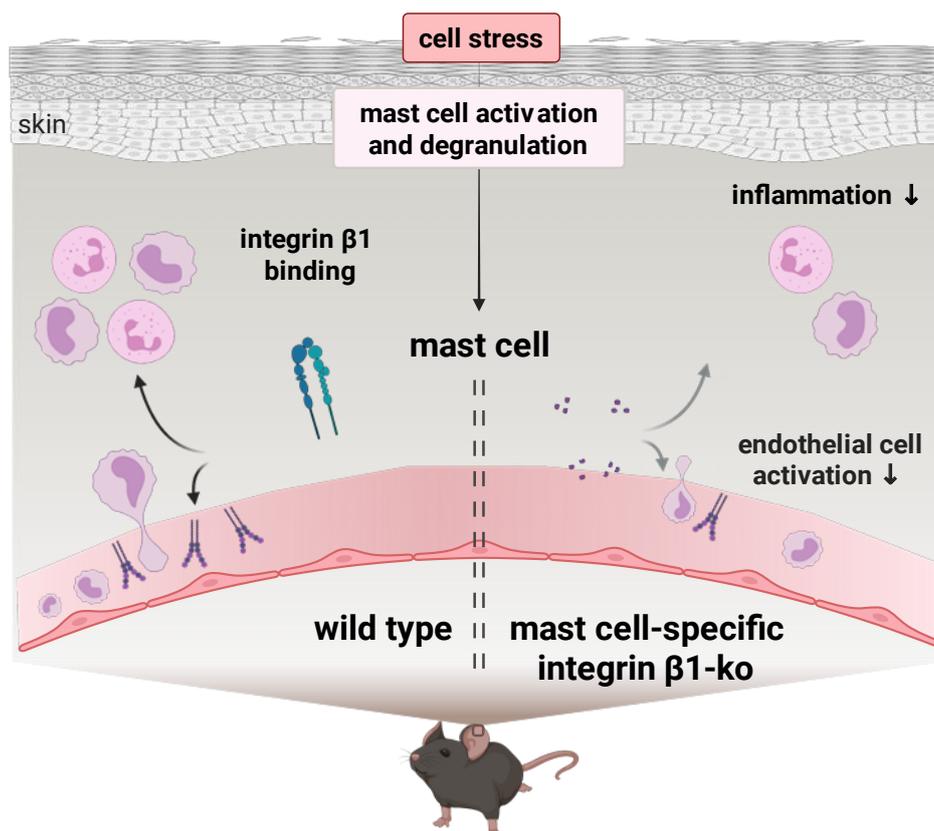
Mast cells (MCs) initiate inflammatory responses via rapid mediator release. Of note, perivascular MCs may play a pivotal role due to intravascular degranulation, yet their characteristics and mechanism of vessel attachment remain unclear.

Results

Using a conditional knockout in MCs, I studied the relevance of the adhesion molecule integrin $\beta 1$ (Itgb1) for perivascular MC vessel attachment and degranulation *in vitro* and *in vivo*. Fluorescence microscopy of murine Itgb1-deficient MCs ($MC^{\Delta Itgb1}$) revealed that Itgb1 is critical for the spindle-like morphology, homogeneous tissue distribution, and perivascular alignment of MCs in the perivascular niche along the blood vessels, particularly at arterioles, at steady state. Moreover, I observed a reduced capacity of $MC^{\Delta Itgb1}$ to degranulate into the blood vessels during skin inflammation. In the contact hypersensitivity (CHS) mouse model, $MC^{\Delta Itgb1}$ mice showed a dramatically reduced ear swelling, accompanied by a significant reduction of infiltrating neutrophils, monocytes, macrophages and $CD8^+$ T cells in the ear skin. Notably, $MC^{\Delta Itgb1}$ mice exhibited an impaired activation of endothelial cells and neutrophils, and a reduced immune cell extravasation from blood to the skin upon CHS. Furthermore, the lack of Itgb1 in MCs resulted in an impaired degranulation efficiency *in vitro*.

Conclusions

The findings reveal the key role of Itgb1 in MC distribution and perivascular alignment along blood vessels, its impact on degranulation and pro-inflammatory function in CHS, and a potential bidirectional cross-talk with endothelial cells.



Impact of T-Cell Oxidative Stress on Prognosis in Acute Myeloid Leukemia

Background

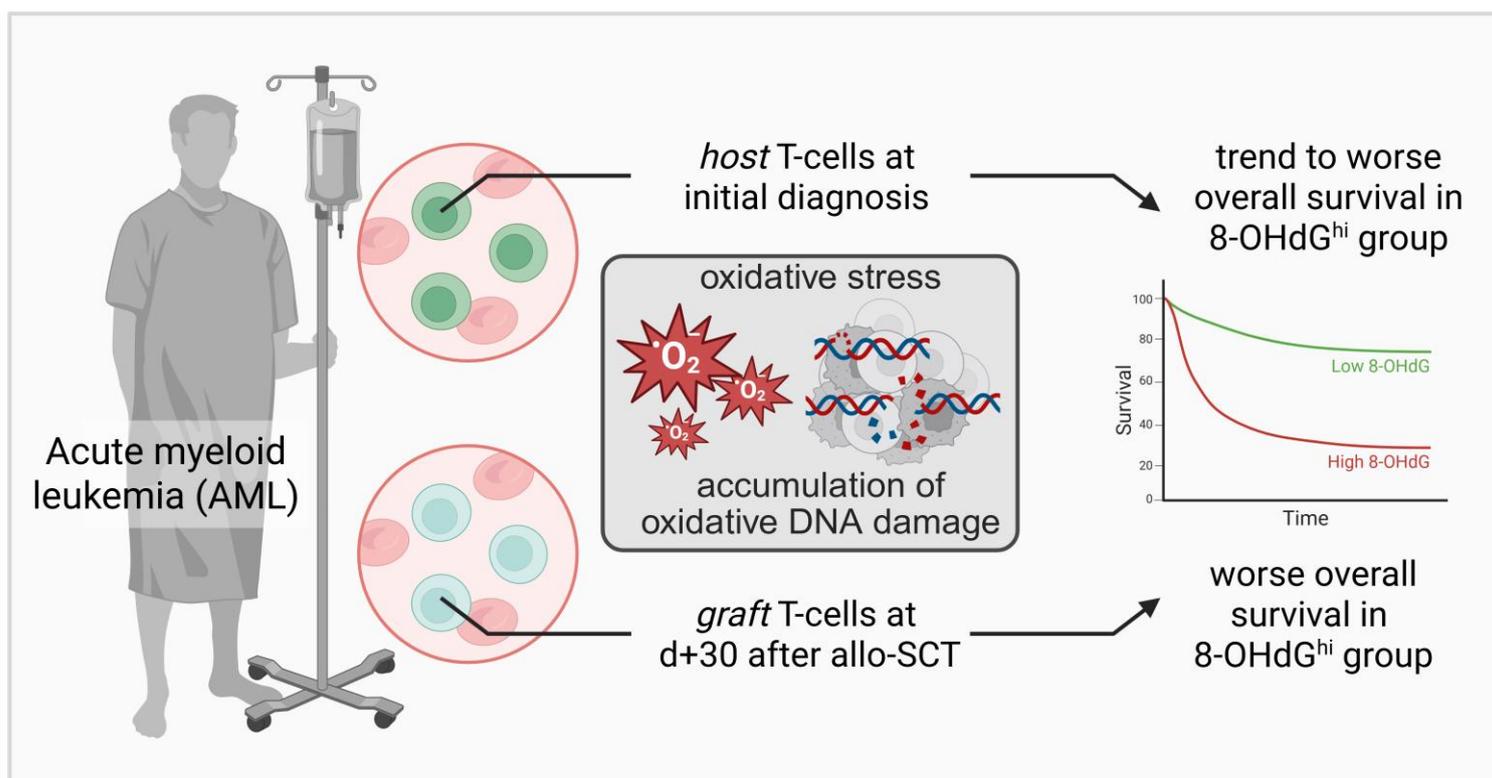
High level of oxidative DNA damage in graft T-cells, characterized by 8-hydroxydeoxyguanosine (8-OHdG), have been associated with a worse event-free and overall survival after allogeneic stem cell transplantation (allo-SCT).

Results

Oxidative stress in peripheral blood T-cells from AML patients (n=70) at initial diagnosis was assessed using flow cytometry. Based on the 8-OHdG level in *host* CD3+ T-cells, patients were separated in 8-OHdG^{lo} and 8-OHdG^{hi} (each n=35). Baseline characteristics were comparable between the two groups. High 8-OHdG levels were linked to patients aged over 80 years and to those classified as intermediate/high risk by the European LeukemiaNet. Oxidative stress in CD3+ T-cells is transferred to T-cell subsets (e.g., CD4+, CD8+) with a corresponding significant correlation. No difference in event-free survival could be observed depending on 8-OHdG levels. However, although not statistically significant, a trend toward poorer overall survival (OS) was evident in the 8-OHdG^{hi} compared to the 8-OHdG^{lo} group. This difference was apparent in the overall cohort (2yOS 40.7% vs. 52.1%, p=0.16) as well as in differently treated subcohorts (cytarabine backbone: 2yOS 47.1% vs. 62.8%, p=0.75; post-allo-SCT: 57.0% vs. 77.5%, p=0.45). Since oxidative stress in *host* CD3+ T cells also appears to influence post-allo-SCT OS (after engraftment of the donor immune system), oxidative imprinting is conceivable.

Conclusions

Elucidating the pivotal impact of T-cell oxidative stress on outcome in AML could emerge patient-specific approaches, since *host*-derived T-cell immunity may exert effects comparable to *graft*-derived T-cell immunity after allo-SCT.



Th2 cell-dependent effects on the airway epithelial barrier during chronic asthma

Background

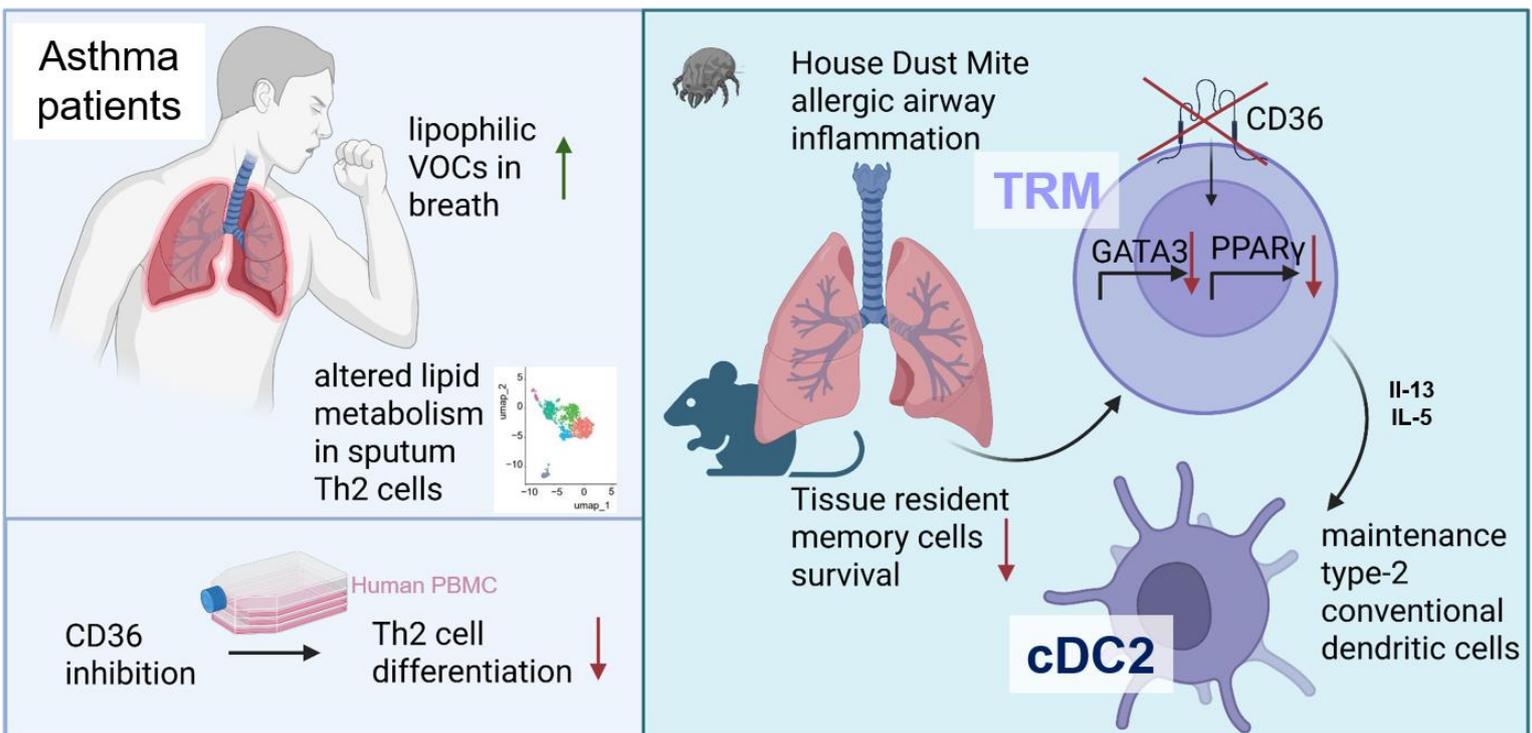
Th2 cells mediate airway inflammation and show a distinct genomic accessibility of lipid metabolism genes, suggesting lipid receptors like CD36 may be crucial for their pathogenic potential.

Results

We found that sputum Th2 cells of asthma patients show an alteration of lipid metabolism genes and an increased amount of lipophilic volatile compounds in their exhaled breath by scRNAseq and proton-transfer-reaction mass spectrometry, respectively. In line with this, mice with a T cell-specific deletion of CD36 have impaired frequencies of Th2 tissue-resident memory (Trm) that show defective PPAR γ and GATA3 signaling, which cumulates in altered conventional dendritic cell type 2 migration during house dust mite allergic airway inflammation. In addition, pharmacological blockade of CD36 in human T cells does not affect activation but terminal Th2 differentiation.

Conclusions

Our study show that CD36 lipid metabolism is a metabolic checkpoint of Th2 memory responses in asthma that could be targeted for therapy.



Analyzing the impact of mesenchymal stromal cells as tolerance promoting barrier in the context of CAR T cell therapies

Background

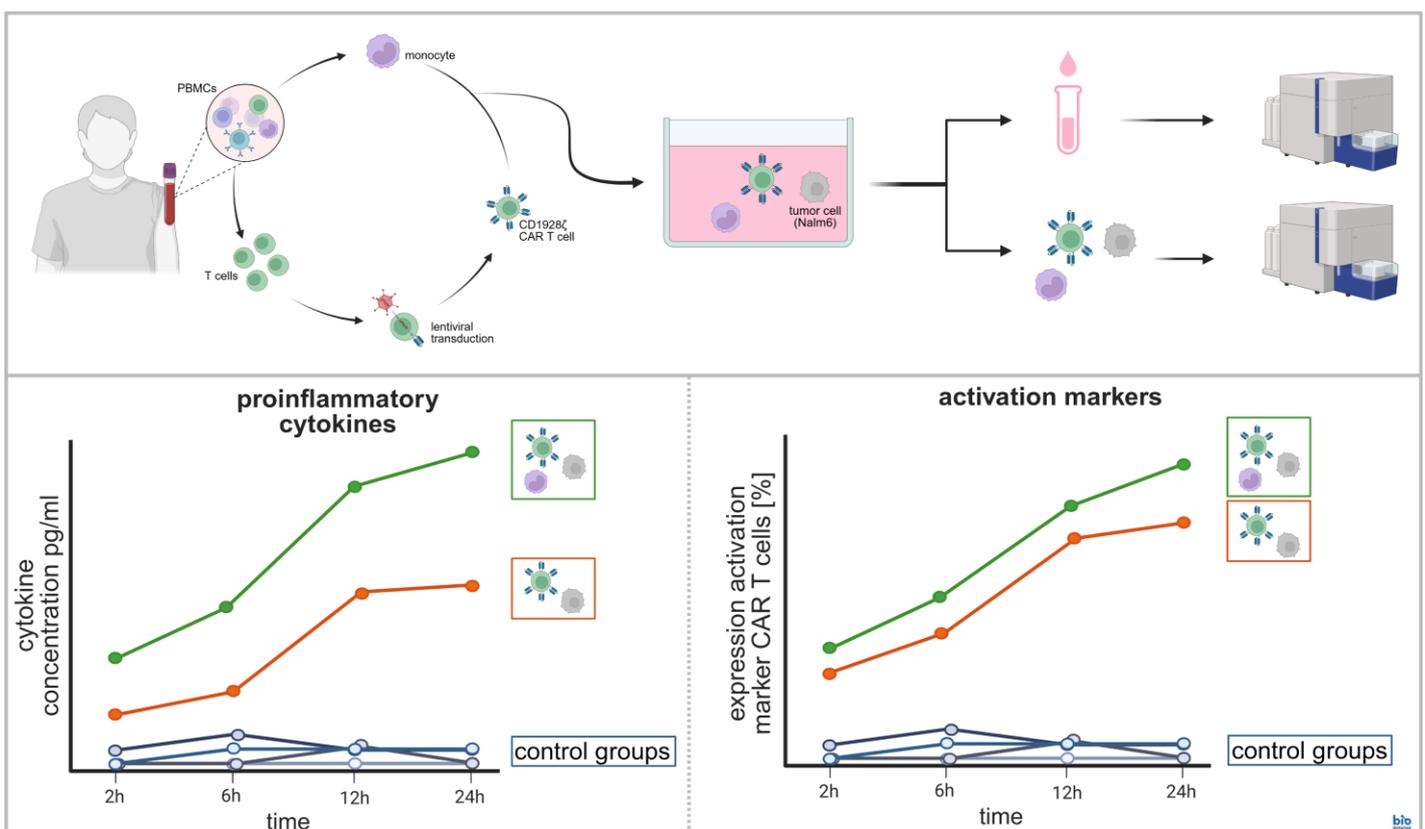
CAR T cell therapy is associated with severe toxicities due to excessive inflammatory immune activation. These toxicities might be alleviated by the immunomodulatory and endothelial-supporting properties of mesenchymal stromal cells (MSCs).

Results

We developed an in vitro model of cytokine release syndrome (CRS) to mimic the development of CRS associated with CAR T (chimeric antigen receptor) cell therapy. The model contains the main players associated with CRS: CAR T cells, tumor cells, and monocytes. We analyzed co-cultures of these cells for typical CRS cytokines and the activation state of the CAR T cells. Compared to single and double cultures, the triple culture exhibited the strongest increase in pro-inflammatory cytokines. These cytokines – IL-6, IFN γ , and TNF – are key mediators known to trigger CRS in patients. We also detected increased concentrations of granzyme B, GM-CSF, and IL-2, indicating CAR T cell activity. In addition to cytokine release, our experiments confirmed the activation of CAR T cells, as evidenced by increased activation markers. These markers include the early activation marker CD69, CD25, as well as the late activation marker CD137. Both the released cytokines and the cellular markers are essential for defining CRS in vitro.

Conclusions

Hence, the data confirm that our in vitro model effectively mimics CRS in patients, providing a reliable foundation for further experiments. Such as testing the impact of MSCs on CRS-related immune overactivation and CAR T cell efficacy.



The role of cold shock proteins in mitochondrial homeostasis and for tubular cell phenotype determination during cell stress

Background

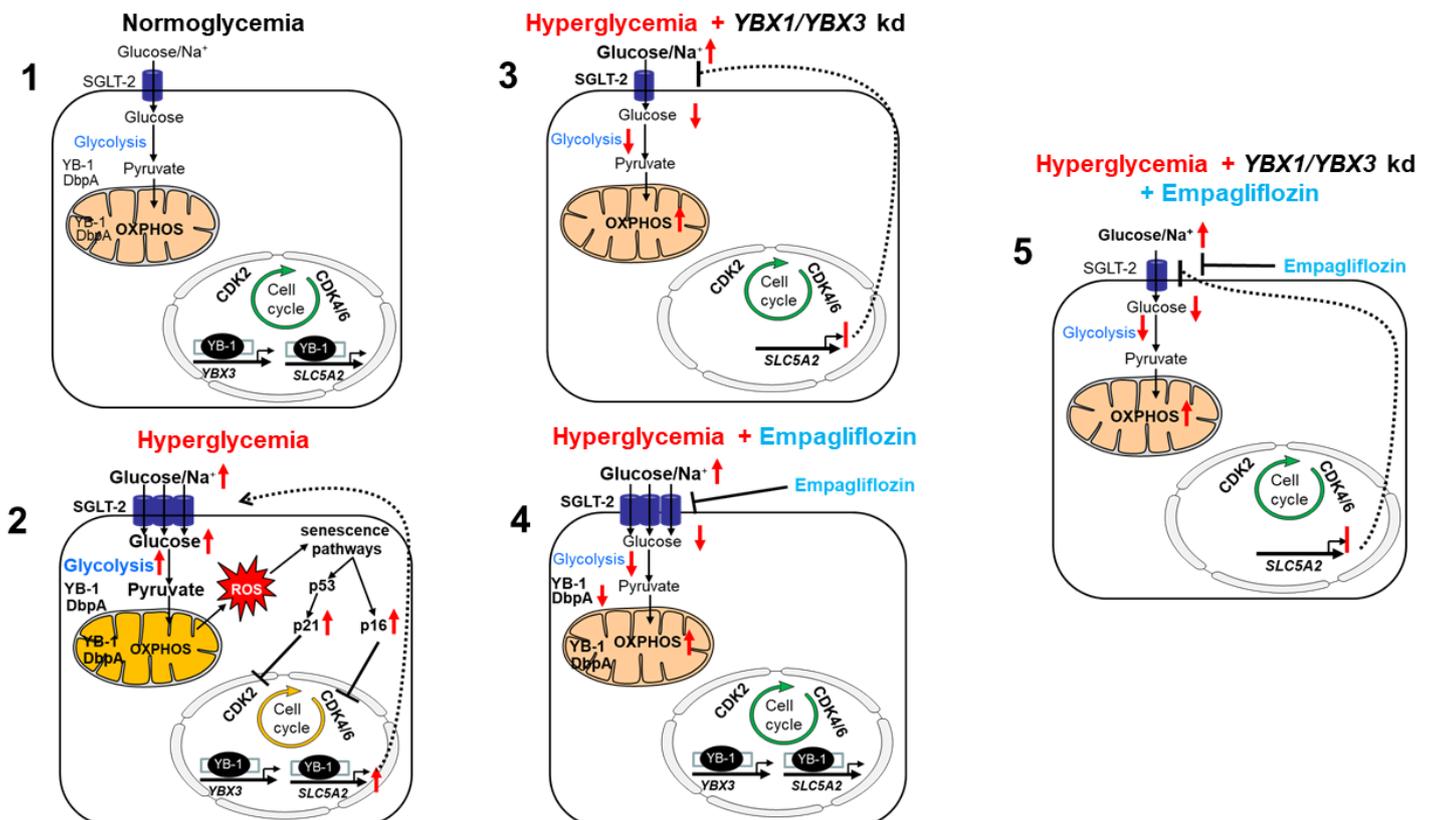
Diabetic kidney disease is the leading cause of renal failure. Hyperglycemia induces irreversible cell cycle arrest, known as senescence. Cold shock proteins YB-1 and DbpA could be crucial due to their role in cell cycle and proliferation.

Results

Kidney primary tubular cells from mice and human embryonic kidney cells (HEK-293) were exposed to high glucose and salt stress in vitro. Hyperglycemic stress upregulated the expression of cold shock proteins (CSP) YB-1 and DbpA, SGLT-2 transporter and cell senescence markers p21 and p16, compared to control. Furthermore, DNA damage (p.H2AX) and fibrosis (α -SMA and fibronectin) markers were also upregulated under hyperglycemia. YB-1 and DbpA knockdown (shYBX1 and shYBX3) in HEK293 cells downregulated the expression of SGLT2 transporter and senescence markers p21 and p16 under hyperglycemic stress compared to control cells expressing YB-1 and DbpA. Furthermore, an intervention with SGLT2 inhibitor empagliflozin has reduced the expression of hyperglycemia induce senescence markers p21 and p16 when administrated at concentration of 500 nM/mL. Expression of cold shock protein DbpA was downregulated under empagliflozin intervention. Combination of SGLT2 inhibition and CSP knockdown significantly reduced the hyperglycemia induced cell senescence.

Conclusions

Kidney tubular cells develop cell senescence under hyperglycemic stress. CSPs YB-1 and DbpA are identified as key regulators of hyperglycemia induce senescence. Blockade of SGLT2 transporters by empagliflozin inhibits DbpA expression.



Local and systemic effects of intestinal inflammation on secondary challenges

Background

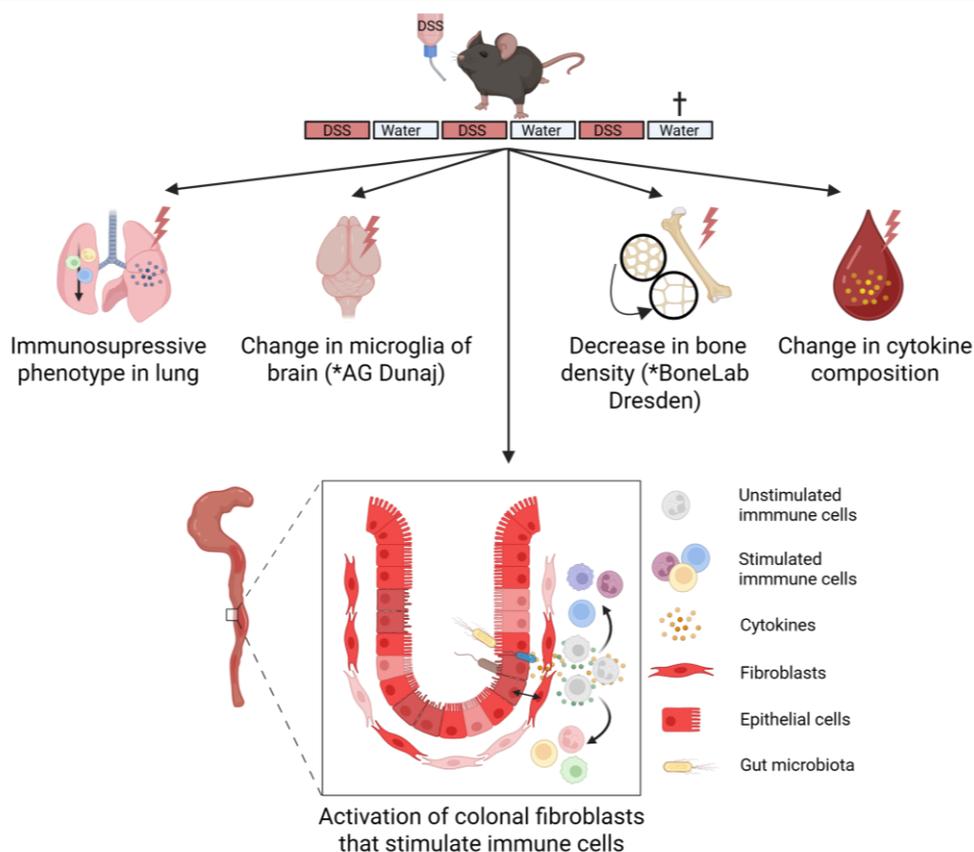
Ulcerative colitis is an autoimmune disease that causes tissue damage to the intestinal tract. It can be modelled in mice by administering dextran sulphate sodium (DSS). Ulcerative colitis is understood not only to be an intestinal disease,

Results

To induce chronic colitis, 11–13-week-old mice received three DSS cycles followed by three weeks of water for remission, while controls had normal water. After sacrifice, colon, lungs, BAL fluid, brain, blood, and bones were analysed. For immune profiling, colon and lung tissues were digested to single-cell suspensions, antibody-stained, and assessed with an ID7000™ Spectral Cell Analyser. DSS colitis caused a marked reduction of CD4+ T cells and alveolar macrophages in lungs, more pronounced during remission, indicating systemic immune effects beyond the gut. In the colon, fibroblasts showed phase-specific changes, with crypt-top and -bottom fibroblasts expanding in acute and remission states. Chronic inflammation altered T cell subsets, with reciprocal iTreg/nTreg shifts during disease that normalised in remission. DSS colitis also reduced bone volume and mineral density with increased trabecular spacing, indicating weaker bone structure. In brain tissue, tight junction protein changes in cortex and hippocampus were accompanied by lymphocyte and myeloid infiltration. Microglia showed condition-dependent morphological and activation marker alterations.

Conclusions

Research into extraintestinal manifestations, particularly in the lungs but also in the brain and bone structure, provides valuable insights into the systemic pathophysiology of the disease.



Exploitation of epithelial/endothelial microenvironment crosstalk

Background

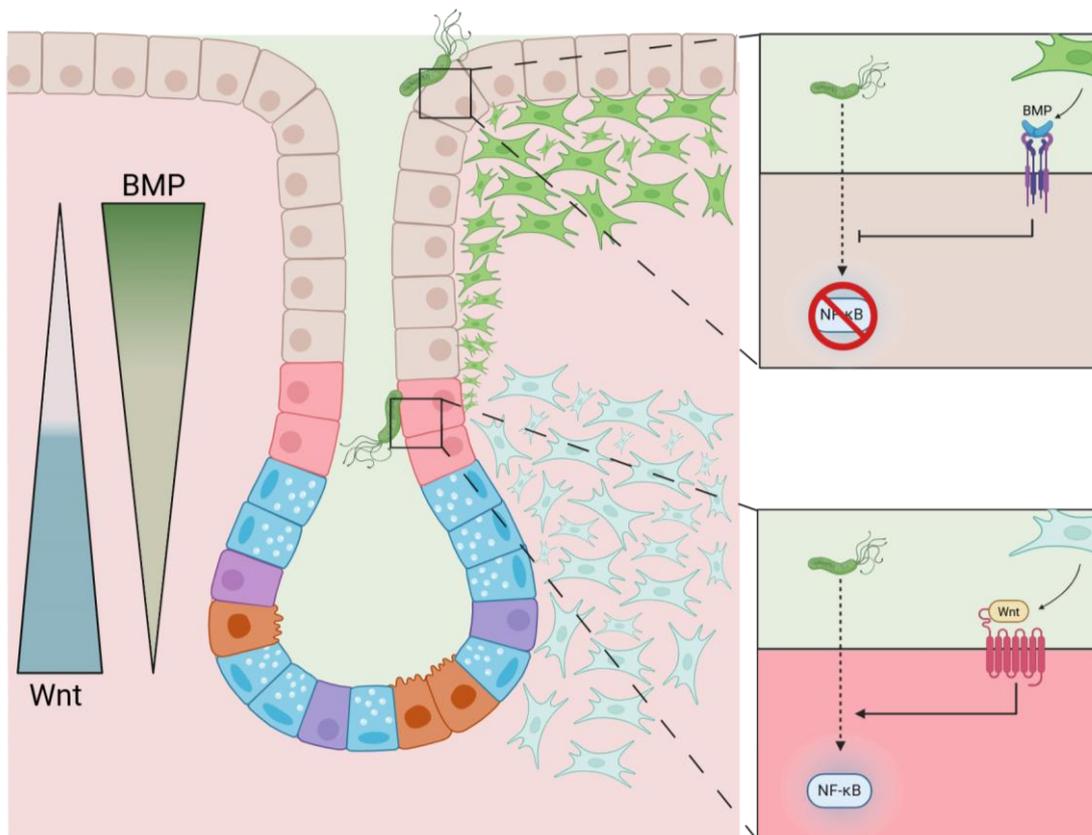
H. pylori triggers responses in gastric stem cells, causing inflammation and mucosal changes, attributed to the crosstalk between NF- κ B and the Wnt/ β -catenin pathway.

Results

Helicobacter pylori triggered NF- κ B activation in gastric mucosoids, and this response was strictly dependent on the stemness factors Wnt3A and RSPO1. In contrast, IL-1 β and TNF-induced NF- κ B activity was independent of these factors and occurred only after basolateral stimulation. Removal of Wnt3A/RSPO1 reduced *H. pylori*-driven NF- κ B activity, stabilized TIFA - an adaptor in the ADP-heptose ALPK1/TIFA pathway and promoted differentiation of mucosoids into pit cells. Alterations in other differentiation pathways, such as the TGF- β pathway, did not affect NF- κ B responses, indicating pathway specificity. Treatment with BMP2, as well as withdrawal of Noggin (a BMP inhibitor), further lowered NF- κ B activity, consistent with BMP signaling promoting differentiation and dampening inflammation. The dependency on Wnt3A and RSPO1 was not observed in gastric cancer cells, highlighting differences between normal and malignant tissue responses.

Conclusions

H. pylori-induced NF- κ B activation in gastric mucosoids requires Wnt3A/RSPO1 and is modulated by BMP signaling. The mechanism behind this needs to be understood and differs from responses in gastric cancer cells.



Optimized models for studying intra- and intercellular crosstalk in biliary diseases

Background

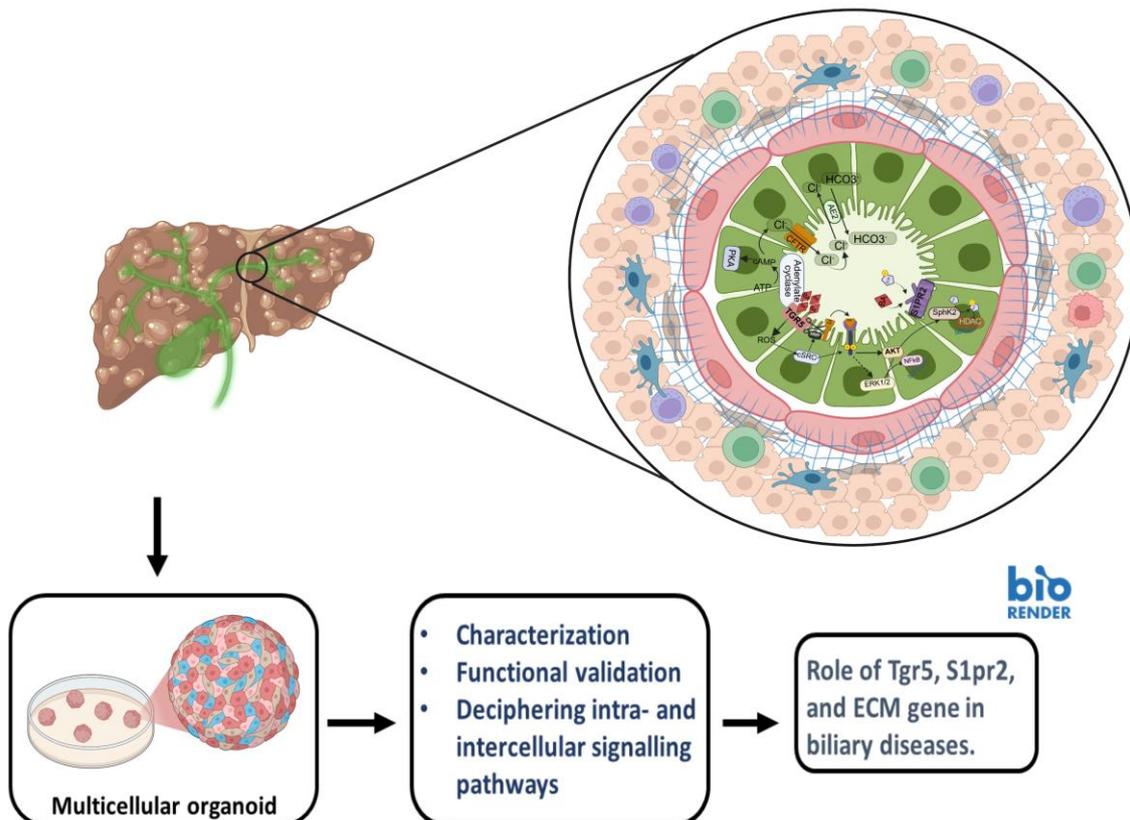
Optimized multicellular organoid models incorporating bile acid signalling and ECM gene networks represent a crucial step towards more physiologically relevant disease modelling and therapeutic development in biliary diseases.

Results

Multicellular liver organoids were successfully generated by isolating distinct cell populations from the mouse liver, starting with liver perfusion. Hepatocytes and non-parenchymal cells (NPCs) were seeded at a 2:1 ratio and cultured for 15-20 days to allow organoid formation. Organoids were initially characterized by haematoxylin and eosin (H&E) staining to distinguish nucleated from non-nucleated cells and to assess overall morphology. To verify cellular composition, immunohistochemistry (IHC) was performed to confirm the presence of different cell types within the organoids, including hepatocytes, liver sinusoidal endothelial cells, Kupffer cells, and hepatic stellate cells (HSCs). Functional validation was carried out using the CellTracker Green CMFDA assay, enabling visualization of bile canaliculi like structures and assessment of transporter activity.

Conclusions

Multicellular liver organoids were successfully established, characterized by H&E and IHC, and functionally validated through CMFDA assay, showing bile canaliculi-like structures.



Mechanisms of induction of classical NF-κB by *H. pylori*

Background

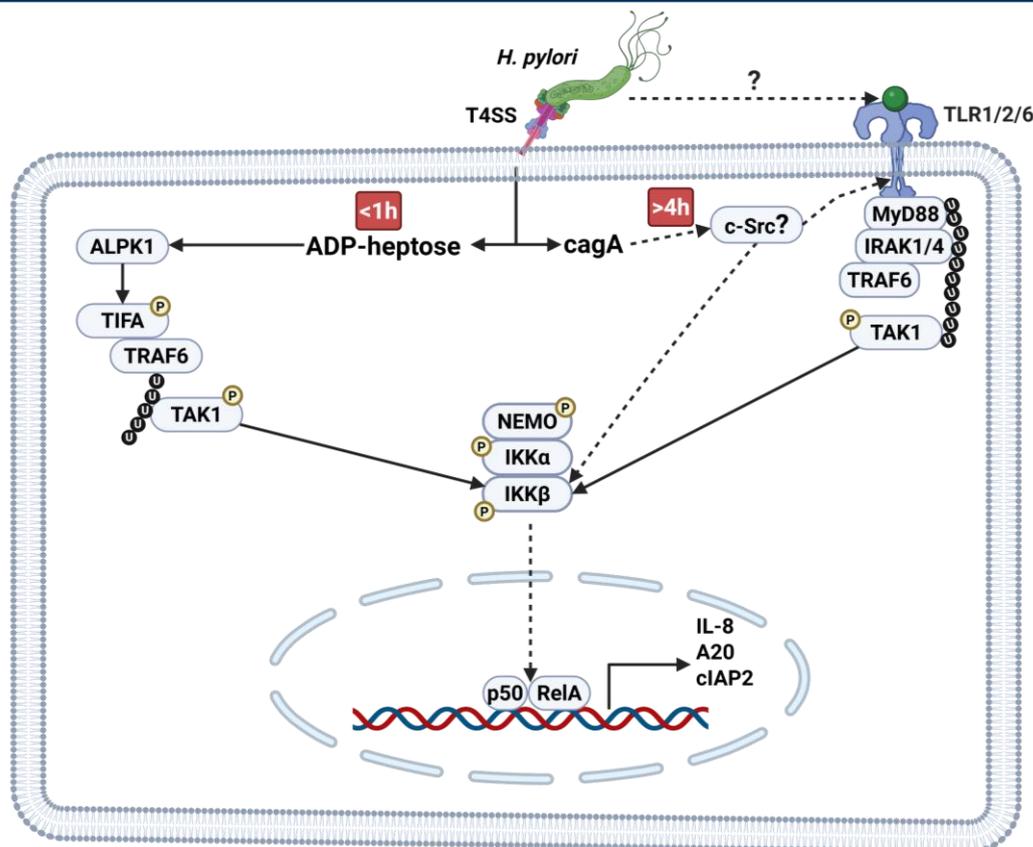
Helicobacter pylori is a gastric pathogen and linked to gastric diseases including cancer. We are investigating diverse molecular mechanisms by which *H. pylori* induces classical NF-κB signaling.

Results

The *H. pylori* effector molecule ADP-glycero-β-D-manno-heptose (ADP-heptose) is a potent and rapid inducer of NF-κB in gastric epithelial cells. In addition to the rapid type IV secretion system-dependent NF-κB response, we identified a delayed NF-κB response, that enables sustained NF-κB activity. Based on the study of isogenic mutants, we suspect that the injected effector protein CagA controls this activity. Furthermore, knockdown experiments showed that MyD88, IRAK1, and TRAF6 are essential for this activity, suggesting the involvement of Toll-like receptors. The role of these molecules and others is currently also being investigated in primary mucosoid cultures. In order to study the microenvironment under conditions that are as close as possible to those *in vivo*, we are planning Transwell co-culture assays with epithelial cells, gastric fibroblasts, and endothelial cells. This system will allow us to assess how these cells contribute to the NF-κB regulation in epithelial cells. Finally, we will perform RNA sequencing of the different cell populations to identify the regulatory molecules responsible for cell communication.

Conclusions

We investigate rapid and sustained NF-κB responses to *H. pylori*, involving distinct virulence factors and host pathways. Co-cultures with different cell populations will address the role of the microenvironment in *H. pylori*-induced NF-κB.



Balancing Immunity and Pathology: Lipid Metabolism and Siglec Signaling in Th2 and Autoimmune Responses

Background

Protective and pathological immune responses often involve similar mechanisms. We investigate Th2 cell metabolism in helminth infection and asthma, and Siglec-mediated immune modulation in *C. jejuni*-triggered Guillain-Barré syndrome.

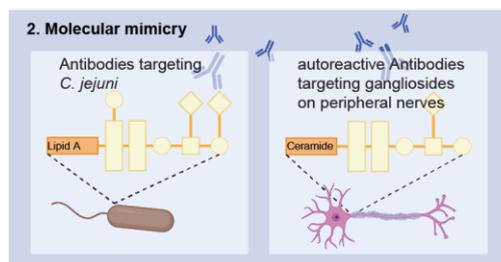
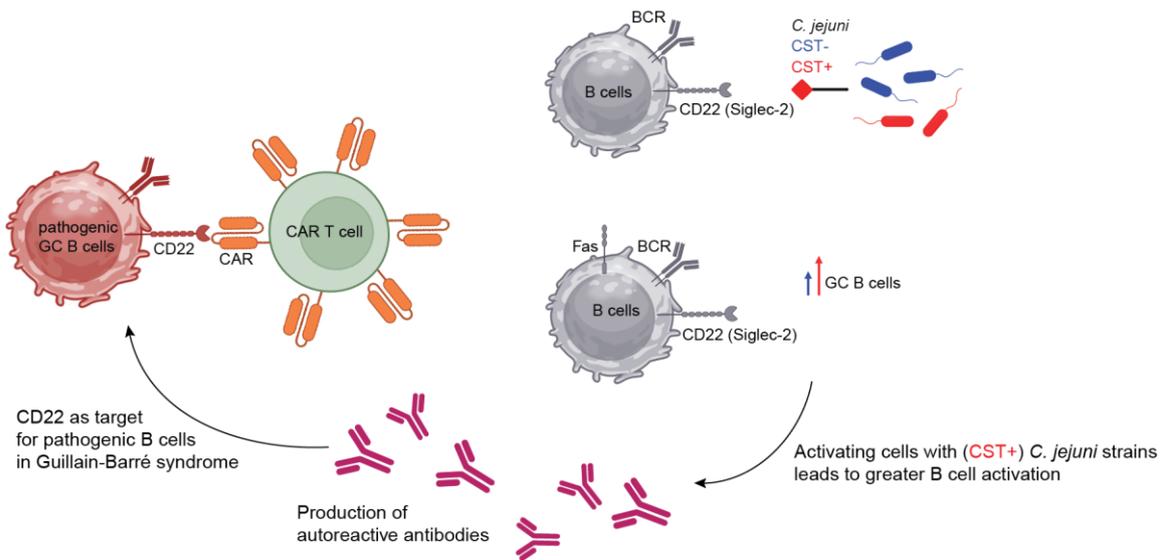
Results

Th2 cells mediate both protective immunity against *Nippostrongylus brasiliensis* and pathological inflammation in allergic asthma. Our preliminary data suggest a strong dependency of Th2 cells on lipid metabolic pathways, particularly during airway inflammation. Using genetically modified mice, bulk RNA-seq, and scRNA-seq, we investigate how lipid receptors modulate Th2 cell function and their interaction with airway epithelial cells during helminth infection versus asthma.

In parallel, Guillain-Barré syndrome (GBS) exemplifies an autoimmune condition driven by molecular mimicry following *Campylobacter jejuni* infection. Sialylated surface structures of *C. jejuni* may be recognized by Siglec receptors on immune cells. Our data show that strains expressing sialyltransferases significantly alter T and B cell frequencies in vitro. We currently analyze the Siglec repertoire of immune cells upon exposure to these strains and perform whole genome sequencing of *C. jejuni* isolates from GBS patients to identify potential molecular triggers.

Conclusions

Our studies aim to uncover shared immunological mechanisms in infection, allergy, and autoimmunity, paving the way for targeted therapies that modulate immune responses without compromising host defense.



Characterization of the genotype underlying hereditary forms of intrahepatic cholestasis

Background

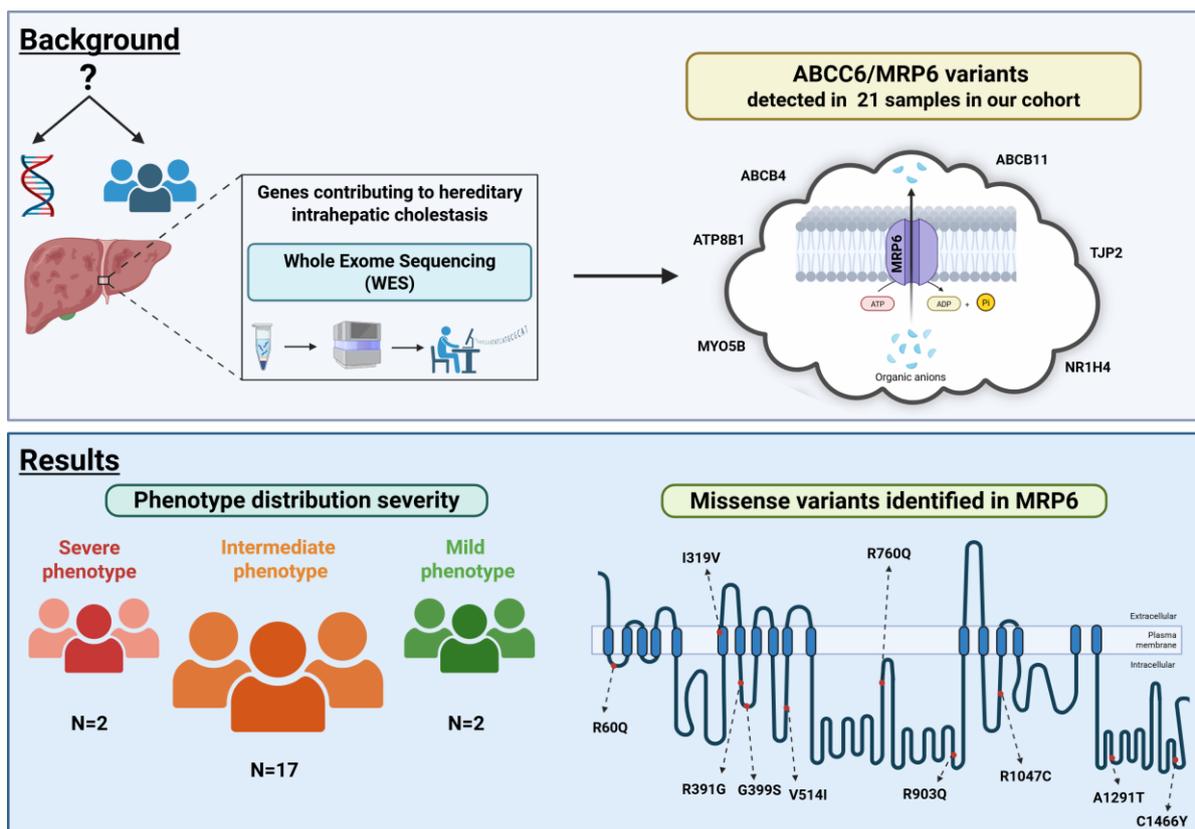
In an index patient with Primary sclerosing cholangitis, we identified a heterozygote candidate variant in the ABCC6 gene (c.2279G>A). ABCC6 variants have been previously described in few cases of hereditary intrahepatic cholestasis.

Results

To identify potentially contributing variants, we carried out variant annotation analysis on the outputs from whole exome sequencing (WES). We analyzed the VCF files from our patient cohort using Exomiser to identify candidate variants relevant to each individual's phenotype. We customized Exomiser to effectively prioritize and annotate genetic variants related to cholestasis. By integrating patient specific phenotype data and tailoring the tool's settings, we enhanced its ability to rank potentially pathogenic variants with potentially clinical relevance. 13 different heterozygote ABCC6 variants were identified in 21 of 286 WES results. Among these, c.179G>A, c.3139C>T, c.2787+1G>T variants were found in patients with low phospholipid-associated cholelithiasis (LPAC). Additionally, c.1171A>G was observed in two related cases: a mother with intrahepatic cholestasis of pregnancy (ICP) and her son with elevated transaminases and jaundice. Moreover, this variant was also detected in two other LPAC patients. c.3871G>A, and c.955A>G were detected in cholestasis and ICP cases, respectively. The splicing variant c.37-1G>A was seen in a patient with progression to cholangiocarcinoma.

Conclusions

These results may point to clinical relevance of ABCC6 variants in intrahepatic cholestasis. Further analysis in a larger cohort is needed, along with in silico and in vitro studies, to determine the association to the patients' phenotypes.



Interleukin-7 dependent infiltration of acute lymphoblastic leukemia across the testicular endothelial barrier

Background

Testis immune privilege is maintained by active immune regulation, not passive exclusion, through mechanisms largely unknown. A disruption of this balance can lead to inflammation and fertility loss or undue tolerance, such as in cancer.

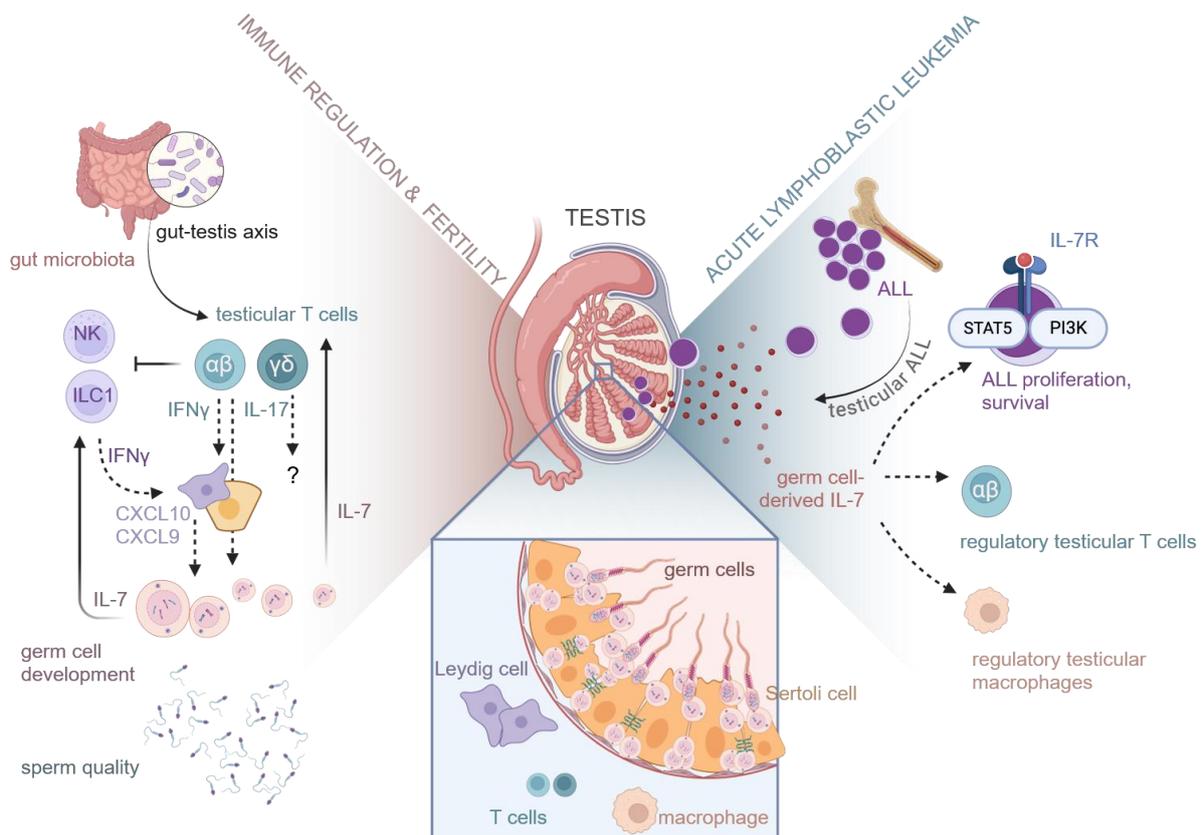
Results

First project part studies the role of testis-derived interleukin-7 (IL-7) in testicular ALL involvement. We determined male germ cells as the cellular source of testicular IL-7 in mice and human. We currently establish an immunocompetent mouse model of testicular ALL to study leukemia progression in mice deficient for germ-cell-derived IL-7 and test therapeutical options targeting IL-7 in testicular ALL (mAb, germ cell depletion etc.).

In parallel, we focus on mechanisms of testicular immune homeostasis. We determined T cells as a major IFN- γ source in the testis under physiological conditions. T cell numbers and levels of IFN- γ and IFN- γ -stimulated genes in testis were dependent on microbiota composition. This changes were associated with alterations in sperm concentration and quality, possibly due to increased germ cell apoptosis. Accumulation of T cells in the testis and IFN- γ production appear to be puberty-driven and antigen-independent. Interestingly, we discovered that, in absence of T cells, other IFN- γ -producing immune cells populations in the testis expand, suggesting a "suppressor" role of testicular T cells.

Conclusions

We uncovered a great complexity of testicular immune landscape. We currently decipher contributions of testicular immune and somatic cell populations to main testicular function i.e. fertility and pathology such as inflammation and cancer.



Investigating the role of mast cells in central and peripheral trained immunity upon skin inflammation

Background

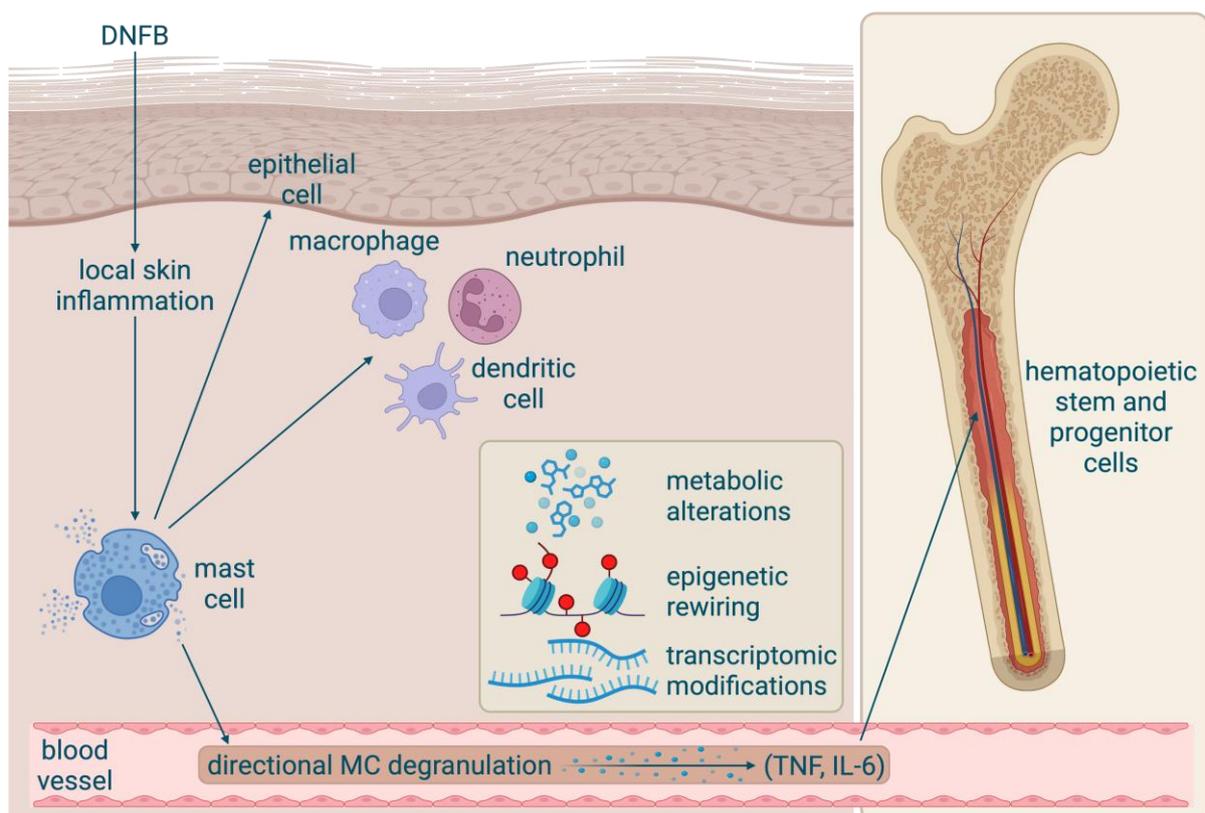
'Trained immunity' is a novel concept that describes long-term changes in innate immunity. We investigate how mast cells (MCs) influence myelopoiesis and peripheral trained immunity in contact hypersensitivity.

Results

Preliminary data from our lab show that skin MCs are relevant for a timely myelopoiesis in the bone marrow following DNFB-induced CHS. Specifically, skin MC-depleted mice displayed an impaired reconstitution of leukocytes, particularly monocytes and macrophages, 24 hours after DNFB exposure. Importantly, using MC-specific TNF knockout mice, we also found significant changes in long-term hematopoietic stem cells in bone marrow 48 hours after DNFB treatment, suggesting early effects of MC-derived signals already on progenitor dynamics. Furthermore, trends in additional hematopoietic progenitors and peripheral innate immune cell populations could be observed. We are currently investigating additional time points and mouse lines either lacking connective-tissue type MCs in their entirety or MC-derived IL-6.

Conclusions

Our findings suggest that MCs and their mediators shape myelopoiesis during DNFB-induced skin inflammation. Furthermore, they might play a role in recruiting and maintaining peripheral innate immune cell populations.



Bidirectional cross-talk between mast cells and endothelial cells in homeostasis and inflammation

Background

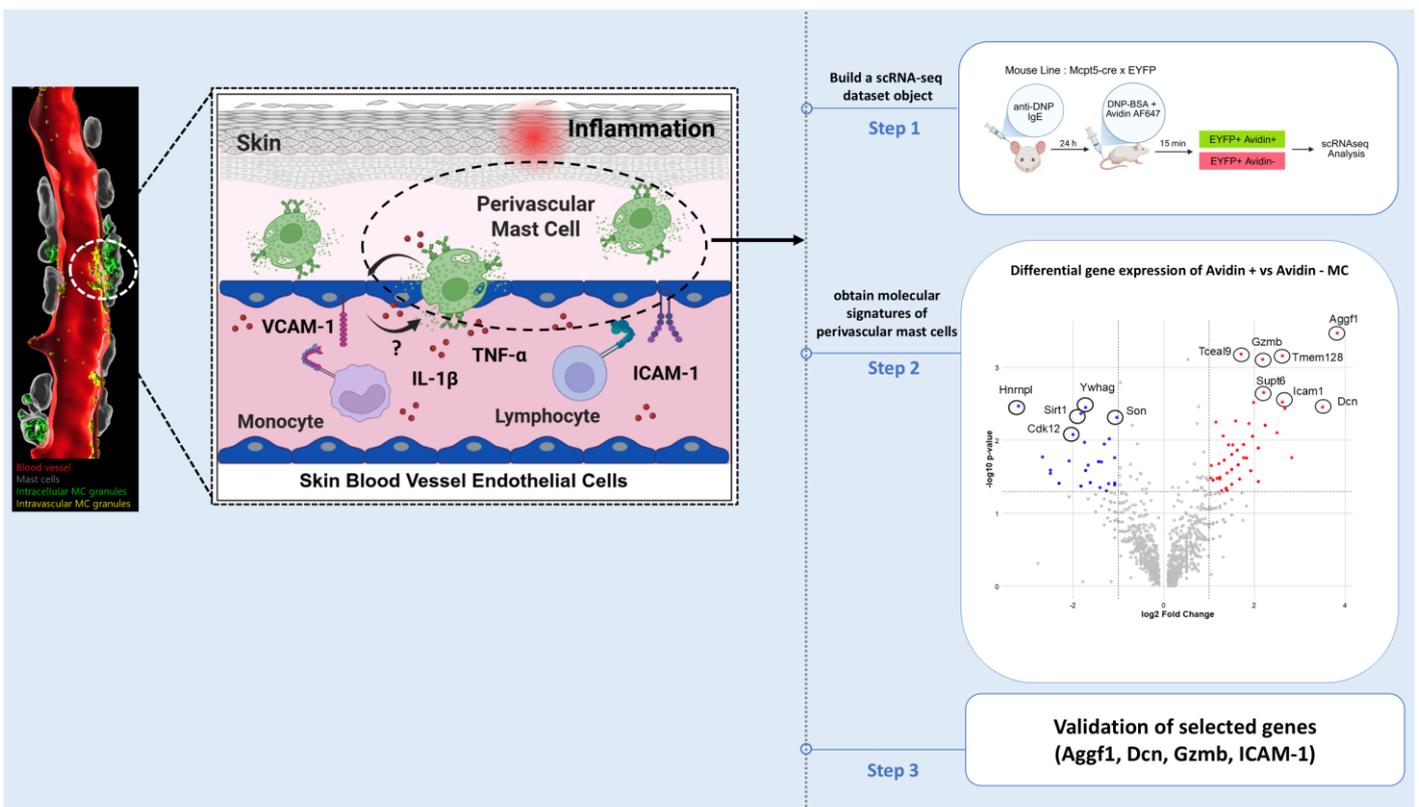
In skin, perivascular MCs (pMCs) are located at blood vessels and pass the endothelial barrier to form intraluminal sheets. Thereby, pMCs can sample and degranulate into the bloodstream and are in direct contact with endothelial cells (ECs).

Results

To characterize pMCs in close contact with ECs, we performed single-cell RNA sequencing (scRNA-seq). Therefore, during a modified passive cutaneous anaphylaxis (PCA) approach, fluorescently-conjugated avidin binding to the heparin structure of MC granules, was intravenously injected, which labeled degranulated pMCs with luminal access (Avi+). In contrast, vessel-distant MC populations remain avidin-negative (Avi-). scRNA-seq enabled high-resolution clustering of MC and pMC populations, revealing six distinct clusters of Avi- MCs, and three transcriptionally defined clusters of Avi+ pMCs. Differential gene expression analysis between the Avi+ and Avi- groups showed high expression of the genes encoding for *Aggf1* (angiogenesis regulator), *Gzmb* (extracellular matrix protease), *Decorin* (extracellular matrix proteoglycan), and *ICAM1* (adhesion molecule) in Avi+ populations. Ongoing work includes the isolation and characterization of ECs to establish co-culture systems with MCs and their granules. To assess EC responses, apoptosis assays are conducted, and dermal sheet staining will be performed to validate gene expression at the protein level to establish in vivo models.

Conclusions

Our data suggest that Avi+ MCs are a transcriptionally distinct subgroup that is adapted to the endothelium and may play a role in angiogenesis, vascular integrity, and immune cell recruitment.



Screening for systemic factors in biliary diseases

Background

Cholangiocarcinoma (CCA) is an aggressive bile duct cancer with rising incidence and limited treatment options. Understanding how serum factors affect tumor behavior is key to discovering better biomarkers and targeted therapies.

Results

To modulate tumor behavior and its microenvironment we established a 3D spheroid model created from cholangiocyte cell lines. ECCA cell lines EGI-1 and TFK-1 and the normal cholangiocyte cell line H69 formed the most stable and globular spheroids and showed the highest growth over time. Blood sera from various CCA and primary sclerosing cholangitis (PSC) patients were collected from our biobank and used to stimulate the spheroids. Spheroids incubated with one of the PSC sera showed significant growth and phenotypic changes after 24 h, as determined by brightfield microscopy. The EGI-1 and TFK-1 spheroids exhibited a 2.5-fold and 4-fold increase in cell viability, respectively, when compared to spheroids exposed to healthy serum. In contrast, H69 spheroids showed lower viability, which is appropriate, as they are normal human cholangiocytes. Serum from another PSC patient showed no effect on morphology or growth. Analysis into disease activity, presence of cholangitis and treatment to compare the patients are ongoing. Further experiments are underway to identify the factors responsible for the observed growth stimulation.

Conclusions

Increased growth and viability of cancerous cholangiocytes in response to PSC serum highlight complex tumor microenvironment interactions and confirms the role of blood-borne factors in promoting tumor proliferation.

