RTG 2408 | Maladaptive processes across physiological barriers in chronic diseases

Annual RETREAT

SEPTEMBER
25–27
2024

RTG 2408

USEFUL INFORMATION

VENUE & ACCOMMODATION

Schlosshotel Villa Westerberge

An den Westerbergen 1

06449 Aschersleben

TIMES

Check-in:	Day 1 from 15:00
Check-out:	Day 3 by 11:00
Breakfast	7:00 – start of program

ROOMS

Breakfast, lunch, dinner:	Restaurant
Talks, meetings, evening events:	Conference room
Meeting point walk on day 2:	In front of hotel

OTTO VON GUERICKE UNIVERSITÄT MAGDEBURG
MED FACULTY OF MEDICINE

Funded by
DFG Deutsche Forschungsgemeinschaft
German Research Foundation

PROGRAM

Day 1 | September 25, 2024

12:00–13:00	Arrival & Welcome Lunch
13:00–13:15	Welcome
Session 1	Chairs: Niklas Heucke, Sohail Ahmad
13:15–13:55	*USP48-dependent regulation of NF-kB in the H. pylori infected gastric mucosa* P1-2 | Lorena Ferino
13:55–14:35	*Th2 cell-dependent effects on the airway epithelial barrier during chronic asthma* P12-2 | Anna Krone
14:35–14:50	Coffee Break
Session 2	Chairs: Sandro Gogia, Eliza von Gehlen
14:50–15:30	*Impact of oxidative stress in T-cell subsets on chemotherapy response and survival in patients with Acute Myeloid Leukemia* CS6 | Tobias Ronny Haage
15:30–16:10	*Interleukin-7 (IL-7) dependent infiltration of acute lymphoblastic leukemia (ALL) across the testicular endothelial barrier* P14-2 |Vladyslava Dovhan
16:10–16:25	Coffee Break
Session 3	Chairs: Arun Kanthasamy, Tobias Ronny Haage
16:25–17:05	*Characterization of the genotype underlying hereditary forms of intrahepatic cholestasis* AP11 | Somayeh Alinaghi Arjas
17:05–17:45	*Characterization of the specific functional relevance of perivascular mast cells in skin inflammation* P4-2 | Aaron Hoffmann
17:45–18:30	Student Meeting, parallel: PI Meeting
18:45–19:45	Dinner
20:00	Pub Quiz

PROGRAM

Day 2 | September 26, 2024

Session 4	Chairs: Vladyslava Dovhan, Aaron Hoffmann
9:00–9:40	*Modes of Helicobacter pylori induced NF-kB activity in the gastric mucosa* AP10 | Arun Kanthasamy
9:40–10:20	*Characterization of the cellular expression pattern of bile acid receptors in intrahepatic cholangiocellular carcinoma* CS5 | Niklas Heucke
10:20–10:35	Coffee Break
Session 5	Chairs: Somayeh Alinaghi Arjas, Lorena Ferino
10:35–11:15	*The role of cold shock proteins in mitochondrial homeostasis and tubular cell phenotype determination during cell stress* P8-2 | Sohail Ahmad
11:15–11:55	*Exploitation of epithelial/endothelial microenvironment crosstalk* P15-2 | Sandro Gogia
12:00–13:00	Lunch Break
Session 6	Chair: Anna Krone
13:00–13:40	*Role of lipid receptor signaling in the differentiation and effector function of pathogenic Th17 cells in central nervous system autoimmunity* MD11 | Eliza von Gehlen
13:40–14:00	Coffee Break
14:20–17:30	Walk and Guided Gallery Tour
17:30–19:00	General Assembly
19:00–20:00	Dinner
20:00	Career Round Tables with PIs

PROGRAM

Day 3 | September 27, 2024

9:00–9:45	Small Group Discussions Discussion on how to get started in RTG in 4 groups: (1) Organization & Administrative Procedures (2) Scientific Introduction (Theoretical & Practical) (3) Personal Development (4) Group Atmosphere & Social Integration
9:45–10:00	Coffee Break
10:00–11:30	Summary Discussion Presentation of group discussions and development of a “Welcome Concept” for the 3rd cohort
11:30–12:15	Farewell Lunch & End

P1-2 | LORENA FERINO

Supervisor: Michael Naumann

Sep 25, 2024

13:15

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

Background

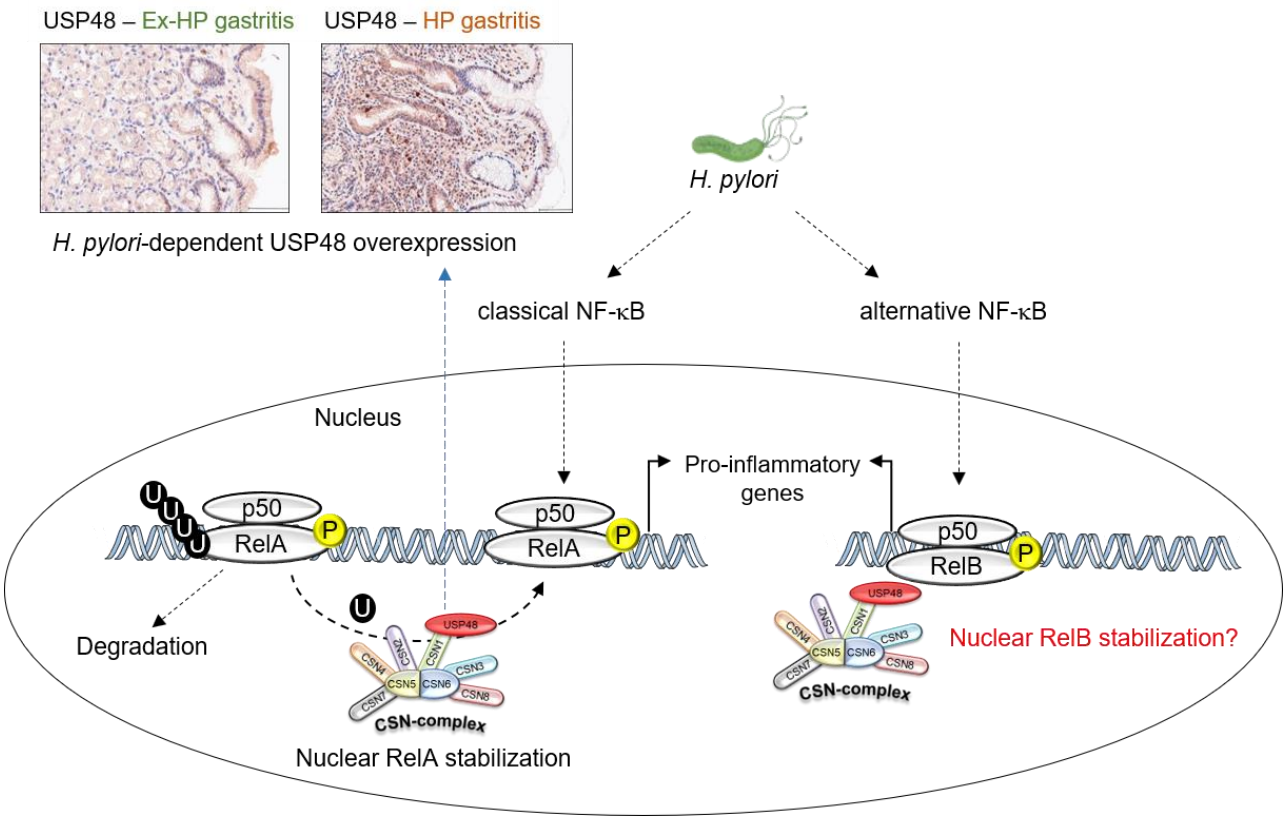
H. pylori induces classical and alternative NF-κB in the gastric epithelium. We showed that CSN-associated USP48 stabilizes nuclear RelA via deubiquitinylation upon infection and aim to extend the research to their role in alternative NF-κB

Results

We observed that the NF-κB regulating deubiquitinylating enzyme (DUB) USP48 is upregulated in *H. pylori*-associated gastritis, and can serve as a prognostic marker. Moreover, *H. pylori*-induced classical NF-κB promotes alternative NF-κB via RelA-dependent transcription and subsequent nuclear translocation of RelB. Therefore, we investigate the mechanistic of *H. pylori* regulated NF-κB signaling by USP48.
Relevant for NF-κB signaling is neddylation, a post-translational modification required for the activation of cullin-RING-E3 ligases that regulate the turnover of κB inhibitors, such as IκBα and p100. Blocking neddylation by a small inhibitor suppresses classical and alternative NF-κB. The multi-protein complex COP-signalosome (CSN) counteracts the cellular control of neddylation. Besides that, CSN-associated USP48 stabilizes nuclear RelA. Knockdown of either USP48 or CSN2 also caused a diminishment of nuclear RelB as well as a decline in NF-κB target gene expression. Moreover, an inducible interaction between USP48, CSN and RelB was detected in co-IP upon infection.

Conclusions

As CSN and USP48 interact 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.



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

Background

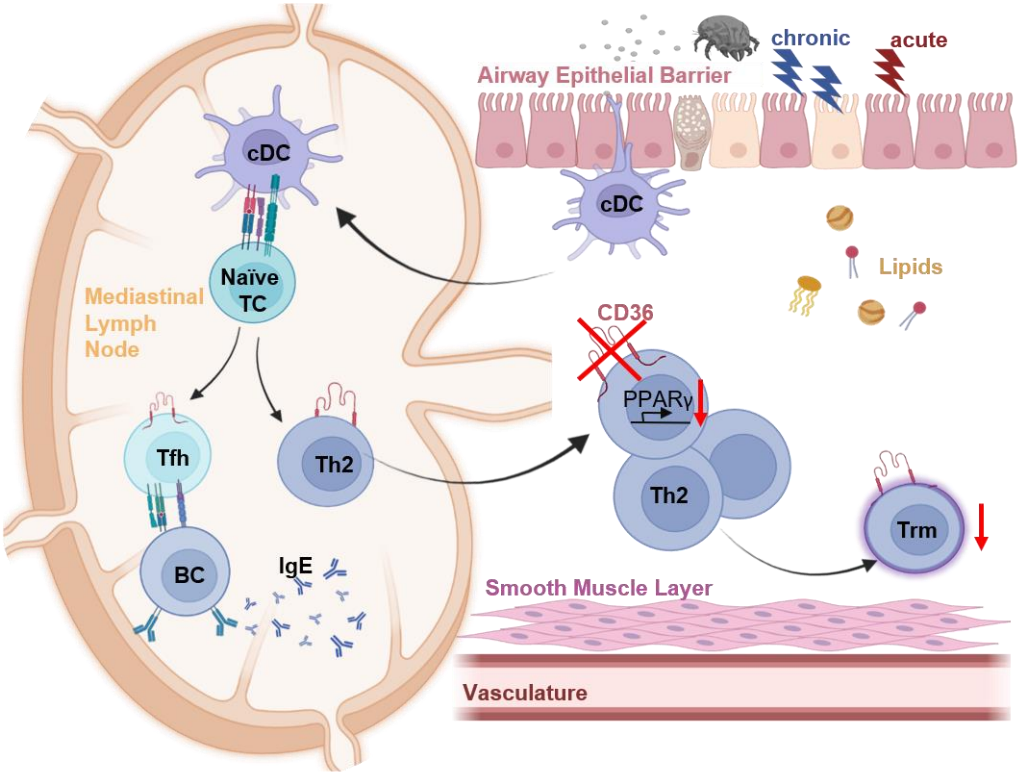
Type 2 T helper cells (Th2) play a crucial role in asthma and rely heavily on lipid metabolism. Notably, asthmatic lungs display elevated lipid levels and the lipid and signaling receptor CD36 has been found to be significantly upregulated.

Results

To investigate the dependence of Th2 cells on lipid receptors during asthma, we induced acute and chronic house dust mite (HDM)-mediated allergic airway inflammation in wild-type mice and mice with a CD4+ T cell-specific deletion of CD36. We analyzed blood, lungs and mediastinal lymph nodes using flow cytometry, ELISA, histological staining and RNA sequencing. Significant differences in IgE levels were observed between the acute and chronic models. Bulk RNAseq revealed a marked downregulation of asthma-related genes, including Il4, Il5 and Il13, which are crucial for Th1/Th2 differentiation. Furthermore, we identified that the transcription factor PPARγ, which acts downstream of CD36, is critical for the development of tissue-resident memory T cells (Trms). These Trms persist in the lung during inflammation and are key drivers of asthma relapses. The deletion of CD36 resulted in a significant reduction of PPARγ expression, accompanied by lower frequencies of Trms in the lung. Additionally, single-cell RNAseq of lung leukocytes revealed an altered gene expression in non-CD4+ cells such as type 2 conventional dendritic cells (cDC2), which are important for Th2 cell polarization.

Conclusions

Th2 cells exhibit a specific dependency on lipid receptors like CD36 during asthma, due to their role in the generation of Trms and their influence on intercellular communication.



CS6 | TOBIAS RONNY HAAGE

Sep 25, 2024
14:50

Supervisor: Dimitrios Mougiakakos

Impact of oxidative stress in T-cell subsets on chemotherapy response and survival in patients with Acute Myeloid Leukemia

Background

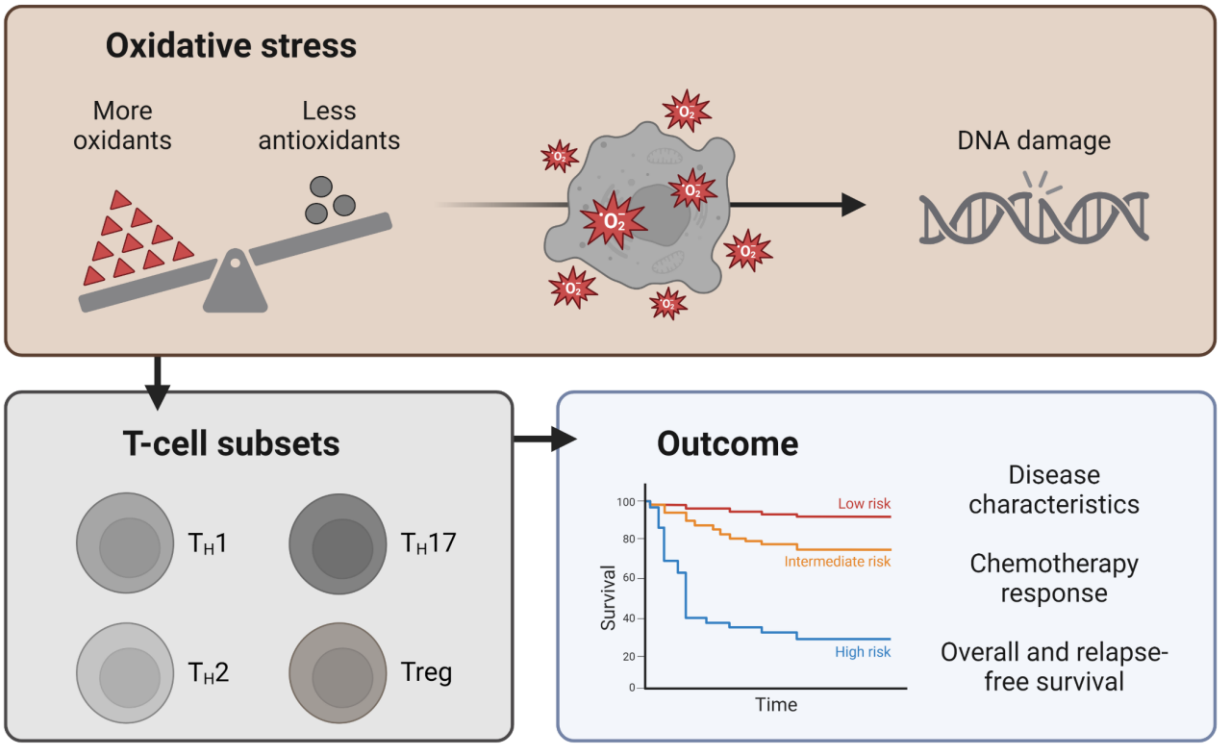
The importance of T-cell immunity in hematologic diseases has recently been demonstrated in various studies for acute lymphoblastic leukemia and after allogeneic stem cell transplantation (allo-HSCT) for acute myeloid leukemia (AML).

Results

T-cells are regarded as a physiological barrier against malignant transformation, a chronic phase of immune attenuation often precedes the acute process. In this project, the influence of different T-cell subsets and in particular the effect of oxidative stress on T-cell immunity in relation to chemotherapy response and survival in patients with AML shall be investigated. For this purpose, patient samples from our biobank are used to unmask differences in T-cell immunity at initial diagnosis using a comprehensive FACS panel and to evaluate their influence on clinical (e.g. blast cells, inflammation, cytogenetics) and survival parameters (overall survival, relapse-free survival). 8-OHdG will be used to examine the influence of oxidative stress on T-cell immunity. The aim is to identify subgroups of AML patients who are associated with a particularly good or particularly poor overall survival, relapse-free survival or therapy response.

Conclusions

A better understanding of this relationship could lead to promising patient-specific treatment approaches, as it is conceivable that host-T-cell immunity has a similar impact as the already examined graft-T-cell immunity after allo-HSCT.



Created with BioRender.com

P14-2 | VLADYSLAVA DOVHAN

Supervisors: Dunja Bruder & Dimitrios Mougiakakos

Sep 25, 2024

15:30

Interleukin-7 (IL-7) dependent infiltration of acute lymphoblastic leukemia (ALL) across the testicular endothelial barrier

Background

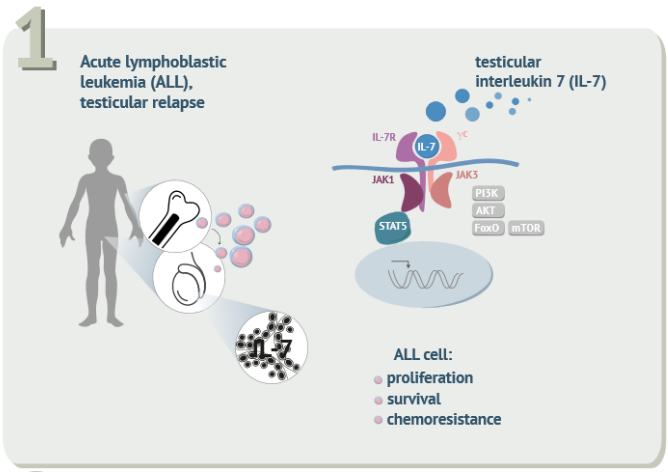
Dysregulated IL-7R signaling can lead to leukemia progression and relapse. Since testis is a site of ALL relapse and a major IL-7 source, this project studies how male gonad-derived IL-7 facilitates testicular ALL involvement.

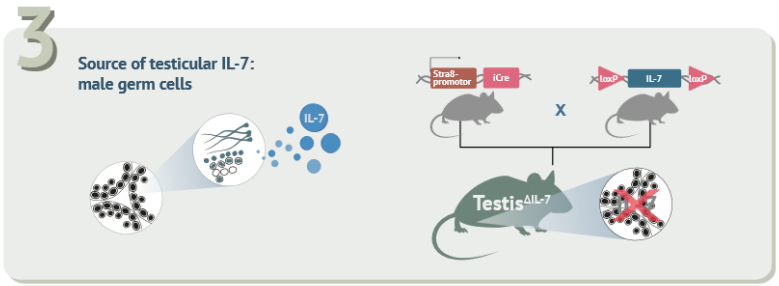
Results

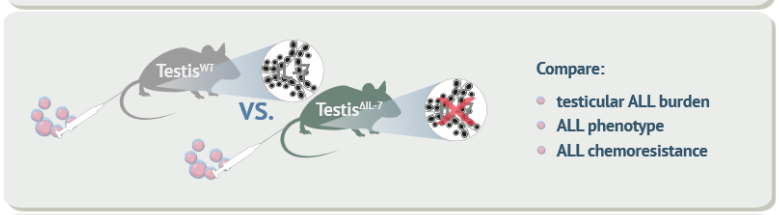
We determined male germ cells as the cellular source of testicular IL-7 in mice using methods of molecular biology and spatial transcriptomics. In line with that, human male germ cells are enriched for IL-7 mRNA reads, predominantly from meiosis onward. To test the role of local IL-7 on testis ALL involvement, we currently establish an animal model for testicular leukemia, which allows for germ cell-specific inactivation of IL-7. To this end, we generated an IL-7-sensitive ALL cell line from murine primary B-ALL on C57Bl/6 background, which allows the use of immunocompetent hosts for transplantation. To inactivate IL-7 specifically in male germ cells, we make use of Stra8-iCre mice. We validated specificity and efficiency (94-97%) of Cre-mediated male germ cell targeting in Stra8-iCrexRosa26RFP reporter mice and thus confirmed feasibility of Stra8-iCrexIL-fl model. After the approval of our animal experiment application, we will transplant Stra8-iCrexIL-fl mice with ALL cells and study the extent and quality of testicular involvement compared to WT littermates. Furthermore, we will test therapeutical options targeting IL-7 in testicular ALL (mAb, germ cell depletion etc.).

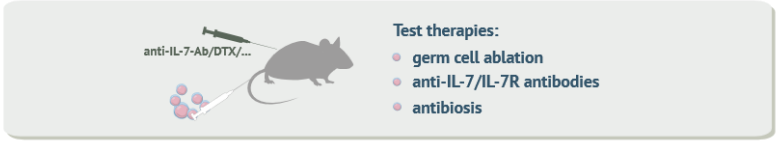
Conclusions

This project investigates the role of testicular niche in IL-7-dependent ALL cell survival, chemoresistance, and metabolic reprogramming. Ultimately, the research strives to develop less invasive treatment strategies for testicular ALL.









AP11 | SOMAYEH ALINAGHI ARJAS
Supervisor: Verena Keitel-Anselmino

Sep 25, 2024
16:25

Characterization of the genotype underlying hereditary forms of intrahepatic cholestasis

Background

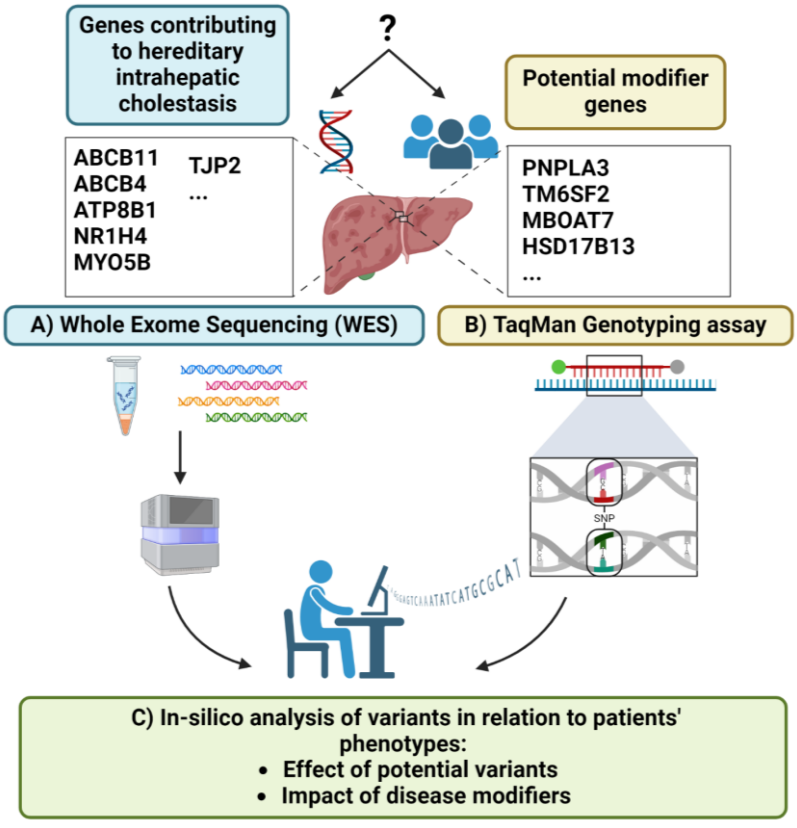
Intrahepatic cholestasis describes defective bile formation comprising a heterogeneous group of liver diseases. Identifying genetic variants contributing to hereditary cholestasis can improve diagnosis and understanding disease progression.

Results

To identify potentially contributing variants, variant evaluation and annotation analysis was carried out on whole exome sequencing (WES) focusing on loss of function and missense variants with an allele frequency of ≤1% in gnomAD. Rare variants in cholestasis-related genes; genes involved in bile acid synthesis, inflammation pathways in liver were analyzed in silico using prediction tools like CADD, LRT, MetaRNN, M-CAP, EIGEN, BayesDel. PhyloP100way and GREP scores were used to assess amino acid conservation. Databases were evaluated for further variant prioritization. In a patient with progressive primary sclerosing cholangitis (PSC), we discovered a possibly relevant heterozygote variant in ABCC6/MRP6 (p.(Arg760Gln)). Analysis of >80 WES results revealed another rare heterozygote MRP6 variant (p.(Arg391Gly)) in our cohort of cholestasis patients. Dysfunctional MRP6 can cause BSEP and ABCG5/G8 overexpression leading to imbalanced bile composition resulting in gallstone formation and cholestasis. ABCC6/MRP6 variants were described in patients with cholelithiasis or intrahepatic cholestasis of pregnancy (PMIDs: 37392836, 33546617).

Conclusions

These results may point to clinical relevance of ABCC6 variants. Further analysis of ABCC6 variants in larger cohorts is needed, along with in silico and in vitro studies, to assess the possible correlations to the patients' phenotypes.



Workflow for genetic analysis in hereditary intrahepatic cholestasis. Variants in genes involved in bile formation are related to hereditary intrahepatic cholestasis. Modifier variants may contribute to disease severity and progression.
A) Whole exome sequencing (WES) is performed to detect potentially relevant genetic variants in the protein-coding and adjacent regions. B) TaqMan genotyping assay is used to identify risk-modifying single nucleotide polymorphisms (SNPs) that may influence disease manifestation.
C) Bioinformatic analysis of WES results is carried out for alignment, annotation, and variant prioritization.

P4-2 | AARON HOFFMANN

Supervisor: Anne Dudeck

Sep 25, 2024
17:05

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

Background

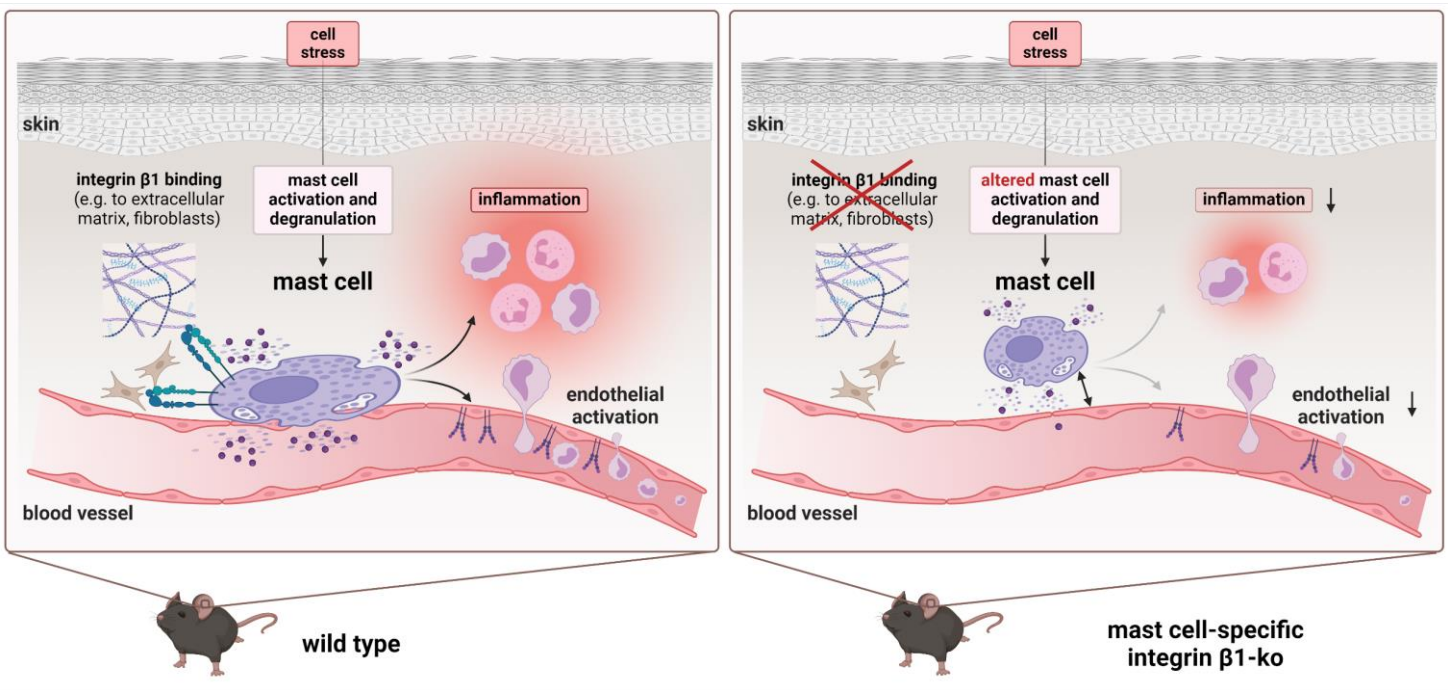
Perivascular mast cells (MC) are pivotal for the onset of innate immune responses by the directional release of MC granules. However, the mechanism of MC attachment to blood vessels, facilitating intraluminal degranulation, remains unclear.

Results

Quantitative high-throughput image analysis of fluorescence microscopy images of integrin β1-deficient MC mice ($MC^{\Delta Itgb1}$) compared to Cre- littermate controls showed that integrin β1 (Itgb1) is essential for the spatial distribution of MCs in the ear skin. In detail, the spindle-like morphology, perivascular alignment and positioning of MCs along the perivascular niche is impaired in $MC^{\Delta Itgb1}$ mice, particularly along arterioles. Moreover, $MC^{\Delta Itgb1}$ mice show a drastically lower ear swelling upon hapten elicitation in the contact hypersensitivity (CHS) model. In fact, there is a significant reduction of leukocyte infiltration into the challenged ear skin of $MC^{\Delta Itgb1}$ mice. In line with increased leukocyte numbers in the blood circulation, an impaired activation of blood endothelial cells could be revealed, potentially leading to a reduced extravasation of leukocytes from the blood to the skin upon CHS. To gain insights into underlying mechanisms, *in vitro* studies including enzymatic approaches, signaling analyses and live cell imaging revealed that the lack of Itgb1 in MCs is associated with an impaired degranulation efficiency upon activation via different stimuli.

Conclusions

Integrin β1 is vital for MC morphology and perivascular alignment in the skin. Further, a potential bidirectional crosstalk between MCs and blood endothelial cells seems to be crucial for the leucocyte extravasation from blood upon CHS.



Modes of *Helicobacter pylori* induced NF-κB activity in the gastric mucosa

Background

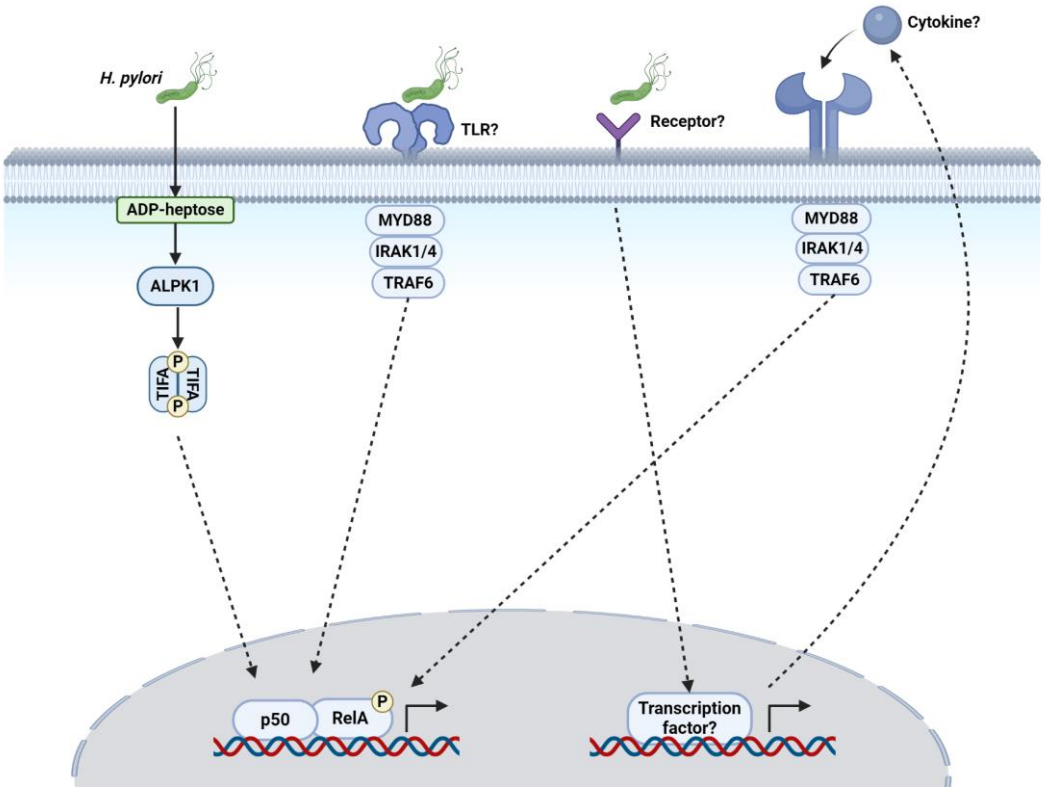
H. pylori triggers delayed NF-κB activity in gastric epithelial cells independent of ADP-heptose, which acts through the ALPK1/TIFA signaling axis. We aim to investigate the factors involved in signal transduction.

Results

In contrast to wild type strains, isogenic mutants of *H. pylori* with deficiency in ADP-heptose synthesis induce delayed and weak activation of NF-κB in epithelial cells. However, ALPK1 or TIFA KO cells showed delayed, but strong NF-κB activity (4 hours post infection).
Preliminary results indicate that the delayed NF-κB activity is dependent on TRAF6, MyD88 and IRAK1 suggesting that Toll-like receptor- (TLR) or interleukin receptor- (IL) signaling pathways are involved. Delayed NF-κB signaling in response to *H. pylori* was also observed in different gastric epithelial cell lines. Neither knockdown of TLR4 nor TLR5 affected the signaling. Thus, we are currently exploring the possibility of other receptors or autocrine induction of the signaling. Further, we plan co-culture experiments to study the influence of the cancer associated fibroblasts in delayed NF-κB signaling induction by *H. pylori.*

Conclusions

Delayed NF-κB signaling is long-lasting and hence this signaling pathway could play a role in chronic inflammatory signaling in the gastric mucosa, which could leads to tumorigenesis.



CS5 | NIKLAS HEUCKE
Supervisor: Verena Keitel-Anselmino

Sep 26, 2024
9:40

Characterization of the cellular expression pattern of bile acid receptors in intrahepatic cholangiocellular carcinoma

Background

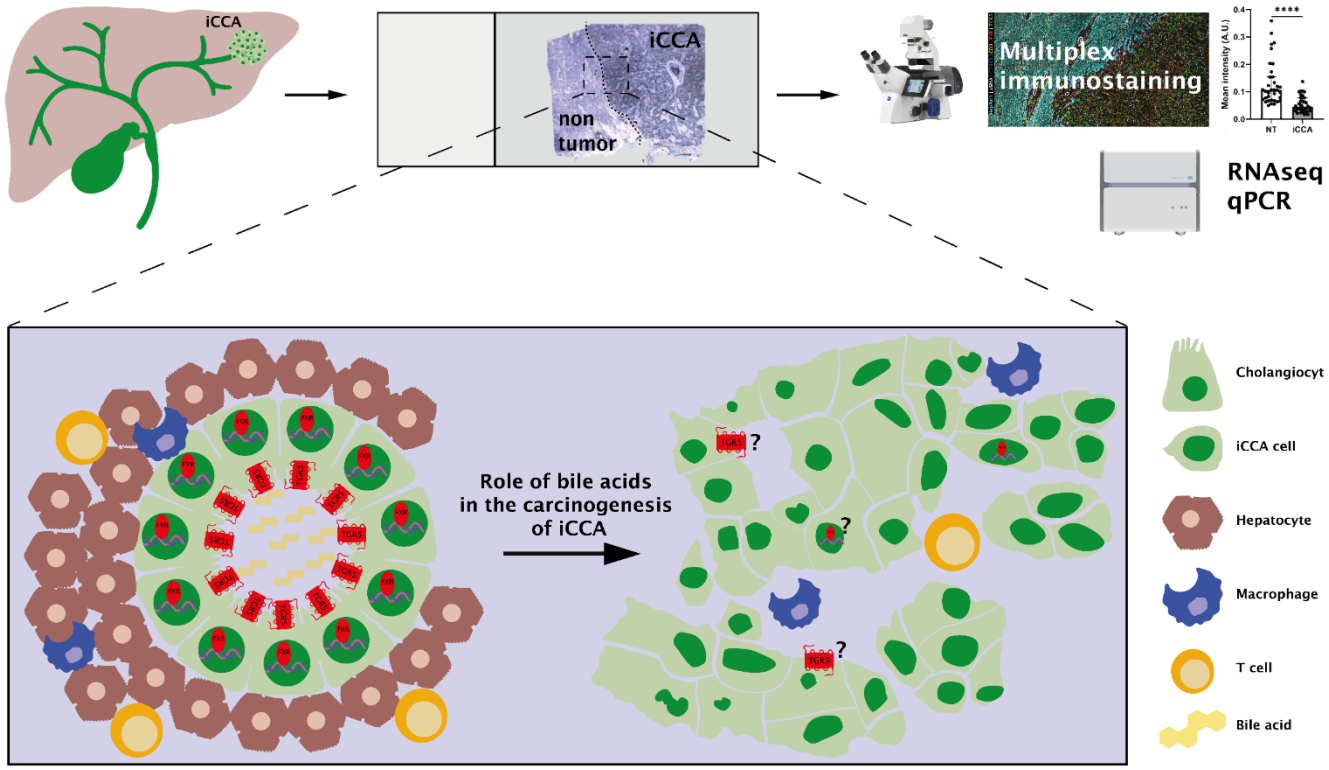
Bile acids have been implicated in the carcinogenesis of intrahepatic cholangiocellular carcinoma (iCCA). However, the spatial expression pattern of bile acid receptors in iCCA is unknown, although they are potential drug targets.

Results

Gene and protein expression of FXR and TGR5 was assessed in 35 iCCA patients using paraffin-embedded tissue. Multiplex immunostaining was used to characterize the cellular landscape using cell-specific markers (CK7, cholangiocytes and iCCA cells; CD163, macrophages; CD3, T cells). FXR mRNA, quantified by PCR, was found to be lower in bulk iCCA samples than in adjacent normal tissue. However, FXR staining intensity did not differ when looking specifically at cholangiocytes and iCCA cells. TGR5 staining intensity was lower in iCCA cells, although no difference was measured at the mRNA level. FXR and TGR5 staining intensities were positively correlated. TGR5 staining intensity was higher in tumors with perineural invasion. No correlation was found between FXR or TGR5 staining levels and disease-free survival. iCCAs with high bile acid receptor staining signals had more intratumoral macrophages compared to low expressing tumors. TGR5 showed three different staining patterns in iCCA, with no relationship to patient survival. However, tumors with high TGR5 staining intensities at the invasion front had fewer T cells and more macrophages throughout the whole tissue section.

Conclusions

iCCAs are characterized by a distinct bile acid receptor expression pattern with a specific associated immune cell landscape. Spatial resolution is therefore crucial to fully unravel the underlying pathomechanism of iCCA carcinogenesis.



P8-2 | SOHAIL AHMAD
Supervisor: Peter Mertens

Sep 26, 2024
10:35

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

Background

High glucose and salt contributes to renal damage. Senescence has been identified as the potential target to prevent renal cell loss. Cold shock proteins YB-1 and DbpA could be crucial due to their role in cell cycle and proliferation.

Results

Kidney tubular cells from (Ybx3+/+ and Ybx3-/-) mice and human embryonic kidney cells (HEK-293 cells) deficient in DbpA and YB-1 were exposed to high glucose and salt stress in vitro. Cellular markers for senescence and expression of SGLT-2 transporter were analyzed. Both mouse primary tubular cells and human HEK-293 cells developed senescence when exposed to high glucose and salt stress in vitro. Key senescence markers (p21, p16 and SA B. galactosidase) and sodium glucose co-transporter 2 (SGLT-2) expression was upregulated in tubular cell from DbpA wild type mice as well as HEK-293 cells. While DbpA knockout tubular cells has shown enhanced cellular senescence under control as well as stress condition when compared wild type. In human HEK-293 cells knockdown of YB-1 and DbpA has improved the high glucose and salt induce senescence, which was evident by downregulation of cell cycle inhibitor p21 and p16. Importantly YB-1 and DbpA knockdown has down-regulated the expression of SGLT-2 transporter in HEK-293 cells.

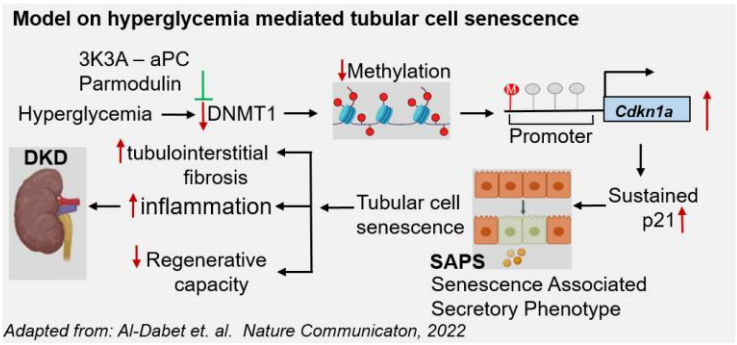
Conclusions

Both mouse primary tubular cells and HEK-293 cells developed senescence under high glucose and salt stress. DbpA is identified as a key regulator for stress induce tubular cell senescence via down-regulation of SGLT-2 transporter.

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

State of the art:

Model on hyperglycemia mediated tubular cell senescence

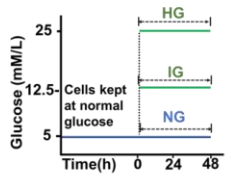


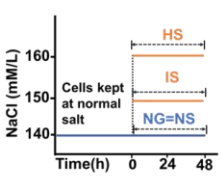
Adapted from: Al-Dabet et. al. Nature Communicaton, 2022

Questions:

- **Is there a cell-specific effect of hyperglycemic memory on cell senescence?**
- **Are cold shock proteins involved in the cellular senescence program?**
- **Are SGLT2 transporter in tubular cells regulated by hyperglycemia *in vitro*?**
- **Is there a link between SGLT2 transporter and cold shock protein expression?**

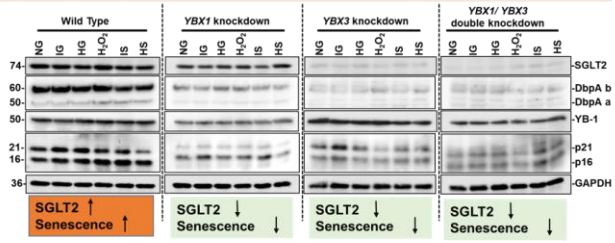
Experimental setup:



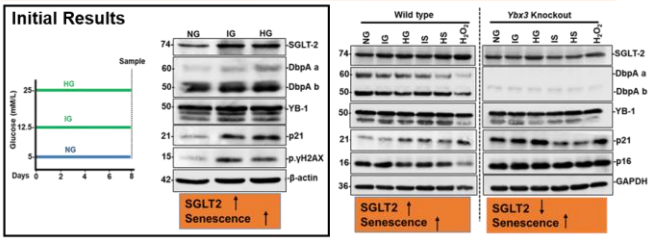


NG = Normal Glucose
IG = Intermediate Glucose
HG = High Glucose
IS = Intermediate Salt
HS = High Salt
H_2O_2 = Hydrogen peroxide (500 µM/ml for 6h)

Results: Human Embryonic Kidney cells (HEK293)



Results: Mouse Primary Tubular cells



Answers:

- **Both human HEK293 cells and murine primary tubular cells develop senescence under hyperglycemic stress.**
- **Cold shock proteins are involved in induction of cellular senescence via SGLT-2 expression regulation.**
- **Expression of SGLT2 transporters upregulated by hyperglycemia in tubular cells.**

P15-2 | SANDRO GOGIA

Supervisor: Michael Naumann

Sep 26, 2024

11:15

Exploitation of epithelial/endothelial microenvironment crosstalk

Background

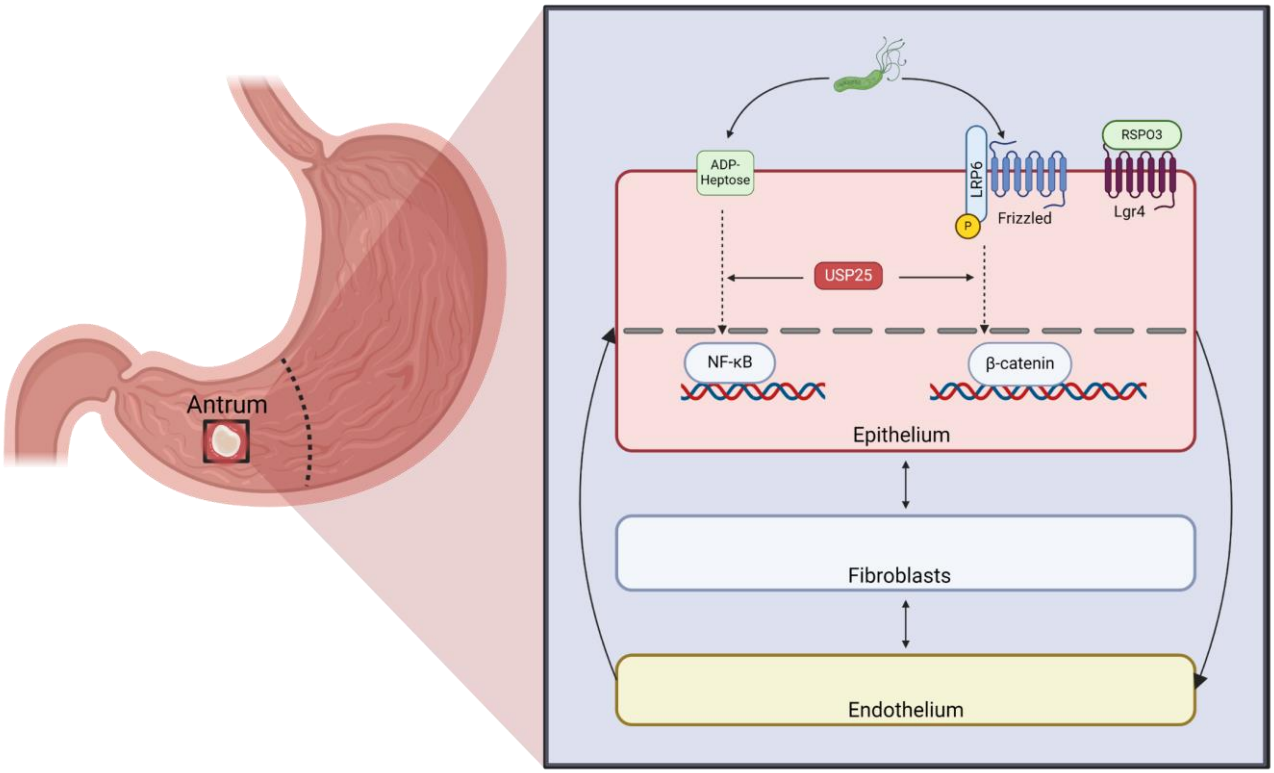
H. pylori triggers responses in gastric stem cells, causing inflammation and mucosal changes, attributed to the crosstalk between NF-κB and the Wnt/β-catenin pathway.

Results

H. pylori was found to activate both NF-κB and Wnt/β-catenin signaling pathways in gastric epithelial cells. To investigate potential crosstalk between these pathways, we tested two candidate molecules. siRNA-mediated knockdown of USP25, a deubiquitinylating enzyme, resulted in a significant down-regulation of both NF-κB and Wnt/β -catenin activity in NCI-N87 cells. In contrast, siRNA-mediated knockdown of TROY (TNFRSF19) did not lead to a noticeable change in NF-κB activity in gastric cancer cells. RSPO3, a key protein in the Wnt/β-catenin signaling pathway, has been demonstrated to be necessary for *H. pylori*-induced NF-κB activity in gastric stem cells. To elucidate the mechanism behind this phenomenon, we performed experiments using a transwell system with gastric primary cells (mucosoids). However, in contrast to *H. pylori*-infection, TNF and IL-1 β-induced NF-κB activation independent of R-spondin.

Conclusions

USP25 might be a potential candidate for crosstalk between the Wnt/β-catenin and NF-κB pathways, influencing both. The mechanism of R-spondin-dependent NF-kB activity in H. pylori infection is so far unresolved.



MD11 | ELIZA VON GEHLEN

Supervisor: Sascha Kahlfuss

Sep 26, 2024

13:00

Role of lipid receptor signaling in the differentiation and effector function of pathogenic Th17 cells in central nervous system autoimmunity

Background

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) that involves Th17 cell dysregulation. We investigate how the lipid receptor CD36 contributes to the pathogenic potential of Th17 cells in mediating MS.

Results

CD36-deficient Th17 cells show a significant reduction in the uptake of palmitic acid (PA), the most abundant fatty acid in the CNS of mice with experimental autoimmune encephalomyelitis (EAE), a mouse model of MS. Importantly, Th17 cells that lack CD36 show higher expression of the chemokine receptor CCR6, which mediates Th17 cell migration across the blood brain barrier (BBB). We currently investigate if acetylation through CD36-mediated PA uptake negatively regulates CCR6 gene expression. On the molecular level, CD36-defective Th17 cells show higher mitochondrial mass and ROS production. The higher ROS production upregulates the expression of the transcription factor NRF2 which regulates the antioxidant genes Gclc, Ho-1, Prx, Slc7a11 (xCT), and Prdx2. We are currently investigating whether Fyn kinase is a negative regulator of NRF2 downstream of CD36.

Conclusions

Together, we postulate that during CNS autoimmunity CD36-mediated fatty acid uptake acts as a negative regulator of Th17 cell gene expression through metabolic-epigenetic mechanisms.