INTESTINAL MICROBIOTA

11th Seeon Conference and Science Camp

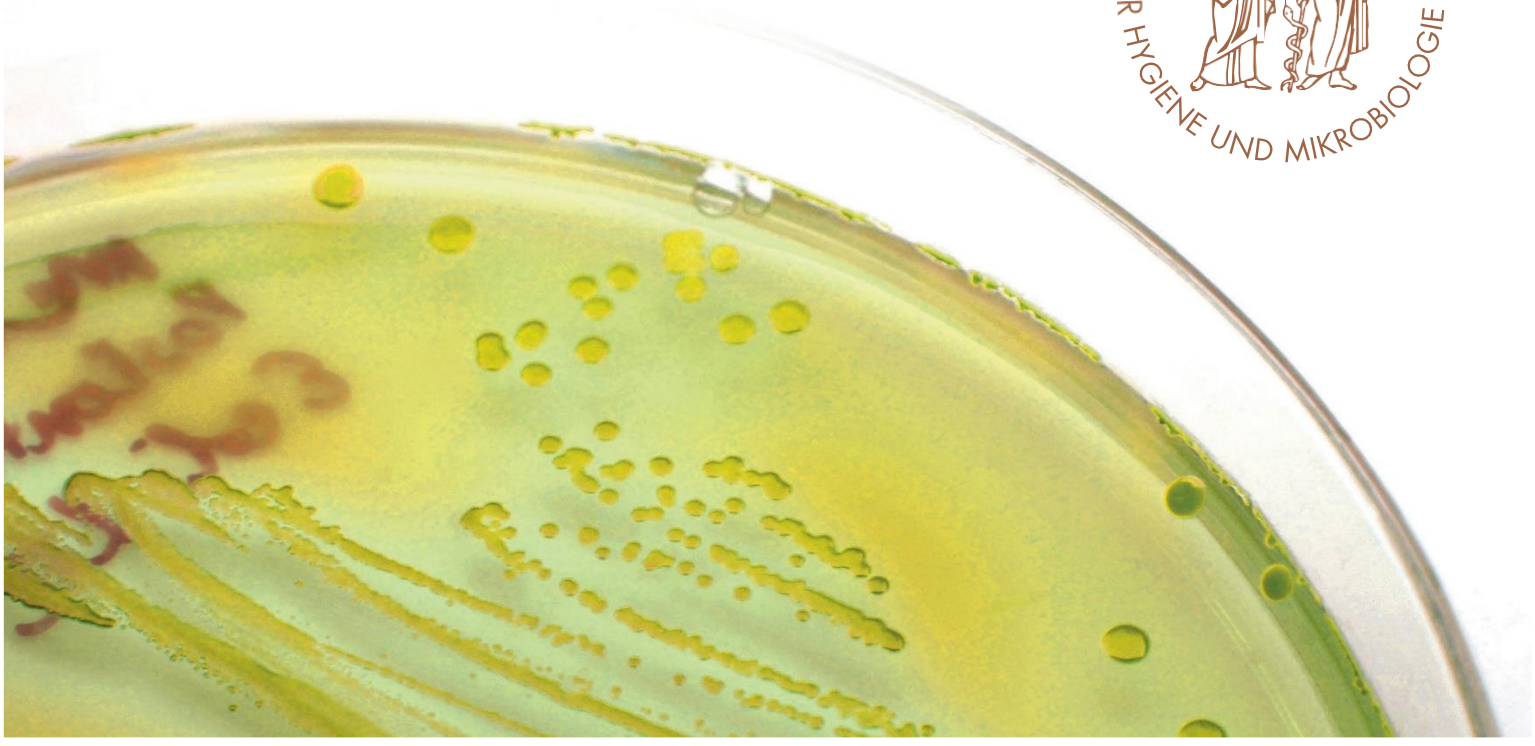
SPP 1656 „From sequencing to function“

06 to 08 July 2018, Conference Center Monastery Seeon

ABSTRACTS

DFG Deutsche Forschungsgemeinschaft

DEUTSCHE GESELLSCHAFT FÜR HYGIENE UND MIKROBIOLOGIE E.V.



July 6th, 2018

Dear Participant,

We are very pleased to welcome you to the **11th Seeon Conference and Science Camp** which is organized for the second time as joint meeting of the **German Society of Hygiene and Microbiology (DGHM)** and the **Priority Program SPP1656** of the German Research Foundation (DFG).

Last year, we celebrated the **10th Anniversary of the Conference "Microbiota, Probiotics and Host"** at the beautiful Seeon Monastery. Besides great science and outstanding keynote-speakers we celebrated the anniversary with an outdoor surprise event organized in group activities culminating in a furious finale (https://youtu.be/vjMK2KYjj2s) which was a great pleasure.

11 years ago, the DGHM section "Microbiota, Probiotics and Host" was founded and since then the "Seeon Conference" has become a vital platform to integrate various disciplines in basic and clinical sciences unified by the aim to understand the human microbiome. The past activities have made an important contribution to the formation of the DFG Priority Programme "MICROBIOTA – a Microbial Ecosystem at the Edge between Immune Homeostasis and Inflammation" (SPP 1656) in 2013.

Over the past 5 years, SPP 1656 has funded over 30 scientists working on projects related to understanding the functional relevance of the intestinal microbiota in health and disease. Many fruitful collaborations have been formed, joint studies have been implemented and new interdisciplinary approaches, technologies and model systems are part of the proud scientific output of this priority programme. In 2018, the **1st Summer School on Microbiome in Health and Disease**, coordinated and funded together with the SPP 1656 took place just before this conference and is planned to become a permanent institution in the future to train and promote young scientists across various disciplines, including gastroenterology, nutritional medicine, immunology, infection research, microbial ecology and computational biology in the area of basic and applied microbiome research.

We are looking forward to fruitful discussions and good science … let's have a great time in Seeon 2018!

Prof. Dr. Bärbel Stecher-Letsch and Prof. Dr. Dirk Haller

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PROGRAM Friday, July 06

14^{00} - 14^{15} Welcome: Bärbel Stecher-Letsch, LMU München
Dirk Haller, Technische Universität München

14^{15} – 15^{00} **1st Keynote Lecture**: **Harry Sokol**, Saint-Antoine Hospital, Paris, France
"Gut microbiota in IBD: from pathogenesis to treatment target"
Chair: Dirk Haller, Technische Universität München

SESSION 1: MICROBIOTA & HOST SIGNALING & INFLAMMATION

15^{00}– 16^{00} Chair: Mathias Hornef, RWTH University, Aachen

Florence Fischer, Institute of Medical Microbiology, University Marburg, Germany
"The impact of dietary fibers on intestinal microbiota and homeostasis"

Amira Metwaly, Chair of Nutrition and Immunology, Technical University of Munich, Germany
"Functional characterization of microbial signatures in inflammatory bowel disease using gnotobiotic humanized mice"

Alex Steimle, Interfacultary Institute of Microbiology and Infection Medicine, University of Tübingen, Germany
"MCPIP-1 in intestinal DCs is crucial for microbiota-dependent induction OR prevention of Th17 immune responses"

Iris Stolzer, Department of Medicine 1, University Hospital Erlangen, University of Erlangen-Nuremberg, Germany
"Role of MLKL mediated regulated necrosis during gastrointestinal infection"

16^{00}-16^{30} Coffee Break

PROGRAM Friday, July 06

16^{30}-17^{15} **2nd Keynote Lecture: Thomas Bosch**, University Kiel
"The holobiont Imperative"
Chair: Bärbel Stecher-Letsch, LMU München

SESSION 2: MICROBIAL ECOLOGY & NOVEL TECHNIQUES

17^{15}– 18^{45} Chair: John Baines, University Kiel/ MPI for Evolutionary Biology

Marcus Höring, Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Germany
"Application of stable isotope labeling high resolution mass spectrometry to study the intestinal lipid metabolism"

Klaus Neuhaus, Core Facility Microbiome/NGS, ZIEL – Institute for Food & Health, Technische Universität München, Freising
"Novel genes in the gut pathogen EHEC discovered by RIBOseq"

Stephanie Schäpe; Helmholtz-Centre for Environmental Research - UFZ, Department of Molecular Systems Biology, Leipzig, Germany
"Simplified human gut microbiota (SIHUMI) community cultivated in in vitro bioreactors shows no changes towards varying nutrient flow rates"

Bruno Sovran, Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France
"Enterobacteriaceae are essential for the modulation of colitis severity by fungi"

Jakob Zimmermann, Gastroenterology & Mucosal Immunology Group, Department for BioMedical Research, University of Bern, Switzerland
"From Meta-omics to single cells: cytometry & sorting of intestinal bacteria"

Theresa Rausch, Department of Gastrointestinal Microbiology, German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany
"Conversion of sulfonates by human intestinal microbiota"

18^{45}-20^{00} Dinner

20^{00}– 20^{45} SPP Member Meeting
and/or
20^{00}– open Bowling at the bar

PROGRAM Saturday, July 07

SESSION 3: MICROBIOME SIGNATURES IN HEALTH AND DISEASE

08^{30} – 10^{00} Chair: Julia Frick, University Tübingen

Marijana Basic, Institute for Laboratory Animal Science and Central Animal Facility, Hannover Medical School, Germany
"Composition of the microbiota determines the outcome of MNV-triggered IBD in mice"

Amélia Camarinha-Silva, Institute of Animal Science, University of Hohenheim, Stuttgart, Germany
"The influence of aging in the active microbial community of the gastrointestinal tract of mice"

Simone Lipinski, Institute of Clinical Molecular Biology, Christian Albrechts University of Kiel, Kiel Germany
"NOD2 influences intestinal microbial resilience after antibiotic perturbation"

Maria Vehreschild, Department I of Internal Medicine, University Hospital of Cologne; German Centre for Infection Research, partner site Bonn-Cologne, Cologne, Germany
"Fecal microbiota transplantation in kidney transplant recipients with recurrent urinary tract infection – 2 cases"

Sören Ocvirk, Division of Gastroenterology, Hepatology and Nutrition, Department of Medicine, University of Pittsburgh, USA; Department of Gastrointestinal Microbiology, German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany
"Altered metabolism of bile acids and short-chain fatty acids by the gut microbiota may contribute to the high risk of colorectal cancer in Alaska native people"

Felix Sommer, Institute of Clinical Molecular Biology, Kiel University, Kiel, Germany
"Effects of protein malnutrition on epithelia-microbe interactions in the intestinal tract of mice"

10^{00}– 10^{30} Coffee Break

10^{30}– 10^{45} Welcome and Introduction for Social Program: Prof. Herwig Stibor
- Learn about the aquatic ecosystem of lake Seeon

10^{45}– 11^{30} **3rd Keynote Lecture: Luis Ferreira Moita**, Instituto Gulbenkian, Portugal
"Homeostasis perturbations: in sickness and in health"
Chair: Dirk Haller, Technische Universität München

11^{30}– 14^{30} Lunch
+ Social Program: Prof. Herwig Stibor - Excursion to learn about the aquatic ecosystem of lake Seeon (outdoor event)

14^{30}– 15^{15} Coffee Break + Poster

15^{15}– 16^{00} **4th Keynote Lecture: Tyrrell Conway**, Oklahoma State University, Stillwater, USA
"The Restaurant Hypothesis"
Chair: Bärbel Stecher-Letsch, LMU München

SESSION 4: MECHANISMS OF MICROBE–HOST INTERPLAY

16^{00}– 17^{00} Chair: Guntram Graßl, Medizinische Hochschule Hannover

Hyun-Dong Chang, German Rheumatism Research Center (DRFZ), Leibniz Institute, Berlin, Germany
"Distinct bacteria of the intestinal microbiota induce mucosal TGF-B-expression and enhance immunglobulin class switch to IgA"

Alibek Galeev, Institute of Medical Microbiology and Hospital Epidemiology, Hannover Medical School and German Center for Infection Research (DZIF), Hannover, Germany
"A sweet bond: Role of epithelial fucosylation in Salmonella infection"

Simone Herp, Max-von-Pettenkofer Institut, LMU München, Germany
"M. schaedleri, a mouse commensal bacterium, protects mice from S. Tm induced colitis"

Yasmina Rodriguez Sillke, Medical Department, Charité, Berlin, Germany; Institute of Nutrition, University of Potsdam, Nuthetal, Germany
"The impact of food antigens on the intestinal homeostasis and inflammation"

17^{00} – 17^{45} **5th Keynote Lecture: Jason M Ridlon**, University of Illinois Urbana-Champaign, Urbana, USA
"Progress in elucidating the human gut sterolbiome"
Chair: Bärbel Stecher-Letsch, LMU München

17^{45}– 19^{15} Dinner

19^{15}– open Poster Slam with Beer and Wine
Chair: Alex Steimle, University of Tübingen

PROGRAM Sunday, July 08

08^{30} – 09^{15} **6th Keynote Lecture: Li Deng**, Helmholtz Zentrum München
"Peering into an unknown viral world: missing pieces in the study of virus-host interaction"
Chair: Dirk Haller, Technische Universität München

09^{15}– 09^{45} Coffee Break

SESSION 5: MICROBIOTA & HOST METABOLISM & CANCER

09^{45}– 11^{15} Chair: Jochen Mattner, FAU Erlangen

Sandra Bierwirth, Chair of Nutrition and Immunology, Technische Universität München, Germany
"ATF6-dependent transcriptional responses in colonic tumorigenesis"

Anja Baumann, Department of Nutritional Sciences, Molecular Nutritional Science, University Vienna, Vienna, Austria
"Metformin treatment in early diet-induced non-alcoholic fatty liver disease: Role of intestinal barrier function and microbiota composition"

Amina Iftekhar, Department of Molecular Biology, Max Planck Institute for Infection Biology, Berlin, Germany
"Role of colibactin in colon carcinogenesis"

Alejandro Ramirez Garcia, Department of Health Sciences and Technology, ETH Zürich, Zürich, Switzerland
"Role of gut microbiota glycerol metabolism in detoxification of diet-derived carcinogens"

Sevana Khaloian, Chair of Nutrition and Immunology, Technische Universität München, Germany
"Intestinal inflammation is associated with mitochondrial dysfunction and appearance of dysfunctional paneth cells"

Alesia Walker, Research Unit Analytical BioGeoChemistry, HMGU, Neuherberg, Germany
"Targeted profiling of bile acids in fecal samples of mice and humans using UHPLC-MS"

11^{15}– 11^{30} Poster Award

11^{30} Lunch and Departure

12^{30} Shuttle Station and Airport

PROGRAM
Friday,
July 06th

Keynote lecture

GUT MICROBIOTA IN IBD: FROM PATHOGENESIS TO TREATMENT TARGET

Harry Sokol

Gastroenterology Department, Saint-Antoine Hospital, Paris, France

The pathogenesis of the inflammatory bowel disease (IBD) is linked to an activation of the gastro-intestinal immune system toward the gut microbiota in genetically susceptible hosts and under the influence of environment. The microbial community in the human gastrointestinal tract is fundamental to the health and is under the influence of both environmental and genetic factors. Loss of the fragile equilibrium within this complex ecosystem, termed dysbiosis, is involved in numerous pathologies, including IBD. Patients with IBD exhibit an altered gut microbiota composition with notably a decreased abundance of anti-inflammatory bacteria such as *Faecalibacterium prausnitzii*. We also observed alteration in the fungal microbiota composition in these patients. The association of several polymorphisms of innate immunity genes involved in microbial sensing with IBD is another argument for the involvement of the gut microbiota in the IBD pathogenesis. Some genetic factors involved in IBD might indeed act through a microbiota effect. We notably demonstrated that this is the case for the IBD susceptibility gene CARD9. Gut microbiota alterations are thus not only a consequence of intestinal inflammation but a key actor in the disease pathogenesis. Fecal microbiota transplantation studies, by showing some efficacy in IBD confirm that the gut microbiota can now be considered as a potential therapeutic target.

MICROBIOTA & HOST SIGNALING & INFLAMMATION

15^{00}– 16^{00} Chair: Mathias Hornef, RWTH University, Aachen

Florence Fischer, Institute of Medical Microbiology, University Marburg, Germany
"The impact of dietary fibers on intestinal microbiota and homeostasis"

Amira Metwaly, Chair of Nutrition and Immunology, Technical University of Munich, Germany
"Functional characterization of microbial signatures in inflammatory bowel disease using gnotobiotic humanized mice"

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"MCPIP-1 in intestinal DCs is crucial for microbiota-dependent induction OR prevention of Th17 immune responses"

Iris Stolzer, Department of Medicine 1, University Hospital Erlangen, University of Erlangen-Nuremberg, Germany
"Role of MLKL mediated regulated necrosis during gastrointestinal infection"

THE IMPACT OF DIETARY FIBERS ON INTESTINAL MICROBIOTA AND HOMEOSTASIS

F. Fischer[1], A. Visekruna[1], A. Walker[2], M. Klein[3], K. Neuhaus[4] and U. Steinhoff[1]

[1] *Institute of Medical Microbiology, University Marburg, Germany*

[2] *Research Unit Analytical Biogeochemistry, Helmholtz Centre Munich, Germany*

[3] *Institute of Immunology, University Mainz, Germany*

[4] *ZIEL - Institute for Food & Health, Core Facility Microbiome/NGS, TU Munich, Germany*

Health benefits of dietary fibers are mainly mediated by the intestinal microbiota, e.g. by the production of short-chain fatty acids. The interaction of the microbiota and the immune system is vital for intestinal homeostasis and disturbance of this complex relation may lead to intestinal as well as extra-intestinal diseases.

The aim of this study was to analyse the mechanism by which cellulose, an insoluble dietary fiber, affects the gut microbiota and intestinal homeostasis. For this purpose, animals were kept on chemically defined diets with or without cellulose.

Animals on cellulose-free diet gained body weight comparable to controls and showed no signs of impaired fitness. However, they were highly susceptible to DSS induced intestinal inflammation, characterized by enhanced expression of pro-inflammatory cytokines and a leaky epithelial gut barrier. 16S rRNA amplicon analysis of the intestinal microbiota revealed that cellulose dramatically influences the development (diversity) of the intestinal microbiota. At young age (eight weeks), a similar diversity of the cecal microbiota was seen, independently of the diet. Interestingly, between week eight and twelve a massive increase in the microbial diversity was exclusively found in mice that were fed a diet containing cellulose. Moreover, the altered microbiota lead to alterations in the cecal metabolome. To further study the mechanism of increased DSS susceptibility in the absence of cellulose, we are currently analyzing the impact of the altered microbiome and metabolome on intestinal immune and epithelial cells.

FUNCTIONAL CHARACTERIZATION OF MICROBIAL SIGNATURES IN INFLAMMATORY BOWEL DISEASE USING GNOTOBIOTIC HUMANIZED MICE

Amira Metwaly[1], Nadine Waldschmitt[1], Ludovica F. Buttó[1], Ilias Lagkouvardos[2], Ana Maria Corraliza[3], Aida Mayorgas[3], Lionel Le Bourhis[4], Sinah Schmidt [5], Andreas Dunkel [5], Thomas Hofmann [5], Matthieu Allez[4], Julian Panes[3], Azucena Salas[3] and Dirk Haller[1, 2]

[1] Chair of Nutrition and Immunology, Technical University of Munich, Germany [2] ZIEL-Institute for Food and Health, Technical University of Munich, Germany [3]Department of Experimental Pathology, Instituto de Investigaciones Biomédicas de Barcelona CSIC, IDIBAPS, CIBERehd Spain [4]APHP, Hôpital Saint Louis, Department of Gastroenterology, INSERM UMRS 1160, Paris Diderot, Sorbonne Paris-Cité University, Paris, France [5] Chair of Food Chemistry and Molecular Sensory Science, Technical University of Munich, Germany

Substantial effort has been dedicated to evaluate the use of microbial signatures as a diagnostic tool for IBD. While cross-sectional studies showed an association between disease activity and an altered microbial composition, a causative role is not yet clear.

We characterized gut microbiota from 3 longitudinal cohorts of IBD patients to identify microbial signatures linked to change of disease state, or response to therapy. Dysbiosis, as measured by community diversity and stability was pronounced in active patients compared to patients in remission. While patients' gut microbiota composition fluctuated dramatically through the course of disease, stratification of patients by disease phenotype, response to therapy or by co-administration of antibiotics led to significant separation between subgroups. In order to address the functional impact of microbial profiles in IBD, we established a humanized IBD model by colonizing germfree IL-10$^{-/-}$ mice with fecal samples from CD patients. We selected 3-paired samples from CD patients representing different disease activities and community clusters. Interestingly, humanized mice recapitulated the disease phenotype and microbial dysbiotic features of their respective human donors after 4 weeks of colonization. 16S rRNA gene sequencing showed that different microbial profiles could drive inflammation in IL10$^{-/-}$ mice humanized with CD-associated microbiota. Consistent with individually diverse microbiota profiles in CD patients, inflammation in gnotobiotic mice was driven by various community profiles, suggesting that different microbial profiles share similar core functions in the susceptible host. Mice colonized with fecal microbiota associated with different disease states could select a distinct range of OTUs to grow in differentially high abundance. Using a machine-learning approach, we could identify a panel of 10 OTUs that discriminates humanized mice by inflammatory status. A microbial signature characterized by an overabundance of *Bacteroides fragillis* and *Desulfovibrio* could classify humanized mice by inflammation with high accuracy. To define the changes in the gut metabolome, we used a targeted metabolomics approach to measure the concentrations of bile acids in fecal samples from human donors and respective humanized mice. Metabolic profiles varied between disease-associated and remission-associated humanized mice, suggesting a microbiota-dependent alteration of metabolic functions in driving disease in the host. The amount of certain metabolites, including Taurocholic acid and Lithocholic acid varied significantly between inflamed and non-inflamed mice.

Our data suggests that IBD pathogenesis involves disruption of the functional diversity and structural complexity of gut microbial ecosystems, and support the translational validity of the gnotobiotic mouse models.

MCPIP-1 IN INTESTINAL DCS IS CRUCIAL FOR MICROBIOTA-DEPENDENT INDUCTION OR PREVENTION OF TH17 IMMUNE RESPONSES

A. Steimle[1], L. Michaelis[1], K. Klees[1], A. Bender[1], JS Frick[1]

[1]*Interfacultary Institute of Microbiology and Infection Medicine, University of Tübingen, Tübingen, Germany*

A so-called Th17 immune response is known to be a prerequisite for the induction of autoimmune diseases1. This certain immune response is characterized and named by an IL-17 secreting T cell subset. Additionally, these IL-17 secreting T cells also express IL-21 and IL-22 supporting the pro-inflammatory functions of IL-17 and their common feature is that they develop into Th17 cells by responding to IL-23 and IL-6 in the surrounding environment.
Certain effector cells, i.e. dendritic cells (DCs), can polarize naïve T cells to become IL-17 secreting Th17 T cells. This can be achieved by secretion of pro-inflammatory cytokines like IL-1β, IL-23 and IL-6 by the effector cells. However, an absence of IL-23, but active secretion of IL-6 can lead to a protective Th17 response promoting, i.e. mucosal defense and barrier tissue integrity.
Here, we demonstrate that intestinal DCs mediate the capability of certain microbiota components to induce a subsequent Th17 response in the host. This is mainly achieved by regulation of Th17 promoting cytokine secretion. For the first time, we report on the impact of the transcription factor IκBζ on the expression of Th17 promoting cytokine secretion in intestinal DCs. IκBζ expression is, in turn, counterregulated by the endogenous endonuclease MCPIP-1. The definite MCPIP-1-to-IκBζ ratio within the intestinal DC decides on the Th17-promoting or even preventing properties of a certain commensal which is sensed by intestinal DCs at mucosal interfaces

ROLE OF MLKL-MEDIATED REGULATED NECROSIS DURING GASTROINTESTINAL INFECTION

Iris Stolzer[1], Mircea T. Chiriac[1], Markus F. Neurath[1], Claudia Günther[1]

[1]Department of Medicine 1, University Hospital Erlangen, University of Erlangen-Nuremberg Germany

In the context of hepatitis, the Mixed lineage kinase domain-like protein (MLKL) expression can be induced by interferons via STAT1. It has been shown that MLKL can execute a previously undescribed type ofRIPK3-independent regulated necrosis and that this IFN-dependent pathway has the potential to influence the pathogenesis of several inflammatory diseases. Regulated necrosis has been implicated in the pathogenesis of gastrointestinal infection, but the precise role of MLKL and interferons in this process remains unclear. In vitro and in vivo, we uncovered that MLKL mediated necrosis is impaired as a result of deletion of STAT1 in the context of gastrointestinal infection. Such a deletion of STAT1 protected Casp8ΔIEC mice against the lethal effects of *Salmonella* Typhimurium infection with a milder course of disease. This protective effect appears to be linked to interferon signaling rather than a reduced number of Paneth Cells and antimicrobial peptides. We further identified that similar to MLKL, expression of the DNA-dependent activator of IFN-regulatory factors (DAI, also known as ZBP1) can be induced particular in intestinal epithelial cells by type I and type III interferons in a STAT1-dependent manner. Restricted ZBP1 levels in Casp8ΔIECStat1-/- mice during infection suggest a hitherto unsuspected link between interferons and regulated necrosis. In summary, our findings suggest a role for interferons in inducing MLKL mediated regulated necrosis potentially by ZBP1 in the context of intestinal infection, but further investigations are required to confirm this.

Keynote lecture

THE HOLOBIONT IMPERATIVE: TOWARDS A HOLISTIC UNTERSTANDING OF COMPLEX LIFE PROCESSES

Thomas C. G. Bosch

Zoologisches Institut, University of Kiel, Germany

In the last decade, biology has made revolutionary advances from century-old debates about the relative importance of non-pathogenic bacteria. Today we know that individuals are not solitary, homogenous entities but consist of complex communities of many species that likely evolved during a billion years of coexistence. Holobionts (hosts and their microbes) and hologenomes (all genomes of the holobiont) are multipartite entities that result from ecological, evolutionary and genetic processes. I propose, therefore, that the health of animals, including humans, is fundamental multi-organismal; that any disturbance within the complex community of host and microbial cells has drastic consequences for the wellbeing of the individual member of this association; and that the microbiome should be viewed as an organ of the host. This newfound awareness of the dependency of phenotypes on other species and environmental conditions presents additional layers of complexity for the life sciences including medicine and evolutionary theory; and raises many questions that are being addressed by new research programmes.

MICROBIAL ECOLOGY & NOVEL TECHNIQUES

17^{15}– 18^{45} Chair: John Baines, University Kiel/ MPI for Evolutionary Biology

Marcus Höring, Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Germany
"Application of stable isotope labeling high resolution mass spectrometry to study the intestinal lipid metabolism"

Klaus Neuhaus, Core Facility Microbiome/NGS, ZIEL – Institute for Food & Health, Technische Universität München, Freising
"Novel genes in the gut pathogen EHEC discovered by RIBOseq"

Stephanie Schäpe; Helmholtz-Centre for Environmental Research - UFZ, Department of Molecular Systems Biology, Leipzig, Germany
"Simplified human gut microbiota (SIHUMI) community cultivated in in vitro bioreactors shows no changes towards varying nutrient flow rates"

Bruno Sovran, Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France
"Enterobacteriaceae are essential for the modulation of colitis severity by fungi"

Jakob Zimmermann, Gastroenterology & Mucosal Immunology Group, Department for BioMedical Research, University of Bern, Switzerland
"From Meta-omics to single cells: cytometry & sorting of intestinal bacteria"

Theresa Rausch, Department of Gastrointestinal Microbiology, German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany
"Conversion of sulfonates by human intestinal microbiota"

APPLICATION OF STABLE ISOTOPE LABELING HIGH RESOLUTION MASS SPECTROMETRY TO STUDY THE INTESTINAL LIPID METABOLISM

Marcus Höring[1], Josef Ecker[2], Gerhard Liebisch[1]

[1]*Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Germany*
[2]*Institute of Nutritional Science, Technical University of Munich, Germany*

Fatty acids (FA) are key molecules for the assembling of various lipid classes. The principally used FA can be obtained through cellular de novo synthesis or by resorption of dietary lipids within the intestine. Therefore the dietary lipids are emulsified by bile acids (BA) and hydrolyzed by lipases in the lumen. After FA uptake into the enterocytes, they are reassembled mainly into glycero(phospho)lipids and released into the circulation. Although it is known that gut microbiota influences the BA profile, it is still unknown whether gut microbiota also affects FA resorption in mammalians. Aim of this work is to develop a flow injection high resolution mass spectrometric (FIA-HR-MS) method to study the intestinal lipid metabolism with stable isotope labeled FAs. This method will be likewise applied to investigate the influence of gut microbiota on these processes.

Labeled FAs were applied to mice by oral gavage of deuterium-labeled triglyceride (TG) D_{93}-tripalmitin. At different time points mice were sacrificed and various sections of the small intestine, liver and blood samples collected. Lipids were extracted in the presence of non-endogenous internal standards and subjected to quantitative FIA-HR-MS.
In first experiments, the incorporation of perdeuterated palmitic acid could be detected in various TG and phosphatidylcholine (PC) species. The most abundant TG and PC species found in the duodenum and jejunum are D_{31}-TG 52:3, D_{31}-TG 52:2, D_{31}-PC 34:2 and D_{31}-PC 34:1. In plasma we could additionally detect the signals of D_{31}-TG 50:1 and D_{31}-PC 36:4. Labeled species showed time-dependent profiles reflecting the lipid flux from gut to circulation followed by hepatic uptake and release. These data also relate to labeled FA profiles determined by GC-MS.

In summary, feeding of stable isotope labeled FAs followed by quantitative FIA-HR-MS offers a valuable tool to trace intestinal FA uptake and metabolism. Ongoing experiments in germ-free mice will provide more understanding how gut microbiota influence these processes.

NOVEL GENES IN THE GUT PATHOGEN EHEC DISCOVERED BY RIBOSEQ

Neuhaus K

Core Facility Microbiome/NGS, ZIEL – Institute for Food & Health, Technische Universität München, Freising

RIBOseq is a relatively recent sequencing technology, which is used to determine mRNA under translation, hence, the so-called 'translatome'. Comparing to proteome data from mass spectrometry there is excellent correlation with high turnover, medium-sized, cytosolic proteins. However, proteins that are membrane bound, small, hydrophobic or strongly charged are difficult to detect via mass spectrometry, consequently, the correlation to RIBOseq data drops substantially.

RIBOseq data were used to detect novel genes in enterohemorrhagic *E. coli* strains (EHEC), which are gut pathogens with an exceptionally low infection dose. These bacteria seem to be a relatively recent, emerging pathogen in developed countries, able to cycle between plants, invertebrates, vertebrates and humans in modern agricultural settings. In two EHEC strains, more than 400 novel genes were detected in intergenic regions, i.e., between already annotated genes. The novel proteins are more often than not also evolutionary young but their role in *E. coli* strain speciation is still unclear. In addition, we found many so-called non-coding RNAs to be covered by RIBOseq reads and, hence, to be coding. Furthermore, novel genes overlapping annotated genes in antisense were detected as well.

In summary, RIBOseq allows adding a substantial number of novel proteins to genome annotations (in the range of 10 to 25%). The role of these often small, possibly membrane bound and / or secreted proteins must await future research. However, we are aiming at RIBOseq data of (minimal) gut consortia for further insights in this important microbial niche.

SIMPLIFIED HUMAN GUT MICROBIOTA (SIHUMI) COMMUNITY CULTIVATED IN IN VITRO BIOREACTORS SHOWS NO CHANGES TOWARDS VARYING NUTRIENT FLOW RATES

Stephanie Serena Schäpe[1], Jannike Lea Krause[2], Gunda Herbert[2], Jean Froment[1], Katarina Fritz[1], Ulrike Rolle-Kampczyk[1], Florian Schattenberg[3], Susann Müller[3], Nico Jehmlich[1], Martin von Bergen[1,4]

[1]Helmholtz-Centre for Environmental Research - UFZ, Department of Molecular Systems Biology, Permoserstr. 15, 04318 Leipzig, Germany; [2]Helmholtz-Centre for Environmental Research - UFZ, Department of Environmental Immunology, Permoserstr. 15, 04318 Leipzig, Germany; [3]Helmholtz-Centre for Environmental Research - UFZ, Department of Environmental Microbiology, Permoserstr. 15, 04318 Leipzig, Germany; [4]Institute of Biochemistry, Faculty of Biosciences, Pharmacy and Psychology, University of Leipzig, Germany

Human gut microbiota play an important role in human health since they are involved in many metabolic interactions with the host. The primary function of the gut microbiota is the conversion of nutrients which will be up taken by the host. The gut microbiota is exposed to many environmental stressors including xenobiotics, infection and life style. These stressors strongly influencing the microbiome:host and the microbe:microbe interactions. Using a Simplified Human Gut Microbiota (SIHUMI) model which consists of eight species reflecting to a large extent the metabolic activities typically found in the human gut ecosystem allows the analysis of its metabolic output. Findings can be extrapolated to specific microbiome:host interactions, and how interactions change in response to environmental stimuli (e.g. diet, food additives, toxic compounds, etc.).

In this study, we investigated the impact of different nutrient concentration by varying the flow rate using an *in vitro* bioreactor under continuous conditions. The influence of medium flow rates of 0,02/ h (12 h), 0,04/ h (24 h) and 0,08/ h (48 h) on SIHUMI over 5 days were tested after the community reached a stable functional state in the bioreactor. Additionally the cultivation was prolonged to see whether the community returns to its prior state after temporary disturbance of the different flow rates. Microbiome analysis was performed by metabolomics, flow cytometry and metaproteome analysis.

The fingerprint tools, metabolomics and flow cytometry revealed that the SIHUMI community reached a stable functional state at day 5. Furthermore, there was no clear change in the community profile within the different flowrates from day 6 to day 10 and from day 10 to 15 suggesting that the community does not change towards varying nutrient concentrations. In addition, it was shown that the function and the taxonomic abundances of SIHUMI are similar between the varying flow rates and on day 15 all community members were still present.

Our results demonstrate that a cultivation of SIHUMI in a continuous bioreactor can be used as an *in vitro* model system for the human gut. Through the simplified community it will be easier to unravel interactions between species and to understand the effect of environmental stressors on the resilience of a gut model community on a specie level.

ENTEROBACTERIACEAE ARE ESSENTIAL FOR THE MODULATION OF COLITIS SEVERITY BY FUNGI

Bruno Sovran[1], Julien Planchais[1], Sarah Jegou[2,3], Marjolene Straube[2,3], Bruno Lamas[1], Jane Mea Natividad[1], Allison Agus[1], Louise Dupraz[1], Jérémy Glodt[1], Grégory Da Costa[1], Marie-Laure Michel[1], Philippe Langella[1], Mathias L. Richard[1] and Harry Sokol[1,2,3]

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The balance between microbes and their host maintains intestinal homeostasis and strongly influence inflammatory conditions such as inflammatory bowel diseases (IBD). Here, we focused on the interactions between bacteria and fungi and their implications for intestinal inflammation, a poorly understood area. In mice, administration of *Saccharomyces boulardii* improved DSS-induced colitis, while *Candida albicans* had the opposite effect. Antibiotic treatment strongly modified the observed phenotype: vancomycin-treated mice were fully protected from colitis, while colistin-treated mice retained the colitis phenotype but were not affected by administration of fungi. Antibacterial treatments not only influenced the bacterial populations but also had an indirect effect on the fungal microbiota. Correlations between bacterial and fungal abundance were dramatically decreased in colistin-treated mice compared to vancomycin-treated and control mice, suggesting that colistin-sensitive bacteria are involved in interactions with fungi. Colistin targets the *Enterobacteriaceae,* among others; besides restoring an *Enterobacteriaceae* population by administrating colistin-resistant *Escherichia coli* is sufficient to reestablish both the beneficial effects of *S. boulardii* and the pathogenic effects of *C. albicans* on colitis severity. The mechanisms involve improved gut colonization of fungi in presence of *Enterobacteriaceae*; this finding provides new insights into the role of inter-kingdom functional interactions in intestinal physiopathology and potentially in IBD.

FROM META-OMICS TO SINGLE CELLS: CYTOMETRY & SORTING OF INTESTINAL BACTERIA

Jakob Zimmermann[1], Andrew Macpherson[1]

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Meta-omics technologies such as next-generation sequencing have revolutionised our understanding of microbiota biology but they do not permit functional characterization of intact bacterial cells. We have developed a high-resolution flow cytometry approach that (1) enables rapid detection of changes in microbiota composition, (2) allows for the sorting of viable bacteria for downstream analyses up to the single-cell level and (3) facilitates determination of absolute bacterial numbers per gram luminal content.

Cecal contents of the 12 member consortium "Oligo-Mouse-Microbiota" [1] were FACS-sorted by high-resolution detection of bacterial light scattering characteristics and nucleic acid content. 16S amplicon sequencing of sorted populations revealed purities of up to 99%. Single cell spotting on agar plates followed by cultivation showed up to 86% viability thus facilitating downstream analyses. Ultimately, our method enabled the determination of absolute bacterial concentrations – difficult to address by NGS – which were in line with previous reports at ~10^{11} bacteria / gram cecal content.

Some downstream analyses such as metabolomics require the rapid sorting of high bacterial numbers – faster than achievable by FACS. For this, we've developed a minimal microbiota that can be separated magnetically. Three representatives of the Phyla abundant in the human intestine (*Lactobacillus reuteri – Firmicutes*, *Bacteroides thetaiotaomicron – Bacteroidetes* and *Escherichia coli – Proteobacteria*) were genetically engineered to be targetable by magnetic beads and thus 10^8 or more cells with purities of 96-99% could be sorted in less than 30 minutes.

Overall, high resolution flow cytometry and cell sorting of intestinal bacteria represent powerful new tools that can complement next-generation sequencing approaches for downstream analysis of intact purified bacterial cells up to the single cell level.

[1] Brugiroux et al., *Nature Microbiology* 1–12 (2016); Uchimura et al., *Genome announcements* **4**: e00951–16–2 (2016).

CONVERSION OF SULFONATES BY HUMAN INTESTINAL MICROBIOTA

Rausch T. [1], Burkhardt, W. [1], Braune A.[1], Blaut M. [1]

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The enrichment of colitogenic bacteria, such as *Bilophila wadsworthia*, is enhanced by dietary components promoting the secretion of bile acids conjugated with taurine in IL-10 deficient mice. *B. wadsworthia* is capable of utilizing the sulfite (SO_3^{2-}) moiety of taurine as electron acceptor resulting in sulfide (S^{2-}) formation, which leads to an enhanced growth of this bacterium. Beside bile acids, diet may also serve as a major source of sulfonated compounds, including taurine, present in meat or fish, or sulfoquinovosyl diacylglycerol (SQDG), present in chloroplasts of leafy vegetables such as spinach or salad. SQDG has been demonstrated to be cleaved by the gut bacterium *Escherichia coli* resulting in the intermediate sulfoquinovose (SQ), which is further metabolized to the final product 2,3-dihydroxypropane-1-sulfonate (DHPS). However, the exact process of SQDG conversion and of other sulfonates in the intestinal environment is unknown. Based on this, we hypothesize that dietary sulfonates and its sulfonated degradation products may serve as a SO_3^{2-} source for *B. wadsworthia* and related bacteria, resulting in a shift to a colitogenic microbiota phenotype.

To investigate the capacity of the human gut microbiota to convert the sulfonates taurine, isethionate, cysteate, 2-mercaptoethanesulfonate (CoM), SQ, DHPS, 3-sulfolactate to the final product S^{2-}, fecal slurries (1%, anoxic 1 x phosphate-buffered saline) of healthy human subjects were incubated with the individual sulfonate under strictly anoxic conditions (N_2/CO_2, 80:20 %) for 96 hours. Results show that human fecal slurries (1 %) converted all tested sulfonates to S^{2-} with formate or lactate as electron donors, except for CoM. Furthermore, gut bacteria utilizing the SO_3^{2-} moiety of taurine for their growth with the formation of S^{2-} were obtained from human fecal slurries by enrichment under strictly anoxic conditions (N_2/CO_2 or H_2/CO_2, 80:20 %). These bacterial isolates were identified based on their 16S rRNA gene sequences and further characterized by Gram staining, motility tests, sequencing of their dissimilatory sulfite reductase (*dsrA*) gene fragment, and their growth behavior. Conversion of sulfonates other than taurine and the immunoregulatory properties of the sulfonate-utilizing isolates will be analyzed in ongoing studies.

PROGRAM
Saturday, July 07th

MICROBIOME SIGNATURES IN HEALTH AND DISEASE

08^{30} – 10^{00} Chair: Julia Frick, University Tübingen

Marijana Basic, Institute for Laboratory Animal Science and Central Animal Facility, Hannover Medical School, Germany
"Composition of the microbiota determines the outcome of MNV-triggered IBD in mice"

Amélia Camarinha-Silva, Institute of Animal Science, University of Hohenheim, Stuttgart, Germany
"The influence of aging in the active microbial community of the gastrointestinal tract of mice"

Simone Lipinski, Institute of Clinical Molecular Biology, Christian Albrechts University of Kiel, Kiel Germany
"NOD2 influences intestinal microbial resilience after antibiotic perturbation"

Maria Vehreschild, Department I of Internal Medicine, University Hospital of Cologne; German Centre for Infection Research, partner site Bonn-Cologne, Cologne, Germany
"Fecal microbiota transplantation in kidney transplant recipients with recurrent urinary tract infection – 2 cases"

Sören Ocvirk, Division of Gastroenterology, Hepatology and Nutrition, Department of Medicine, University of Pittsburgh, USA; Department of Gastrointestinal Microbiology, German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany
"Altered metabolism of bile acids and short-chain fatty acids by the gut microbiota may contribute to the high risk of colorectal cancer in Alaska native people"

Felix Sommer, Institute of Clinical Molecular Biology, Kiel University, Kiel, Germany
"Effects of protein malnutrition on epithelia-microbe interactions in the intestinal tract of mice"

COMPOSITION OF THE MICROBIOTA DETERMINES THE OUTCOME OF MNV-TRIGGERED IBD IN MICE

S. Bolsega[1], M. Basic[1], A. Smoczek[1], B. Stecher[2] and A. Bleich[1]

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Intestinal microbiota has been shown to play an important role in the development of various pathophysiological conditions including inflammatory bowel disease (IBD). IBD is a chronic inflammatory disorder of the gastrointestinal tract with diverse factors being involved in its development such as genetic susceptibility of the host, inappropriate immune response towards intestinal microbiota and environmental factors. Though the exact mechanisms are not fully understood, microbiota composition is crucial in IBD development. To unravel highly complex host-microbiota interactions, gnotobiotic animal models colonized with selected bacteria or defined bacterial consortia are well suited. Using these, we have previously shown that murine norovirus (MNV) triggers intestinal inflammation in the IL10-deficient (*Il10*^{tm1Cgn}, *Il10*$^{-/-}$) mouse model of IBD depending on bacterial colonization. Thus, the aim of this study was to assess the ability of specific representatives of commensal microflora to modulate MNV induced intestinal barrier disruption and thereby the development of the experimental IBD by using two defined bacterial consortia (Altered Schaedler Flora - ASF and Oligo Mouse Microbiota - OMM) as well as segmented filamentous bacteria (SFB).

Gnotobiotic *Il10*$^{-/-}$ mice associated with OMM showed mild intestinal inflammation predominantly located in the proximal colon, whereas in mice carrying ASF only few inflammatory lesions were observed. MNV infection exacerbated the pathological changes in the colon only in mice carrying ASF, but not OMM. The intestinal pathology was characterized by epithelial hyperplasia, infiltration of inflammatory cells and disturbed intestinal barrier as well as increased production of pro-inflammatory cytokines. Furthermore, when these mice were additionally co-colonized with segmented filamentous bacteria (SFB), MNV was not able to trigger the intestinal inflammation in ASF colonized mice. However, in animals carrying OMM, SFB co-colonization did not induce the protective effect. SFB presence was confirmed only in mice colonized with ASF, but not OMM, which likely explains the absence of SFB mediated protective effects. These effects were associated with boosted immune response and upregulated expression of factors determining the intestinal barrier including antimicrobial peptides, mucus and tight junction production in both chronic and acute phase of MNV infection.

Altogether, our results showed that the colitogenic stimulus of MNV depends on the presence of particular bacterial species. Furthermore, SFB presence prevented MNV triggered disruption of the intestinal barrier by strengthening the intestinal barrier and activating the host immune system without causing intestinal lesions. Moreover, SFB presence itself is dependent on microbiota composition.

THE INFLUENCE OF AGING IN THE ACTIVE MICROBIAL COMMUNITY OF THE GASTROINTESTINAL TRACT OF MICE

A. Hernandez-Arriaga[1], A. Baumann[2], O.W. Witte[3], C. Frahm[3], I. Bergheim[2], A. Camarinha-Silva[1]

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Intestinal microbiota is known as a key factor of gastrointestinal homeostasis and human gut health. It has been suggested that aging may be associated with alterations in intestinal microbiota and those changes may affect the aging process and be linked to chronic and inflammatory diseases. However, the associations between microbial ecology and the aging process are not yet revealed. This study aimed to understand the microbial shifts of mice mouth and intestine during aging. Samples from C57Bl/6 mice aged 2, 9, 15, 24 and 30 months were collected from mouth, duodenum and colon. The active microbial community was characterized with target amplicon sequencing. The overall microbiota exposed an age-related variation between samples (*p*-value=0.004). A statistical difference was detected between all analysed sections of the gastrointestinal tract (*p*-value=0.001). At phylum level, Firmicutes was predominant in mouth and duodenum while Proteobacteria was present in higher abundance in the colon. However, phylum fluctuations were observed across the age. The main OTUs were assigned to different strains of *Lactobacillus*, including *Lactobacillus reuteri*, a common probiotic colonizer of mammalian species, was predominant in duodenal samples. *Helicobacter hepaticus*, a pathosymbiont associated with inflammatory responses, was the most abundant bacterial species detected in the samples. This species increased its abundance with age. Our findings show that the active microbial community of the gastrointestinal tract is affected by aging and might influence disease development.

NOD2 INFLUENCES INTESTINAL MICROBIAL RESILIENCE AFTER ANTIBIOTIC PERTURBATION

Jacqueline Moltzau Anderson[1], Simone Lipinski[1], Wei-Hung Pan[1], Ateequr Rehman[1], Maren Falk-Paulsen[1], Robert Häsler[1], Richa Bharti[1], Sven Künzel[2], Felix Sommer[1], John F. Baines[2,3] and Philip Rosenstiel[1]

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[2]*Max-Planck-Institute for Evolutionary Biology, Evolutionary Genomics, August-Thienemann-Str. 2, 24306, Plön, Germany*
[3]*Institute for Experimental Medicine, Christian Albrechts University of Kiel, Kiel, Germany*

Background: Loss-of-function variants in the nucleotide-binding oligomerization domain-2 (NOD2) gene, impairing the recognition of the bacterial cell wall component muramyl-dipeptide, are associated with an increased risk for developing Crohn's disease (CD). A disturbed control of gut microbial communities is hypothesized as a causative mechanism contributing to increased susceptibility for chronic intestinal inflammation through this genetic variation. Here, we show the influence of NOD2 on the longitudinal dynamics of the intestinal microbiota using wild-type (WT) C57BL/6J and knock-out (KO) NOD2 mice treated with broad-spectrum antibiotics, and investigate germ-free (GF) mice colonized with WT and KO donor microbiota. **Results:** Sequencing of the 16S rRNA gene and ITS1 region revealed that antibiotics caused significant long-term shifts in the bacterial and fungal community composition. Importantly, we demonstrate a phenotypic variation, where the NOD2 genotype impairs resilience of the bacterial gut microbiota leading to a delayed recovery. Furthermore, colonization of GF mice with WT and KO donor microbiota demonstrated a post-antibiotic inflammatory effect of the microbiota derived from NOD2-deficient animals. While bacterial diversity decreased in both genotypes, fungal diversity increased and remained altered throughout the study. **Conclusions:** These results provide evidence of the role of NOD2 for controlling resilience of the intestinal microbiota and suggest that uncontrolled dynamics of intestinal microbiota after perturbation could be involved in the etiology of IBD.

FECAL MICROBIOTA TRANSPLANTATION IN KIDNEY TRANSPLANT RECIPIENTS WITH RECURRENT URINARY TRACT INFECTION - 2 CASES

L. M. Biehl[1,2], A. Tsakmaklis[1,2], R. Cruz-Aguilar[1,2], F. Farowski[1,2], W. Hahn[3], A. Nowag[4,5], H. Wisplinghoff[4,5,6], M. J. G. T. Vehreschild[1,2]

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Background: Recurrent urinary tract infections (rUTIs) are a frequent medical problem among women. The pathogenesis is commonly explained by ascending intestinal bacteria. However, new findings also suggest an important role of the vaginal, urinary and intestinal microbiota in the regulation of disease activity. A recently published study could show a decrease in the number of rUTIs after performance of fecal microbiota transfer (FMT) for the treatment of recurrent *Clostridium difficile* infection (CDI).

Methods: We report on two kidney transplant recipients with a history of rUTI being treated with FMT in the absence of CDI. For one case, a comprehensive urinary, fecal and vaginal microbiota analysis was performed. In this case, we collected midstream urine, stool and a vaginal swab prior to FMT and three times during follow-up (day 14, day 39 and day 84). Microbiota composition was analysed by 16S rRNA gene amplicon sequencing.

Results/Cases: Both patients had a history of rUTIs with culture positive, dysuric episodes approximately four times per year and were under ongoing immunosuppressive treatment after kidney transplantation three (patient 1) respectively twelve (patient 2) years ago. FMT was performed via oral administration of frozen capsulized microbiota (patient 1) or via distal colonic enema (patient 2). Both patients remained without symptoms after FMT and all urinary specimens collected during follow-up remained culture negative. In patient 1, we observed a gradual decrease in Enterobacteriaceae over the follow-up time in urine specimens. Of note, the causative pathogen during rUTIs in this patient belonged to the family of Enterobacteriacaeae. Furthermore, alpha diversity indices from urine, stool and vaginal swab specimens were higher 14 days after FMT as compared to scores from specimens taken immediately before FMT.

Conclusions: Our observations hint at the possibility of a successful modification of the urinary microbiota by FMT and an influential role of the intestinal microbiota composition in the pathogenesis of rUTIs. Underlying mechanisms of action need to be addressed in depth by future research.

ALTERED METABOLISM OF BILE ACIDS AND SHORT-CHAIN FATTY ACIDS BY THE GUT MICROBIOTA MAY CONTRIBUTE TO THE HIGH RISK OF COLORECTAL CANCER IN ALASKA NATIVE PEOPLE

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Sporadic CRC is predominantly driven by environmental factors, particularly diet. A high consumption of fat is suggested to promote colorectal cancer (CRC) risk by stimulating hepatic synthesis of bile acids (BA) and their delivery to the colon, where they undergo conversion by the microbiota to secondary BA that show tumorigenic activity, especially deoxycholic acid (DCA). In contrast, dietary fiber is suggested to reduce CRC risk by promoting the luminal synthesis of short-chain fatty acids (SCFA), especially butyrate, which regulates cell cycle progression and proliferation of the colonic epithelium. Alaska Native (AN) people have the highest recorded incidence for CRC (~100:100,000), whereas rural African (RA) people were shown to have the lowest risk for CRC (<5:100,000). Since the diet of AN people is rich in fat and low in fiber, we investigated how this shapes the BA and SCFA pools and metabolic functions of the gut microbiota, which may collectively promote CRC risk in AN people, and compared it to RA people who have a low-fat, high-fiber diet.

We collected fecal samples from 32 AN and 21 RA healthy 40-65 year old volunteers. The fecal BA pool was analyzed using LC-MS and fecal SCFA detected using GC-FID. Abundance of functional microbial genes involved in BA conversion or butyrate synthesis of the fecal microbiota were assessed by qPCR. 16S rRNA sequencing was performed to analyze the fecal microbiome.

Fecal levels of all major (un-)conjugated primary and secondary BA were significantly increased in AN in comparison to RA participants. AN participants exhibited a more than two-fold increase of tumor-promoting DCA in feces compared to RA (26.73 vs. 11.00 µmol/g feces; $p<0.01$). This was associated with significantly increased abundance of 7α-dehydroxylating bacteria in AN fecal samples (61 gene copies per 20ng DNA vs. below detection limit; $p<0.001$). Fecal levels of most SCFA were significantly lower in AN than RA people (butyric acid: 22.51 vs. 47.24 µmol/g feces: $p<0.01$). The gut microbiota of AN participants showed an overall reduced diversity, but was enriched for *Blautia*, which includes species closely linked to 7α-dehydroxylating bacteria detected in humans. Consistently, genera that cover species expressing bile salt hydrolases, involved in deconjugation of BA, were more abundant in AN fecal samples. In contrast, *Prevotella* and *Ruminococcus* were the dominant genera present in RA fecal microbiota.

A high-fat, low-fiber diet promotes changes in the BA and SCFA pools, which are mediated by the gut microbiota and have an impact on their metabolic capacities. These interactions between diet, the gut microbiota and their metabolites create a colonic environment with tumor-promoting activity, which may contribute to the high rate of CRC in AN people.

EFFECTS OF PROTEIN MALNUTRITION ON EPITHELIA-MICROBE INTERACTIONS IN THE INTESTINAL TRACT OF MICE

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Maintaining health requires a homeostatic equilibrium between the microbial community and the intestinal epithelium that serves as the regulatory interface with the host. Nutritional stressors such as malnutrition, starvation or caloric restriction may disturb this equilibrium and thus provoke changes in the function of the intestinal microbiota and intestinal mucosa. We aim to elucidate the mechanisms underlying this complex tripartite interplay between microbiota, the intestinal epithelium and the environment (nutritional stressors). We therefore developed experimental protein energy malnutrition (PEM) models to investigate the effects of adult, postnatal and prenatal PEM on the microbiota and host physiology. We find that PEM induced drastic modifications of the host's gastrointestinal tract but also systemic immunity and metabolism. PEM extensively changed the overall microbiota composition enriching for Gammaproteobacteria and Bacteroidaceae while depleting for Actinobacteria, and Verrucomicrobia. The intestinal epithelial transcriptome was also largely reprogrammed in response to PEM. PEM-induced changes in the microbiota and epithelial transcriptome partially remained upon switching to control diet, which could account for the "memory effect" seen in individuals who underwent a period of malnutrition. Ongoing experiments aim at characterizing the functional responses of the microbiome using metagenomics and will determine whether PEM phenotypes are transmissible, for example from the mother to offspring. Finally, therapeutic interventions using cultures of the PEM-responsive bacteria will be investigated by prebiotic colonizations under a PEM regime.

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Keynote lecture

HOMEOSTASIS PERTURBATIONS: IN SICKNESS AND IN HEALTH

Luís Ferreira Moita

Innate Immunity and Inflammation Laboratory, Instituto Gulbenkian de Ciência, Portugal

Sepsis is a life-threatening condition most often initiated by a bacterial infection. It is the leading cause of death in intensive care units and the third cause of overall hospital mortality. The pathophysiology and molecular basis of sepsis remain poorly understood. The urgently needed novel therapies for sepsis can only be inspired by new insights into the molecular basis of multiple organ failure and endogenous tissue protective mechanisms. There are two evolutionarily conserved defense strategies against infection that can limit host disease severity. One relies on reducing pathogen load, i.e. resistance to infection that courses with and requires inflammation, while the other provides host tissue damage control, limiting disease severity irrespectively of pathogen load, i.e. tolerance to infection (Nat Rev Immunol. 2017 Feb;17(2):83-96).

Recently, our laboratory used a drug screen to identify the clinically approved group of anthracyclines as potent *in vitro* inhibitors of two key initiators of sepsis, tumor necrosis factor (TNF) and interleukin (IL)-1β (*Immunity*. 2013 Nov 14;39(5):874-84). *In vivo*, *anthracyclines confer strong protection against severe sepsis* in mice. This protective effect relies on the induction of DNA damage responses (DDR), autophagy and on an anti-inflammatory program that increase the tolerance to infection without reducing bacterial burden. Using an shRNA-based screen we identified Ataxia Telangiectasia Mutated (ATM) gene as a mediator of the protective effect of anthracyclines. ATM-deficient ($Atm^{-/-}$) mice are refractory to this protective effect succumbing to severe sepsis with similar kinetics to the non-treated wild-type mice. Based on our recent discovery of anthracyclines as potent effective drugs in mouse models of sepsis, without modifying the bacterial burden and their dependence on the activation of DDR for protection, we propose that *the identification of the molecular events downstream of the DDR has the potential to provide a first molecular framework to explain the mechanisms of tissue tolerance* to inflammation and other deleterious or lethal stressors. I will discuss our recent progress towards this end.

Keynote lecture

"THE RESTAURANT HYPOTHESIS"

Conway Tyrrell

Microbiology and Molecular Genetics, Oklahoma State University, USA

Competition for resources defines community structure and determines the success or failure of introduced species. According to David Tillman's theory of equilibrium competition, stable co-existence of two species is possible when competing for two resources. Rolf Freter extended Tillman's theory to explain how competition for resources in the intestine results in stable multispecies communities. He postulated that for many species to coexist each must use one limiting nutrient better than all others. According to Freter's nutrient-niche hypothesis, successful invaders might use nutrients not used by colonized residents or outcompete them for the same nutrients. However, we isolated an *E. coli* mutant from the mouse intestine that grows much slower than its parent strain in vitro on mucus, yet is a 10-fold better colonizer. This suggests that the mutant occupies a niche that is distinct from the one occupied by its parent. We therefore postulated that *E. coli* resides in "**restaurants**". According to Paul Cohen's Restaurant Hypothesis, the resident microbiota resides in mixed biofilms, taking up nutrients as they are released by adjacent polysaccharide degrading anaerobes. Consequently, only small amounts of sugars that escape the mixed biofilms would be available to invading pathogens. However, the invaders have to compete directly with planktonic residents that have left the biofilms. Therefore, an invader might initially compete in a "Freter-like" niche, in which nutrients are mixed and equally available to invaders and planktonic residents alike, and if it can remain long enough in the intestine, then enter a "restaurant" to stably colonize. According to the Restaurant Hypothesis, both invading pathogens and commensals must compete with the microbiota for nutrients needed to initiate infection or engage in the succession of microorganisms that make up a stable, healthy gut microbial community.

MECHANISMS OF MICROBE–HOST INTERPLAY

16^{00}– 17^{00} Chair: Guntram Graßl, Medizinische Hochschule Hannover

Hyun-Dong Chang, German Rheumatism Research Center (DRFZ), Leibniz Institute, Berlin, Germany
"Distinct bacteria of the intestinal microbiota induce mucosal TGF-B-expression and enhance immunglobulin class switch to IgA"

Alibek Galeev, Institute of Medical Microbiology and Hospital Epidemiology, Hannover Medical School and German Center for Infection Research (DZIF), Hannover, Germany
"A sweet bond: Role of epithelial fucosylation in Salmonella infection"

Simone Herp, Max-von-Pettenkofer Institut, LMU München, Germany
"M. schaedleri, a mouse commensal bacterium, protects mice from S. Tm induced colitis"

Yasmina Rodriguez Sillke, Medical Department, Charité, Berlin, Germany; Institute of Nutrition, University of Potsdam, Nuthetal, Germany
"The impact of food antigens on the intestinal homeostasis and inflammation"

DISTINCT BACTERIA OF THE INTESTINAL MICROBIOTA INDUCE MUCOSAL TGF-B-EXPRESSION AND ENHANCE IMMUNGLOBULIN CLASS SWITCH TO IGA

Alexander Beller[1], Andrey Kruglov[1,2], Pawel Durek[1], Katharina Werner[1], Victoria von Goetze[1], Gitta Anne Heinz[1], Justus Ninnemann[1], Katrin Lehmann[1], René Maier[1], Ute Hoffmann[1], René Riedel[3], Kevin Heiking[1], Britta Siegmund[4], Mir-Farzin Mashreghi[1], Andreas Radbruch[1], Hyun-Dong Chang[1]

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Immunoglobulin A (IgA) is a predominant immunoglobulin class in mucosal immune responses in both healthy humans and wild type mice. IgA plays a central role in the interplay between the host cells and microbiota at the mucosal surfaces. The production of IgA as such is controlled by microbiota and requires the cytokine “transforming growth factor beta” (TGF-β), which induces IgA switch transcripts. However it has not been clear which bacteria direct production of IgA and how they do it.

We now show that not the colonization of the intestinal tract and the stimulation of the intestinal immune with bacteria as such, but rather distinct microbial species lead to the preferential induction of intestinal IgA. Our data indicate that the bacteria of the genus Anaeroplasma increase numbers of IgA secreting plasma cells in the lamina propria of the small intestine, and significantly enhance mucosal IgA levels. Anaeroplasma controls IgA expression by inducing expression of the IgA class switch-inducing cytokine TGF-β in T follicular helper cells of Peyer´s patches. Its anti-inflammatory properties of inducing the immune regulatory cytokine TGF-β, strengthening the intestinal barrier by enhancing mucosal IgA, make it an interesting probiotic for the prevention and treatment of intestinal inflammation.

A SWEET BOND: ROLE OF EPITHELIAL FUCOSYLATION IN SALMONELLA INFECTION

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Approximately 20% of Caucasians ("non-secretors") lack terminally fucosylated glycan structures of ABO and Lewis histo-blood group antigens expressed on epithelial cells of the gastrointestinal tract and on secreted glycoproteins in mucus. *FUT2* gene encodes an enzyme facilitating terminal α-1,2-fucosylation, this fucosyltransferase is inactive in "non-secretors". *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium) is a pathogen employing multiple virulence factors, such as type 3 secretion systems, flagella, and fimbriae. Fimbriae are thin proteinous filaments expressed on the bacterial surface which mediate adhesion of *Salmonella*. To date, 14 different fimbriae have been identified in *S*. Typhimurium; importantly, the core π-class Std fimbriae encoded by the *std* fimbrial operon specifically bind terminal α-1,2 fucose. We investigated the role of *Fut2*-dependent glycans in *Salmonella* infection. *Fut2*$^{+/+}$ and *Fut2*$^{-/-}$ mice were infected with either *S*. Typhimurium Δ*aroA* or Δ*aroA*Δ*stdA* strains and sacrificed at day 7 post infection. Interestingly, *Fut2*$^{-/-}$ mice had significantly lower bacterial colonization and decreased histopathological changes in the colon and cecum compared to *Fut2*$^{+/+}$ mice. Notably, the higher bacterial colonization and gut inflammation in *Fut2*$^{+/+}$ mice was abrogated upon infection with a *S*. Typhimurium *stdA*-deficient strain. The role of Std fimbriae *in vivo* has been further confirmed by competitive index assays: the *S*. Typhimurium Δ*aroA*Δ*stdA* strain was significantly outcompeted by the Δ*aroA* strain in the intestine of *Fut2*$^{+/+}$ mice, but not in *Fut2*$^{-/-}$ mice. Additionally, a spatial distribution of Std fimbriae expression was observed in the large intestine of the infected mice. In agreement with *in vivo* data, Std-expressing bacteria adhered significantly better to fucosylated cell lines compared to Std-negative control. Accordingly, an association of bacteria expressing Std fimbriae with α-1,2-fucose residues was observed in primary epithelial organoids, while Std-deficient bacteria were randomly distributed. Overall, these results indicate that epithelial fucosylation is important for *Salmonella* colonization and intestinal inflammation.

M. SCHAEDLERI, A MOUSE COMMENSAL BACTERIUM, PROTECTS MICE FROM S. TM INDUCED COLITIS

Simone Herp[1], Markus Beutler[1], Diana Ring[1], Sandrine Brugiroux[1], Buck Hanson[2], Saib Hussain[1], Michaela Steinberger[2], Alesia Walker[3], Lara Jochum[1], Philippe Schmitt-Kopplin[3], David Berry[2] and Bärbel Stecher[1]*

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The mammalian gut harbors a complex consortium of bacteria which contribute to our health in several ways. They play a role in food digestion and nutrition, mediate balanced immune system maturation and, most importantly, protect their host from enteric infections. The interactions of single bacterial species and their host leading to protection are incompletely understood. Using gnotobiotic mouse models we analyzed the contribution of individual bacterial species to colonization resistance, prevention of dysbiosis and inflammation during enteric *Salmonella* Typhimurium (*S*. Tm) infection. We found that *Mucispirillum schaedleri*, a mouse commensal bacterium which is closely associated with the mucus layer, protects efficiently against *S*. Tm infection in different gnotobiotic mouse models. To get a deeper understanding of the underlying mechanisms we analyzed *M. schaedleri* associated mice with respect to differences in mucosal gene expression and metabolite production. By understanding the interaction between *M. schaedleri*, *S*. Tm, the gut microbiota and the host we expect to identify new approaches for preventing pathogen-induced intestinal inflammation.

THE IMPACT OF FOOD ANTIGENS ON THE INTESTINAL HOMEOSTASIS AND INFLAMMATION

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One of the hallmarks of inflammatory bowel disease (IBD) is a dysregulation of the intestinal immune system. The combination of the genetic predisposition and environmental factors, as microbiota and food antigens, seems to provoke the development of the disease. Although nutritional therapy with the elemental diet is effective, little is known about its mechanism. Here, the mucosal immune system is linked to food antigens through the Peyer´s patches (PP) in the terminal ileum. Murine data indicate that food antigens induce an activation and subsequent apoptosis of the $CD4^+$ T cells in the PP thus maintaining the healthy balance of the mucosal immune system.
Besides Crohn´s disease (CD) and ulcerative colitis (UC), the pathogenesis of celiac disease (CeD), is also linked to the loss of the intestinal tolerance and barrier function, while CeD is based on an autoimmune reaction towards gluten.
T cells isolated of human PP were characterized for their activation, phenotype, survival and apoptosis between CD, UC patients and healthy controls. Gluten served as a model food antigen. Thus gluten-activated $CD4^+$ T cells in the peripheral blood of CD, UC and CeD patients were analysed by a magnetic enrichment of $CD154^+$ cells and a novel subsequent cytometric antigen-reactive T cell analysis ("ARTE" technology).
$CD4^+$ T cells isolated from PP of CD patients revealed a significantly reduced apoptotic rate compared to UC patients and healthy controls. This was accompanied by an increased expression of the survival marker Bcl-2. Further characterization identified an up-regulation of FoxP3 as marker for regulatory T cells, as well as the activation marker Helios in CD patients. In addition, there was a higher frequency of gluten antigen-specific T cells ($CD4^+CD154^+$) in the peripheral blood of active CD and CeD patients compared to UC, CeD on a gluten-free diet (GFD) and healthy controls. These cells were characterized by up-regulation of the pro-inflammatory cytokines IFN-γ, IL-17A and TNF-α.
The decreased apoptosis in parallel to an enhanced Helios expression of $CD4^+$ T cells in PP of CD patients suggests a pathological T cell hyperactivation followed by a disturbed immune homeostasis. The analysis of antigen-specific T cells in the peripheral blood leads to a diagnostic characterisation within the different patients groups in IBD. Additionally, these results demonstrate the modulation of the immune compartment, even in the periphery, by intestinal barrier disruption.

Keynote lecture

PROGRESS IN ELUCIDATING THE HUMAN STEROLBIOME

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The human body harbors trillions of microbes whose genetic potential dwarfs that of the host and is considered “the second human genome”. Much of the host-microbe interactions are facilitated through the action of small molecule metabolites generated from endogenous and exogenous sources. Steroids represent a particularly important class of small molecule involved in inter-kingdom signaling. Recent research into microbial metabolism of bile acids and glucocorticoids illustrates the complexity and potentially far-reaching implications of microbial metabolism. Furthermore, the discovery of sterolbiome pathways in the gut has led to identification of organisms inhabiting other body sites with the same biochemical function. We apply a combination of next generation sequencing, enzymology, structural biology, and gnotobiotics to uncover important sterolbiome pathways and propose novel hypotheses for their importance in host-microbe interactions. In particular, we have identified the key players in steroid-17,20-desmolase, a pathway involved in conversion of cortisol to androgens. Genes in this pathway have been discovered, kinetic analysis and substrate specificity of the enzymes determined, as well as much of the structural biology of the protein enzymes have been completed. We have also reported discovery of several novel pyridine nucleotide-dependent hydroxysteroid dehydrogenases (HSDH) involved in bile acid metabolism. Further, a flavoprotein catalyzing the reductive steps in the bile acid 7α-dehydroxylation pathway has been identified and characterized. We have also determined that bile acid 7α-dehydroxylating bacteria express NADPH-dependent 12α-HSDH. We conclude with gnotobiotic experiments as an approach to understanding the role of sterolbiome genes in host physiology

PROGRAM
Sunday,
July 08th

Keynote lecture

PEERING INTO AN UNKNOWN VIRAL WORLD: MISSING PIECES IN THE STUDY OF VIRUS-HOST INTERACTION

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Human virome, including those of bacteria (bacteriophages) have received an increasing attention recently, owing to the rapid developments in human microbiome research and the awareness of the far-reaching influence of microbiomes on health and disease. Viral communities are emerging as fundamental drivers of microenvironment by profoundly shaping microbial populations and processes that go beyond mortality and gene transfer to also include direct manipulation of metabolic pathways integral to host-cell function. Nevertheless, human viromes are still underrepresented in literature making viruses a virtually untapped resource of diversity, functional and physiological information. The newly founded Emmy Noether group aims to develop methods for target analysis of human vriome both at the wet lab front, as well as the bioinformatics, including the human virome protein cluster database as an effort to improve functional annotation and characterization of human viromes. We present here a powerful toolkit – from the concentration and purification of viral particles to the amplification of the resulting DNA for sequencing preparation – for studying viral communities in the 'omics' era. In addition, "Viral-Tagging" is a high-throughput method to link wild viruses to specific host cells for screening and sequencing, thus allow for much greater access to the tagged viral community to study virus-host interactions in complex communities. We are equipped to study viral ecology by quantitatively linking objectively defined environmental viral populations, and their genomes, to their hosts, thus can better elucidate the processes that drive the population structure of virus and their host in nature. Using these new tools, we are currently studying the viral community from both environment (e.g. water) and human (e.g. lung and gut).

MICROBIOTA & HOST METABOLISM & CANCER

09^{45}– 11^{15} Chair: Jochen Mattner, FAU Erlangen

Sandra Bierwirth, Chair of Nutrition and Immunology, Technische Universität München, Germany
"ATF6-dependent transcriptional responses in colonic tumorigenesis"

Anja Baumann, Department of Nutritional Sciences, Molecular Nutritional Science, University Vienna, Vienna, Austria
"Metformin treatment in early diet-induced non-alcoholic fatty liver disease: Role of intestinal barrier function and microbiota composition"

Amina Iftekhar, Department of Molecular Biology, Max Planck Institute for Infection Biology, Berlin, Germany
"Role of colibactin in colon carcinogenesis"

Alejandro Ramirez Garcia, Department of Health Sciences and Technology, ETH Zürich, Zürich, Switzerland
"Role of gut microbiota glycerol metabolism in detoxification of diet-derived carcinogens"

Sevana Khaloian, Chair of Nutrition and Immunology, Technische Universität München, Germany
"Intestinal inflammation is associated with mitochondrial dysfunction and appearance of dysfunctional paneth cells"

Alesia Walker, Research Unit Analytical BioGeoChemistry, HMGU, Neuherberg, Germany
"Targeted profiling of bile acids in fecal samples of mice and humans using UHPLC-MS"

ATF6-DEPENDENT TRANSCRIPTIONAL RESPONSES IN COLONIC TUMORIGENESIS

S Bierwirth[1], OI Coleman[1], EM Lobner[1], KA Dyar[3], E Glaab[4], P Giansanti[5], NH Uhlenhaut[3], B Kuster[5], P Wilmes[4], P Rosenstiel[6], KP Janssen[7], D Haller[1,2]

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The unfolded protein response of the endoplasmic reticulum (ER-UPR) has been implicated in the pathogenesis of colon cancer (CRC) and inflammatory bowel diseases, yet mechanistic insights remain unknown. Tissue-specific expression of activated ATF6 (nATF6) initiated inflammation-independent tumorigenesis in homozygous nATF6$^{IEC\ tg/tg}$ (tg/tg) mice, although nATF6$^{IEC\ tg/wt}$ (tg/wt) mice are tumor-free. Tumor formation requires microbal signals, however the contribution of the transcriptional program activated by ATF6 in colon cancer remains unknown. To address this, we performed transcriptome and proteome analyses at a pre-tumor stage in the transgenic mouse. RNAseq analyses identified 3735 transcripts only regulated in tg/tg mice in a pre-tumorous stage, 287 only regulated in tg/wt mice and 866 transcripts expressed in both genotypes. Whereas in tg/wt mice pathway analyses revealed activated ER stress with protein processing in the ER as the main cellular pathway represented, a switch to pathways associated with cell cycle and viral carcinogenesis is evident in homozygous mice, indicating initiation of tumorigenic mechanisms. Transcriptome analyses of tg/tg mice housed under germ-free conditions showed 2255 regulated transcripts, revealing an enrichment of genes related to ER protein processing and protein export, and a loss of regulated genes associated with cell cycle and cellular pathways. 246 regulated proteins in tg/tg mice, and therefore associated with a pre-tumor stage were identified by proteome analysis. In line with the transcriptome analyses, proteins regulated in the tg/wt mice (313 proteins) are associated with ER stress. First ChIP-seq experiments of tg/tg mice at a tumor-stage show a direct ATF6-regulation of ER-UPR genes. Analyses of relevant ATF6-dependent signatures on RNA and protein level showed 28 co-downregulated and 25 co-upregulated target genes. Kaplan-Meier analysis of a published CRC cohort revealed significantly reduced disease-free survival, validating prognostic significance of these ATF6-signatures. Within the upregulated targets six genes were significantly co-expressed in a human colorectal cancer cohort of which three genes are already associated with different cancer types: *serpinh1/hsp47* (glioma, esophageal squamous cell carcinoma, renal cell carcinoma and colorectal cancer*), mthfd2* (leukemia and colorectal cancer) and *oip5* (gastric and colorectal cancer). To gain further insight into the transcriptional profile of nATF6, we are currently performing further ChIP-seq and *ex vivo* intestinal organoid experiments.

METFORMIN TREATMENT IN EARLY DIET-INDUCED NON-ALCOHOLIC FATTY LIVER DISEASE: ROLE OF INTESTINAL BARRIER FUNCTION AND MICROBIOTA COMPOSITION

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Besides genetic predisposition, general overnutrition and a nutrition rich in sugar and fat, alterations of intestinal barrier function and microbiota composition may be critical in the development of non-alcoholic liver disease (NAFLD). Results of clinical studies suggest that the oral insulin sensitizer metformin affects metabolic parameters and liver function but also intestinal homeostasis. However, underlying mechanisms involved have not been fully understood. Here, we aimed to determine the effect of an oral metformin treatment on early diet-induced NAFLD and its associations with changes of intestinal microbiota composition and barrier function in small intestine. Female C57Bl/6J mice aged 6-8 weeks were pair-fed a liquid control diet or a Western-style diet (WSD) +/- metformin (300 mg/kg bw) for 4 days. Indices of liver damage and inflammation as well as makers of intestinal barrier function and microbiota composition were analysed in blood, liver and small intestine. The 4 day intake of WSD was associated with early signs of hepatic steatosis and inflammation. These alterations were associated with a loss of tight junction proteins in small intestine and increased levels of endotoxin in the portal vein. Metformin treatment significantly attenuated most of these WSD-induced alterations. The beneficial effects of metformin on WSD-induced alterations were associated with significant changes of microbial community structure. At level of genus control diet+metformin-fed mice showed higher abundance of *Akkermansia* compared to control and WSD-fed mice. In conclusion, our data suggest that an oral metformin treatment may change intestinal microbiota composition in small intestine within only 4 days and that is associated with a protection against early signs of NAFLD.

ROLE OF COLIBACTIN IN COLON CARCINOGENESIS

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Colorectal cancer is the third most common cause of cancer related deaths worldwide. The B2 *E. coli* strain found in the large intestine can harbor the *pks* genomic island coding for the synthesis of colibactin genotoxin. B2 *E. coli* strains are found in 70% of human colorectal tumours compared to 20% in controls. It is known that colibactin can lead to alkylation of DNA and inter-strand crosslinks. This results in DNA damage and cell cycle arrest in G2/M phase. A stringent causal link has not been demonstrated between B2 *E. coli* strain and tumorigenesis in the colon. We therefore are investigating the role colibactin plays in colon epithelial transformation. For this, we have established the colon organoid model derived from murine primary epithelial cells. We have observed that *pks+ E. coli* cause DNA damage in colon organoids and this is not seen with the mutant strain defective for *pks*. We have also established an air liquid interface model with the epithelial cells derived from organoids. This model resembles the *in vivo* colon epithelium closely and provides an advanced *in vitro* model for studying the interaction of the bacterium with the epithelial cells. Infection with *pks+ E. coli* results in increased proliferation and upregulation of Wnt signaling in colon organoids. DNA sequencing revealed that *pks+ E. coli* infected colon organoids have chromosomal aberrations and high mutational load. Further, we wish to do *in vivo* work in mice to characterize the sites of colonization and potential changes in proliferation and hyperplasia in the colon. Our data aims to provide a better understanding of how colibactin could contribute to malignant transformations and facilitate cancer progression.

ROLE OF GUT MICROBIOTA GLYCEROL METABOLISM IN DETOXIFICATION OF DIET-DERIVED CARCINOGENS

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HCA are carcinogens formed during high-temperature cooking of meat. Red meat consumption has been linked to risk for colorectal cancer. HCA can react with acrolein, a metabolite of bacterial glycerol reduction catalysed by cobalamin-dependent glycerol/diol dehydratases (GDH) to form less cytotoxic/mutagenic glycerol conjugates. Therefore, glycerol reduction driven HCA transformation has the potential to reduce HCA exposure of the host. Gut microbial communities have been shown to transform HCA in the presence of glycerol, but how different taxa with GDH and their competition for glycerol utilization impact HCA transformation has not been investigated. The aim of our study was to determine the relationship between glycerol utilization pathways, abundance and diversity of taxa with *gdh,* and HCA conjugation. So far, we used five different *ex vivo* cultivated colonic microbiota (CM) obtained from the PolyFermS continuous fermentation model, performed batch fermentations with different glycerol concentrations (0, 10 and 100 mM) and determined glycerol utilization and HCA transformation by HPLC-RI and nano-LC MS/MS, respectively. Abundance of selected bacterial groups harbouring *gdh* was assessed using a newly developed quantitative PCR assay.

Our results show that all five colonic microbiota (CM1-CM5) were able to metabolize glycerol. About 100 and 60% glycerol were utilized when 10 and 100 mM were provided, respectively. Three /5 were able to transform HCA (CM1 ~60%, CM4 ~25%, CM5 ~35%) in the presence of 100 mM glycerol while HCA transformation was not observed with 0 and 10 mM glycerol. qPCR results showed that *gdh* assigned to *Eubacterium hallii, Veillonella dispar,* and *Lactobacillus reuteri* was highly abundant in samples with high HCA transformation (CM1). Whereas *Klebsiella* and *Citrobacter gdh* was more abundant in samples with lower HCA transformation (CM4, CM5). *Ruminococcus obeum, Ruminococcus gnavus,* and *Flavonifractor plautii gdh* was present all samples showing HCA transformation. *gdh* was not detected in the two microbiota without HCA transformation (CM2, CM3).

These findings suggest that the presence of specific taxa with *gdh* might predict the proportion of HCA transformed. Glycerol consumption seems not to be a suitable indicator of HCA transformation, because both reductive and oxidative pathways can occur simultaneously. Our model studies will help to correlate gut microbial HCA transformation capacity with colon cancer risk status associated with meat consumption.

INTESTINAL INFLAMMATION IS ASSOCIATED WITH MITOCHONDRIAL DYSFUNCTION AND APPEARANCE OF DYSFUNCTIONAL PANETH CELLS

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Chronic intestinal pathologies like Inflammatory Bowel Disease (IBD) and cancer are associated with changes in intestinal epithelial cell (IEC) homeostasis, altered mitochondrial function and activation of the mitochondrial unfolded protein responses (MT-UPR). We have previously shown that disease severity in $TNF^{\Delta ARE}$ mice, a model for IBD, is associated with loss of Paneth cells. Here, we present evidence that impaired mitochondrial function in intestinal stem cells (ISCs) or IECs is sufficient to drive differentiation of $Lgr5^+$ stem cells into dysfunctional Paneth cells. To prove our hypothesis, we used mice lacking Hsp60 in ISCs and IECs as well as $TNF^{\Delta ARE}$ mice. Consequences of gene deletion and respiratory chain inhibition on IEC homeostasis were further investigated using ex vivo intestinal organoid culture. Further characterization of $TNF^{\Delta ARE}$ mice showed reduced granularity, diffuse cytoplasmic Lyz staining, and finally loss of Paneth cells with increasing inflammation. Additionally, $TNF^{\Delta ARE}$ mice displayed reduced $Lgr5^+$ stem cells and niche factors, indicating the impact of inflammatory conditions on Paneth cell appearance and their function in maintaining stem cells. Similarly, in a mouse model of mitochondrial dysfunction, loss of ISC-specific Hsp60 resulted in a transient drop in Lgr5 expression paralleling with appearance of dysfunctional Paneth cells. CC3 and TUNEL staining illustrated no enhanced cell death in stem as well as Paneth cells. Reduced numbers of $Lgr5^+$ stem cells were further paralleled with increased numbers of $Lgr5^+$-Lyz^+ cells and elevated numbers of $Hsp60^-$-Lyz^+ cells, indicating differentiation of ISC into Paneth cells. $Lgr5^+$- Lyz^+ cells displayed a dysfunctional phenotype paralleling our data from $TNF^{\Delta ARE}$ mice, suggesting a role of mitochondrial dysfunction on Paneth cell homeostasis. Additionally, ex vivo Oligomycin treatment, a chemical inhibitor of the respiratory chain complex V, resulted in an increase in CHOP expression, illustrating alteration in mitochondrial function and diminished Lgr5 and Lyz expressions, confirming our results from IEC and ISC-specific Hsp60 deletion. Our results indicate that mitochondrial function not only controls IEC phenotypic changes but seems to be the driving force in epithelial cell differentiation. Mitochondrial function might therefore represent a key player at the edge of intestinal tissue homeostasis and repair/healing processes in the context of diseases.

TARGETED PROFILING OF BILE ACIDS IN FECAL SAMPLES OF MICE AND HUMANS USING UHPLC-MS

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Bile acids (BAs) are synthesized from cholesterol in the liver and participating in the digestion of dietary lipids through mixed micelles formation. They act as signaling molecules, are antimicrobial and are studied as a function of health or disease. Moreover, BAs are classical compounds, undergoing co-microbial metabolism in the gut environment, such as deconjugation, dehydroxylation and epimerization, resulting in variety of secondary BAs. Here, we present a method to quantify 45 BAs using ultra-high pressure liquid chromatography coupled to time of flight or ion trap mass spectrometry (UHPLC-MS). We primarily applied this approach to investigate BAs profiles in fecal samples of mice and humans. Our targeted assay contains one sulfated (S), 7 glycine (G), 10 taurine (T), 27 unconjugated (u) bile acids. For example, mouse stool samples are represented by several muricholic acids (αβω), including their taurine conjugates, which are completely absent in human stool. Major BAs of human stool are cholic, chenodeoxycholic and deoxycholic acid, together with their glycine conjugated which are under the limit of detection in mouse samples. Detailed evaluation of human stool samples allowed us to reveal that several BAs are conjugated with sulfate, as for example deoxycholic acid. Hence, there are no purchasable authentic chemical standards; we are currently performing chemical synthesis of six different sulfobile acids.

The applicability of our validated method was tested in a study about the role of metformin on BA metabolism in a diabetic mouse model. Single treatment of metformin resulted in most notable increase of 7-oxodeoxycholic, 3-dehydrocholic and cholic acid. Additionally, we could determine that cecal samples of mice contain so called allo BAs (5α), especially free and taurine conjugated allocholic acid. Since, the origin of allo BAs is under investigation, we profiled fecal samples of germfree mice and proved that taurine conjugate of allocholic acid is present in microbiota free host.

POSTER

1 THE IMPACT OF PARTIAL ENTERAL NUTRITION ON GUT MICROBIOTA COMPOSITION IN PAEDIATRIC CROHN'S DISEASE PATIENTS

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Dysregulated immune responses towards the gut microbiota are considered to play a key role in Crohn's disease pathology (CD). Exclusive Enteral Nutrition (EEN) is an highly effective treatment for active luminal inflammation in paediatric CD patients and therefore recommended as first line therapy. However, due to very demanding adherence and exclusion of all regular foods, EEN as long-term treatment is not possible. Partial Enteral Nutrition (PEN) providing patients with 25% of their daily energy, may be an attractive adjuvant to improve remission maintenance and benefit on growth, but data on its efficacy and its mechanism of action is still lacking. The impact of PEN as an adjuvant therapy on growth, remission maintenance and gut microbiota composition was examined through a two centre non-randomized controlled intervention study over one year in paediatric CD patients, who were in remission or had mild disease activity at inclusion time. Number of relapse did not differ between groups in the study period (PEN 4/22 vs. Non-PEN 4/20). However, PEN significantly improved z-scores for height and BMI over time, which was not observed in Non-PEN controls. 16S rRNA gene amplicon sequencing was performed on stool samples, in order to identify PEN microbial signatures. Samples were collected from patients at baseline and three, six, nine and twelve months for both the PEN-intervention and the control (no PEN) groups. Microbial profiles did not differ between both groups at baseline, however, analysis of pooled samples (3, 6, 9, 12 months) between the groups showed significant but minor changes in community structure, supporting the fact that PEN affects microbial composition. There was a considerable variation in the microbial profiles between patients throughout the study and across individual disease course (inter-individual variation). Diversity levels, at the level of species richness, showed a small but significant reduction for the PEN group. In conclusion, beta diversity significantly differed between groups but displayed minor changes in the overall ecosystem.

2 ROLE OF SEGMENTED FILAMENTOUS BACTERIA IN CROHN'S DISEASE-LIKE ILEITIS

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Changes in gut microbial composition (dysbiosis) of the intestinal microbiota are associated with ileal Crohn´s disease (CD). Causal link between dysbiosis and disease development in experimental mouse model of CD-like ileitis in specific pathogen free (SPF) and gnotobiotic environment has been previously reported. SPF mice developed heterogeneous CD-like ileitis phenotype, progressing with age, independently of cage and litter effect. Herein, it has been further shown that severity of ileitis was related to the presence of segmented filamentous bacteria (SFB). We used the genetically-predisposed TNFΔARE mouse model of spontaneous chronic CD-like ileitis in different housing conditions to understand the contribution of this commensal to ileal inflammation.

TNFΔARE and wildtype littermates were housed in SPF or gnotobiotic conditions. Germ-free (GF) TNFΔARE mice showed no signs of intestinal inflammation. TNFΔARE mice monoassociated with SFB exhibited severe enterocolitis accompanied with TNF-driven generation of pathogenic Th17 cells. Notably, ileal disease phenotype coincided with abundant adhesion of SFB to caecal and colonic epithelium (confirmed by transmission electron microscopy (TEM) and was characterized by high numbers of mucosa-infiltrating immune cells. Th17 cell induction was confirmed by FACS and qPCR. Reduced lysozyme expression in Paneth cells was associated with increased granulocyte numbers and CXCL1 transcript levels in inflamed TNFΔARE mice. Notably, introduction of SFB to TNFΔARE mice with mild ileal disease phenotype and complex SFB-free microbial communities, resulted in SFB expansion and severe ileitis development throughout subsequent generations, suggesting SFB as a key player in microbe-host interactions in chronic intestinal inflammation setting.

For the first time, we demonstrate that commensal bacterium SFB, induces strong inflammatory response in CD-ileitis model. Identification and therapeutic targeting of other SFB-functionally analogous bacterial species from human gut in the context in TNF-driven inflammatory conditions would have substantial clinical implications in the future.

3 ROLE OF DIETARY SULFONATES IN THE STIMULATION OF INTESTINAL BACTERIA PROMOTING GUT INFLAMMATION

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The interplay between the intestinal microbiota and the host has increasingly been recognized as a major factor impacting health. Diet is probably the most influential determinant affecting the gut microbiota. A diet rich in saturated fat was shown to promote the growth of the colitogenic bacterium *Bilophila wadsworthia* by enhancing the secretion of the bile acid taurocholate (TC), whose sulfonated taurine moiety is utilized as a substrate by *B. wadsworthia*. This bloom of *B. wadsworthia* was accompanied by an increased incidence and severity of colitis in interleukin (IL)-10 deficient mice, which are genetically prone to develop intestinal inflammation.

Dietary sources of sulfonated molecules include chloroplasts in green vegetables and cyanobacteria, which contain the sulfolipid sulfoquinovosyl diacylglycerol (SQDG) in considerable amounts. Interestingly, the gut bacterium *Escherichia coli* has recently been reported to convert the sulfoquinovose (SQ) moiety of SQDG to 2,3-dihydroxypropane-1-sulfonate, whose sulfite moiety potentially serves as an electron acceptor for *B. wadsworthia*.

Based on these findings, we hypothesize that the dietary intake of sulfonated compounds stimulates the growth of colitogenic gut bacteria such as *B. wadsworthia* and thereby leads to intestinal inflammation in genetically susceptible mice. To investigate whether dietary sulfonates stimulate the growth of colitogenic gut bacteria in a complex bacterial community, we use specific pathogen-free (SPF) IL-10 deficient mice. They are fed either a control diet, a diet containing cyanobacteria rich in SQDG or are gavaged daily with SQ, TC or water. After three weeks of treatment, the mice are killed and their blood and intestinal tissue are analyzed for inflammatory markers and histopathologically scored. Sulfonate and sulfide concentrations and the abundance of *B. wadsworthia* and other sulfite-reducing bacteria are determined.

Preliminary results from experiments with SPF IL-10 deficient mice gavaged with SQ, TC or water indicated no increased cell numbers of *B. wadsworthia* or other sulfite-reducing bacteria in either test group. Additionally, there were no macroscopic signs of intestinal inflammation in these mice or any changes in gut permeability or organ weights. Analysis of inflammatory markers in selected serum samples revealed no increase in either group. From these findings we conclude that dietary intake of SQ or TC does not influence the growth of colitogenic gut bacteria, but further analysis will be conducted to elucidate the fate of dietary sulfonates in the digestive tract and their impact on the microbiota.

4 THE PROBIOTIC E. COLI STRAIN NISSLE 1917 COMBATS STX-PHAGES

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In the year 2011 Germany was hit by a devastating epidemic of a newly evolved *E. coli* O104:H4 strain carrying the *shiga toxin 2* (*stx2*) lambdoid bacteriophage genome. This pathogenic producer of the virulence factor Shiga Toxin (Stx) can induce the development of a severe gastrointestinal disease and life threatening complications such as HUS and caused thereby the death of 53 people. This example of fast evolution demonstrates the high importance to combat not only the production of the toxin but also the *stx* carrying phages, which can turn harmless bacteria into life-threatening pathogens. With our studies, we could demonstrate that the probiotic *E. coli* strain Nissle 1917 (EcN) cannot get infected by *stx*-phages. This resistance was traced back to be not based on the lack or mutation of the phage repressor, rather the expression of a phage repressor (*pr*) gene of a defective prophage in EcN was determined to be involved in this resistance. Moreover, we unraveled that EcN cannot only efficiently reduce the Stx level of Shiga toxin producing *E. coli* (STEC) strains but also interfered with the *stx*-phage infection of *E. coli* K-12 strains. We investigated the protective ability of EcN by employing co- and triculture studies of STEC strains with EcN and K-12 strains. STEC + *E. coli* K-12 coculture studies showed a dramatic increase of the Stx level (+ 250 %) upon *stx*-phage infection of the K-12 strain. On the contrary, when EcN was added to this experimental set up the Stx level was reduced by 90 % compared to the STEC monoculture. Simultaneously to the strong reduction of the toxin level the *stx*-phage titer was up to 1,000-fold reduced in the presence of EcN. This phage-neutralizing outcome was equally shown after incubation of EcN with isolated *stx*-phages. A thermostable protein on the surface of EcN was identified to be involved in this phage inactivation (Bury et al., 2018). Our results indicate EcN is resistant towards the *stx*-phage infection. We identified that STEC strains can convert harmless K-12 strains into strong Stx producers. We could furthermore show that the increase of the toxin level and the *stx*-phage titer can be prevented by the presence of the probiotic strain EcN, from which we can conclude an interference with the K-12 infection. Additionally, our findings from coculture studies of EcN with *stx*-phages demonstrated for the first time a phage neutralizing effect of probiotics on phages. These findings support the idea of using EcN as a medication in the treatment of human STEC infections.

Bury, S., Soundararajan, M., Bharti, R., Bünau, R., Förstner, K. U., & Oelschlaeger, T. A. (2018). The probiotic *Escherichia coli* strain Nissle 1917 combats lambdoid bacteriophages *stx* and *λ*. *Frontiers in Microbiology*.

5 MECHANISM OF E. COLI-MEDIATED COLONIZATION RESISTANCE AGAINST SALMONELLA TYPHIMURIUM IN OLIGO-MM^{12} MICE

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The mammalian gut microbiota fulfills many beneficial tasks for its host, such as nutrient degradation, training of the immune system and protection against enteric pathogens, a phenomenon termed colonization resistance (CR). Due to the high diversity of the gut microbiota, it is challenging to pin down the contribution of individual bacteria to CR and to identify protective species. Therefore, we used a gnotobiotic mouse model (Oligo-MM^{12}) with reduced microbial complexity to investigate the functions of individual bacteria during enteric *Salmonella* Typhimurium (*S*.Tm) infections. This minimal microbiota possesses intermediate CR against an avirulent *S*.Tm strain in comparison to mice colonized with the Altered Schaedler Flora (ASF) and mice with conventional microbiota. By genome-informed design, an improved version of the Oligo-MM^{12} was created by adding three facultative anaerobic bacteria (*Escherichia coli, Streptococcus danieliae* and *Staphylococcus xylosus*) and this consortium provided conventional-like CR (Brugiroux et al., Nature Microbiology 2016). We further dissected the role of facultative anaerobic bacteria in CR and found out that *E. coli* is solely responsible for the restored CR against *S*.Tm in this model, while *S. danieliae* and *S. xylosus* are dispensable. The future aim is to unravel the mechanism underlying *E. coli* mediated CR in Oligo-MM^{12} mice.

6 MICROBIOTA-HOST-ENVIRONMENT INTERPLAY AND ITS INFLUENCE ON SUSCEPTIBILITY TO S. SUIS DISEASE

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European efforts to reduce the preventative use of antibiotics in livestock farming and lack of effective vaccines has led to an emergence of *Streptococcus suis*, a Gram-positive bacterium which causes meningitis, sepsis, and other diseases in swine. *S. suis* is also frequently associated with porcine respiratory disease complex which is one of the major causes of mortality in pigs. In polymicrobial respiratory infections *S. suis* is considered as an opportunist invader and the interactions with other pathogens and the effects on the host's immune system are not well understood. *S. suis* is also a zoonotic pathogen and human infections worldwide have increased significantly in the past years, with most cases originating in Southeast Asia.

Colonisation of the palatine tonsils with piglet microbiota may have an impact on colonisation of *S. suis* and risk of invasive disease. To identify bacterial taxa positively or negatively correlated with *S. suis* abundance on the palantine tonsils, we are collecting microbiological samples from the tonsil and small intestine of piglets around weaning. Bacterial taxa with strong co- and anti-occurrences with *S. suis* have already been identified, suggesting the ecosystem may occur in alternative states separated by 'tipping points' linked to other components of the environment and physiology of individual animals. Notably, opportunistic pathogens from the genera *Actinobacillus*, *Actinomyces*, *Fusobacterium* and *Streptococcus* co-occurred with *S. suis* in tonsil samples suggesting this ecological state supports abundance of *S. suis*, on the tonsils around weaning.

A culturomics approach is being used to identify commensals which in inhibit growth of *S. suis*. This has already led to the identification of a mammalian commensal producing an antibiotic ionophore via a non-ribosomal peptide synthetase (NRPS) gene cluster (Gaiser et al., submitted). Such small molecules may be responsible for direct microbial antagonism and as such could play a role in colonisation resistance by commensal species against pathogenic microbes. Key abundant bacterial species present in the oropharyngeal biofilms of healthy animals on farms without a recent history of problems with *S. suis* will be tested for their capacity to drive the development of microbial communities that provide colonisation resistance against pathogens in the weaning period.

7 KORA – THE INTESTINAL MICROBIOME OF A PROSPECTIVE POPULATION-BASED COHORT

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The relevance of shifts in the intestinal microbiome in disease states has been confounded by underpowered analyses with low numbers of individuals. Several ongoing population-based studies are describing microbiome profiles in large-scale manners and in relation to host health. KORA-FF4 is one of these studies with primary focus on cardiometabolic health and a prospective design. Fecal microbial communities were analysed in 2,076 participants by high-throughput sequencing of 16S rRNA genes (V3-V4 regions). An average of 13,724 ± 7,098 high-quality reads were generated per sample, representing 384 ± 77 OTUs (Operational Taxonomic Units) per individual. We noted a significant decrease in richness with increasing BMI (up to 41 OTUs less on average in obese subjects). Fourteen bacterial phyla were detected, with members of the phylum *Firmicutes* representing the majority of sequence diversity (56% ± 13% relative abundance). Unsupervised k-means clustering performed on prevalent OTUs (present in >30% individuals) generated four main groups of individuals based on the phylogenetic makeup of their fecal microbiome, driven by differential relative abundances of species belonging to the genera *Prevotella*, *Bacteroides*, and *Ruminococcus*. Metabolic related variables and prevalence of T2D cases were significantly different between these microbiota-driven clusters: the highest proportion of TD2 cases (17%) was observed within the *Bacteroides* cluster, while the microbiome of individuals without metabolic disturbance was dominated by *Prevotella*. The majority of pre-diabetic cases (67%) was found within the two microbiota clusters in which *Ruminococcus* spp. occurred at higher relative abundances. Prospective sampling of a sub-cohort of these KORA-FF4 subjects (n = 850) is ongoing and will allow following the development of their gut microbiomes over a period of five years and assessing the impact on metabolic health in a predictive manner.

8 MITOCHONDRIAL DYSFUNCTION IN INTESTINAL EPITHELIAL CELLS INFLUENCES REGENERATIVE AND NEOPLASTIC PROCESSES

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Tissue homeostasis in the intestinal epithelium requires a fine-tuned regulation of cell proliferation and differentiation. Phenotypic transitions of intestinal epithelial cells (IECs) occur along the crypt-villus axis, which are reflected by altered mitochondrial activity. Disturbances in mitochondrial function, metabolism and mitochondrial unfolded protein response (MT-UPR) have been implicated in various pathologies including inflammatory bowel disease (IBD) and cancer. We have recently shown that deletion of the mitochondrial chaperone Hsp60 in IECs activates MT-UPR and controls proliferation. Here, additional mouse models were used to further characterize mitochondrial dysfunction in disease development. Underlying the relevance of MT-UPR and associated Hsp60 in healing processes, an induction of Hsp60 in hyperproliferative wound areas following mechanical and chemical wounding (DSS) was shown. This was accompanied by an increased expression of the growth factor Wnt10a. Immunofluorescence staining of Hsp60 further illustrated its induction in inflammation-associated ($IL10^{-/-}$ mice) and genetically-driven tumor models. Reflecting the importance of these signaling processes, Cre-mediated loss of Hsp60 in colonic IEC ($Hsp60^{\Delta/\Delta IEC}$ mice) was followed by occurance of Hsp60+, Ki67+, BrdU+ hyperproliferative IEC foci, repopulating the colonic epithelium from day 5 after end of tamoxifen treatment. Concomitantly, an increased recruitment of immune cells, including macrophages, was observed. Moreover, IEC-specific deletion of the MT-UPR associated mitochondrial protease *ClpP* ($ClpP^{\Delta/\Delta IEC}$ mice) and mitochondrial metabolism-associated *Ckmt1* ($Ckmt1^{-/-}$ mice) impacted epithelial differentiation and subpopulations. Specifically, highly secretory Lyz+ Paneth cells and mucin+ goblet cells were affected by mitochondrial dysfunction, suggesting alterations in the intestinal stem cell niche and impaired production of antimicrobial peptides. These data demonstrate a strong link between mitochondrial function, healing processes and neoplastic changes in intestinal epithelial cells. To better illustrate MT-UPR as a promising target in the context of disease, we will further analyze mitochondrial dysfunction in mouse models of sporadic and inflammation-associated cancer.

9 GETTING CLOSER TO IN VIVO: A 3D CELL CULTURE MODEL TO STUDY THE MOLECULAR IMPACT OF PROBIOTIC E. COLI NISSLE 1917 AND NISSLE VARIANTS ON THE HOST

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Infectious diseases are still a major cause for death worldwide. With regard to the globally increasing problems of antibiotic resistance, there is an urgent need for the development of novel therapeutics and/or new preventive strategies (e.g. probiotics). To speed up the development, new *in vitro* cell culture models are necessary that bridge the gap between cell culture based research and more complex *in vivo* animal models. There has been a recent shift from the original "flat biology" approach (2D) to three-dimensional models (3D) of human intestinal epithelia which more precisely mimic the *in vivo*-like host-microbe interactions characterized by cell polarity, barrier function, and mucin production. 3D models offer an *"in vivtro"* possibility of a meaningful dissection of the local bacterial/cellular crosstalk.
Employing the mucus-producing human colonic cell line HT-29 MTX E12, we compared cellular responses after co-incubation with the probiotic *E. coli* strain Nissle 1917 and a first set of Nissle variants (*fliM*, *ycgR* and *opgH* gene), re-isolated from infants up to two years after the first inoculation (described on poster by Haleluya Wami).
Growing HT29 MTX-E12 cells on Transwell® Inserts results in polarization and the formation of functional tight junctions with a three dimensional architecture resembling colonic crypts [1]. Nevertheless, gastrointestinal epithelial cells grown in standard transwell cultures still do not mimic a complex mucosal surface. A wide range of 3D cell culture systems exist by now. We employ the NASA-designed rotating wall vessel (RWV) bioreactor, which has been used for studying the cellular and molecular responses of hosts and microbes [2]. The RWV allows dynamic culture conditions of epithelial cells under low physiological fluid shear stress which influences cellular differentiation and development. Furthermore, cells growing on Cytodex-3 micro-carrier beads (collagen type-I-coated) express a 3D tissue-like matrix.
Our preliminary results derived from gene expression profiles of HT-29 MTX E12 cells (RT2 Profiler Arrays, Qiagen), revealed distinct differences comparing the varying cell culture models. We put a strong emphasis on the fine tuning of host responses by miRNA regulation. These differences might provide a better understanding of the fundamental mechanisms governing host-microbe (probiotic) interactions, ultimately correlating changes in the microbiome composition with intestinal disease development and in the end, pave the way for a faster translation "from bench to bedside" (application of probiotics).

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10 DISEASE-RELATED CHANGES IN BACTERIAL PROTEIN ABUNDANCE AND FUNCTIONS IN A REFINED MODEL OF DSS-INDUCED COLITIS

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Only little is known about the microbial changes and more importantly their functional impact in the chronic dextran sulfate sodium (DSS)-induced colitis model. We used a refined model of chronic DSS-induced colitis that reflects typical symptoms of the human disease without detrimental weight loss usually observed in DSS models. We sampled cecum and colon content as well as colon mucus from healthy and diseased mouse cohorts (n=12) and applied 16S rRNA gene sequencing and metaproteomics. A reduction of Actinobacteria was observed in the colon mucus of DSS-treated mice, as well as an increase in the *Prevotella sp*. in both colon content and mucus. Functional differences were observed between sample types demonstrating the importance of separately sampling lumen content and mucus. We observed a decrease in tryptophan metabolism in DSS-treated animals. This pathway was also negatively correlated to the histopathological score. In addition, there was a decrease in abundance of the known tryptophan metabolizers Lactobacilli and Allobaculum in DSS-treated animals. In the colon mucus, Lachnoclostridium, another tryptophan metabolizer, was positively correlated both to tryptophan metabolism and vitamin B6 metabolism. Tryptophan metabolites are known to foster protection against inflammation. Furthermore, a positive correlation between the lipopolysaccharide biosynthesis pathway and the histopathological score was observed. Lipopolysaccharide is an important factor involved in the onset and progression of inflammation. In conclusion, functional changes in the distal colon caused by DSS treatment were more pronounced in the mucus-associated microbiota than in the microbiota present in the distal colon content. The results suggest that inflammation induced in the DSS model is worsened by the resulting dysbiosis of the microbiota on the taxonomic and functional level.

11 IMPACT OF STEM CELL DYNAMICS DURING INJURY AND REGENRATION ON STEM CELL-MICROBE INTERACTIONS

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The colonic epithelium is organized into crypts that represent clonal units, which are constantly repopulated by stem cells located in the crypt base.

Thus, long-lived cells are located in the base, far away from the lumen that contain various potentially toxic bacteria and their metabolites, whereas differentiated short-lived cells are closer to the lumen and are being constantly shed into the lumen. This anatomical organization results in the fact that stem cells are less likely to be in direct contact with bacteria that colonize the lumen. Additionally to this physical protection, several other mechanisms such as expression of antimicrobial proteins have been shown to specifically enable stem cell protection.

We would like to characterize the mechanisms of stem cell protection in more detail and understand under which conditions stem cells are exposed to the microbiota.

As a first step, we characterized colonic stem cells. We established a model to visualize the epithelial stem cells and performed lineage-tracing experiments to follow the fate of the stem cell-derived clones. Moreover, this model allows a quantification of turnover dynamics in the crypt.

Next we asked which factors can affect stem cell number and proliferation. Using germ-free stem cell reporter mice, we find that these animals have a reduced number of stem cells and a more primitive epithelial crypts. We now are analyzing these stem cells in more detail to get mechanistic insights into how stem cells are affected by the microbiota.

In parallel, we characterized stem cell dynamics in the context of colonic injury and find that stem cell quantity, identity, as well as epithelial turnover dynamics are significantly rearranged during injury. While these processes appear to be required to maintain epithelial integrity, they appear to increase the risk of stem cell exposure to luminal bacteria.

12 *IN VITRO* ACTIVATION OF HUMAN TOLL-LIKE RECEPTOR 9 BY BACTERIAL GENOMIC DNA

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Human Toll-like receptor 9 (TLR9), an endosomal pattern recognition receptor of the innate immune system, responds to specific DNA motifs, such as dimers of unmethylated cytosine and guanine (CpG), which are more abundant in bacterial than human genomes. Previous studies demonstrated activation of TLR9 by phosphorothioate backbone-containing synthetic DNA molecules (ODNs) in different types of immune and non-immune cells. The human TLR9 response is also influenced by the bases surrounding the CpG dimer and the length of the CpG-containing fragment. To better understand the role of microbial DNA in TLR9-mediated immune activation, we stimulated human peripheral blood mononuclear cells (PBMCs) and reporter cell lines (Invivogen's HEK-Dual TLR9 and Ramos Blue B Cells) with synthetic phosphorothioate (ODN-2006 + control), and short phosphodiester synthetic oligonucleotides (sODNs: TCGTT and TTTTT), as well as bacterial genomic DNA isolates, *in vitro*. Our results suggest that the relative abundance of the 5-mer TCGTT is a better predictor of the bacterial genome's potential to activate TLR9 than of the 2-mer CG. We also found the same isolated 5-mer, as sODNs, while not activating on its own, to boost stimulation by low concentrations of ODNs and bacterial genomic DNA if added to the reaction. Furthermore, DNase I-digested genomic DNA induced stronger TLR9 activation compared to untreated genomic DNA and similar to sODNs, this digested bacterial DNA could also be used to boost TLR9 activation by low concentrations of ODNs. Our results demonstrate that bacterial genomic DNA might both directly induce activation of TLR9 and, in the form of sODNs, indirectly amplify activation by other ligands, which may be relevant as a mechanism to differentiate between conditions of high and low microbial abundance and genomic DNA integrity.

13 DOES CLOSTRIDIUM RAMOSUM PROMOTE OBESITY IN CONVENTIONAL C57BL/6J MICE?

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Globally obesity has reached pandemic extent with 2.8 million people dying each year. The World Health Organization reported for 2016 more than 1.9 billion people to be overweight (BMI ≥ 25) and 650 million to be obese (BMI ≥ 30) with an increasing tendency. Obesity is mainly caused by inadequate nutrition and reduced physical activity. However host genetics and the gut microbiome have recently been identified as factors influencing obesity development. Interestingly, human studies revealed a positive correlation between obesity-related symptoms and the abundance of fecal *Clostridium ramosum* (Qin et al. 2012, Karlsson et al. 2013, Le Chatelier et al. 2013). *C. ramosum* (Cra) is a Gram-positive, anaerobic spore-forming bacterium belonging to the Erysipelotrichi, a class within the phylum Firmicutes. Woting et al. 2014 demonstrated that feeding gnotobiotic mice a high-fat diet stimulates the growth of *C. ramosum*. More importantly, the presence of this organism promoted obesity development and led to increased hepatic triglyceride levels in mice associated with a simplified human intestinal microbiota (SIHUMI) compared to SIHUMI mice without Cra.

To investigate whether Cra would also promote obesity in a complex gut microbiota we fed conventional C57BL/6J mice either a low-fat diet (LFD) or a high-fat diet (HFD) and orally applied either live Cra or heat inactivated (dead) Cra cells for 21 weeks. All mice fed a HFD gain more body weight compared with mice fed a LFD, no matter whether the mice were treated with live or dead Cra cells. Furthermore, presence of Cra did not influence the bodyfat mass after 21 weeks of treatment (LFD_control: 9.24 %, LFD_live: 9.46 %, LFD_dead: 9.93 %, HFD_control: 20.09 %, HFD_live: 21.40 %, HFD_dead: 22.65 % of body weight, respectively) or relative organs weight. Measurement of glucose absorption and glucose oxidation led us to conclude that the mice were not diabetic or insulin-resistant. Lipid absorption was estimated by measuring triglyceride levels in blood and β-oxidation of free fatty acids. Both parameters were elevated in all mice fed a HFD and these effects were independent of the presence of live or dead *C ramosum.*

It is striking that both body-weight change and body fat were highly variable among the HFD-fed mice (HFD_control: 9.18 % - 36 %, HFD_live: 7.87 % - 40.93 %, HFD_dead: 11.06 % – 33.05 % of body fat mass, respectively) with or without Cra application. We speculate that epigenetics or individual gut microbiota composition of the mice might cause such a high variability. Ongoing experiments are expected to confirm the above results which indicate that *C. ramosum* does not promote obesity in conventional C57BL/6J mice harboring a complex gut microbiota.

14 DEVELOPMENT OF LOVO CELL LINES OVEREXPRESSING HUMAN ISOFORMS OF GLYCOPROTEIN 2

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Glycoprotein 2 is the most abundant major pancreatic zymogen granule membrane protein. It is secreted from pancreas on the membrane of zymogen granule, but recent studies also showed wide presence in mucosal products of tubular organs facing the environment. Besides, it is expressed on the membrane of M-cells, where it acts as a transcytotic receptor, internalizing and presenting pathogenic antigens to the immune system. GP2 is a binding partner of a bacterial fimbrial tip protein FimH. Number of GP2 isoforms varies among species, humans have four different isoforms. Isoforms 1 and 4 are longer, containing 537 (534) amino acids, while shorter isoforms 2 and 3 lack GPI anchor. LoVo cell line is derived from the colonic adenocarcinoma. Stable LoVo cell lines overexpressing the human isoforms 2 and 4 were created using the lentiviral transduction. Expression and localization of the proteins was confirmed by indirect immunofluorescence and mRNA PCR. Cell lines are designed for adhesion and infection assays in order to asses binding properties of various FimH expressing bacteria. Subsequently, sequencing of bacterial genomes and FimH genes is planned to link the adhesive characteristics to the genotype.

15 *MUCISPIRILLUM SCHAEDLERI DELAYS ONSET OF SALMONELLA INDUCED COLITIS IN GNOTOBIOTIC MICE*

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During infection with enteric pathogens the interactions between the gut microbiota and pathogenic organisms play a crucial role for resistance and/or severity of the infection. However, the mechanisms underlying these interactions and modifying the outcome are poorly understood. It is further unknown which members of the conventional gut microbiota are essential in providing those functions.

We identified *Mucispirillum schaedleri* (*M. schaedleri*), a mucus dwelling bacterium commonly found in mice, as candidate for delaying the onset of *S*. Tm induced colitis in mice, despite not interfering with *Salmonella enterica* serovar Typhimurium (*S*. Tm) colonization of in the mouse gut. We then used RNA sequencing to elucidate if the presence *M. schaedleri* modifies the transcriptome of *S*. Tm in mice harboring a consortium of three members of the Altered Schaedler Flora, *M. schaedleri* and/or *S*. Tm. We also looked for transcriptional indications of competition for common nutrients or energy resources such as nitrate, which may be used as terminal electron acceptor by both bacteria. The presence of *M. schaedleri* led to differential downregulation of about hundred genes in *S*. Tm, involving energy metabolism, nutrient acquisition and ribosomal protein synthesis. Further experimental validation of these first preliminary results will give more detailed insights into the mechanisms of *M. schaedleri* mediated interference with *Salmonella* induced colitis.

16 INTESTINAL IGA SHOWS BROAD BUT SPECIFIC BINDING TO DIVERSE BACTERIA

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Secretory immunoglobulin A (SIgA) is a key component in gut homeostasis. SIgA binds to luminal and gut epithelial surface associated bacteria, thereby contributing to intestinal host defense against pathogens and maintaining a stable microbiota composition. Considering the vast changes in the microbiota consortium depending on diet, medical treatment and infection, it remains elusive how the host immune system can generate and regulate beneficial SIgA responses to a highly dynamic commensal setting.

This study sought to profile the binding spectrum of monoclonal IgA antibodies (mABs) derived from human healthy donors and inflammatory bowel disease (IBD) patients by flow cytometry. Screening of almost 200 monoclonal IgA antibodies revealed an unexpected high frequency of mABs with substantial microbiota reactivity. In order to determine the binding spectrum of microbiota reactive mABs, we used these mABs to stain bacteria isolated from $RAG^{-/-}$ feces with subsequent 16S rDNA sequencing of bound and unbound bacterial fractions. Individual mABs bound a diverse spectrum of commensals rather than showing reactivity to single taxa. This suggests that single monoclonal mABs functionally bind a relevant fraction of different intestinal bacteria *in vivo*. Unlike recent reports, we did not observe a correlation of high microbiota reactivity and polyreactivity of respective mABs. In addition, while mABs with microbiota reactivity showed frequent somatic mutations, germ-line variants of previously high binding mABs showed decreased microbiota binding or an altered binding profile. We therefore speculate that ongoing somatic hypermutation selects for intestinal IgA with broad, yet specific binding to different bacterial taxa.

17 COMBINATION OF DIFFERENT FLUORESCENT STAINS ON CELL LINES AND BACTERIA FOR QUANTIFICATION OF ADHESIONS/INFECTIONS OF SALMONELLA ON HUMAN AND CHICKEN INTESTINAL CELL LINES

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Adhesion to the host cell is one of the most vital steps in bacterial strategies for successful colonization and onward infection. The study was designed to quantify the adhesion/infection of *Salmonella* on intestinal cell lines of human (LoVo) and chicken (C8E11). Chicken isolates belonging to *Salmonella enterica* serovar *S. infantis*, *S. typhimurium* and *S. entritidis* representing 33 different pulse field gel electrophoresis (PFGE) clones were used in this study. The LoVo and C8E11 cells were allowed to grow as monolayers on 96-well plates and the bacteria were subjected to adhere/infect these monolayers. The plates were washed, fixed and the adhered/infected bacteria were stained with Fluorescence *In Situ* Hybridization (FISH) probes. The stained bacteria were counted by fluorescent microscope coupled with automated video-scan technology. The monolayers were counterstained with 4',6-diamidino-2-phenylindole (DAPI) to ensure that these were still intact and that *Salmonella* was attached to the cell lines, and not the surface of the plate. Unlike *E. coli,* which only adheres to the cells, *Salmonella* has the ability to infect in addition to its adherence that leads to the disruption of monolayer. Therefore, DAPI staining, which only stains the nuclei of the cells and not the cytoplasm, is not enough to ensure that the counting of FISH stained bacteria carried out by video scan is correct. DAPI staining also did not provide us the information about the location of FISH stained *Salmonella*. Phalloidin was used to stain actin filaments in the cytoplasm in combination with DAPI and FISH probes to ensure the counting of FISH stained bacteria on the monolayer. The protocol for the combination of fluorescent stains was optimized and applied to study the adherence/infection of *Salmonella* on the cell lines.

18 BACTERIUM-HOST INTERACTION IN THE GUT: COMPARING THE TRANSCRIPTIONAL RESPONSE OF AN INTESTINAL EPITHELIAL CELL LINE TO PROBIOTIC AND PATHOGENIC ESCHERICHIA COLI

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Probiotic *E. coli* strain Nissle 1917, uropathogenic *E. coli* strain CFT073 and asymptomatic bacteriuria (ABU) *E. coli* isolate 83972 are in spite of their different environmental origins and dispositions closely related. Their outcomes of infection divide between remissive effects on ulcerative colitis to causing bladder infection. To understand the molecular mechanisms by which probiotic bacteria are effectively recovering gastrointestinal diseases that involve inflammation, we need cell culture models that bridge the gap between the insights we obtain from 2D cell culture models and *in vivo* studies.

Transwell cell culture systems are used for culturing cells into polarised cell structures that better mimic the morphological and functional *in vivo* situation than *in vitro* cell monolayers do. Epithelial cells in the colon are covered by an overlying mucus layer, which shields the cells from direct contact with colonic bacteria. We culture mucin-producing HT29-MTX-E12 cells in a semi-wet manner under continuous shaking to allow for the secretion and formation of an adherent mucosal layer. This method has been described to create polarization, formation of functional tight junctions, a three-dimensional architecture approximating colonic crypts and production of an adherent mucus layer. Using *E. coli* Nissle 1917 derivatives and clonal *E. coli* strains, which differ in pathogenicity, we test for differential transcriptional responses of the epithelial cells upon interaction with microbes. Our preliminary results suggest that an infection of our mucus secreting cell layer, which prohibits direct adherence of bacteria to cells, elicits meaningful host responses towards the microbes. The tested *E. coli* strains also seem to differ in their adhesive properties to mucus, which might be essential for their effect on the epithelial barrier. We believe that these transcriptomic data give an insight into antibacterial responses that gastrointestinal epithelial cells during an *in vivo* encounter might send.

19 PATHOGENIC OR BENEFICIAL: FUNCTIONAL RELEVANCE OF ENTEROCOCCUS FAECALIS IN CHRONIC COLITIS IS DETERMINED BY THE MICROBIAL ENVIRONMENT

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The commensal *Enterococcus faecalis* (*E.f*) has been linked to the pathogenesis of inflammatory bowel diseases and induces chronic colitis in IL10-/- mice. This dualistic character makes *E.f* an exemplary model to study disease relevant microbe-host interactions. We could show that the colitogenic activity of *E.f* in monoassociated mice can be assigned to certain bacterial structures (Ocvirk et al., 2015), but little is known about the functional relevance of *E.f* and disease-associated bacterial genes in a complex bacterial environment.

E.f function in a bacterial community was addressed by colonizing mice with a colitis-inducing microbial consortium (SIHUMI). Surprisingly, colonization of IL-10-/- mice with SIHUMI in the absence of low abundant *E.f* not only induced inflammation, but resulted in an aggravated inflammatory host response. This suggests that the colitogenic activity of *E.f* is compensated by other members of the consortium and the presence of a colitogenic strain might even be protective in a certain bacterial community. The marked contrast between the colitogenic and protective effect of *E.f* colonization depending on the environment indicates that microbe-microbe interactions can affect microbe-host interactions relevant for colitis development. A massive pro-inflammatory response of reactivated MLN cells to *E. coli* lysate stimulation and a positive correlation between histological score and *E. coli* abundance points to this bacterium as main driver of SIHUMI mediated colitis.

E.f disease-associated gene expression was assessed via RNA sequencing of bacteria isolated from monoassociated and SIHUMI colonized mice. Under inflammatory conditions, 30 bacterial genes were differentially expressed in both association environments, whereas 209 were only regulated in *E.f* isolated from monoassociated mice and 129 only in bacteria isolated from SIHUMI colonized mice. This indicates that the microbial environment has a strong influence on *E.f.* gene expression. Surprisingly, expression levels of most known *E.f* virulence genes were not altered (Ocvirk et al, 2015), but several genes relevant for bacterial fitness were differentially expressed. Among the highest upregulated genes in monoassociated mice, a major facilitator superfamily transporter (Mfs) and genes of the ethanolamine utilization locus were identified. Despite their considerable upregulation in inflamed hosts, deletion of Mfs and ethanolamine utilization genes had no major influence on *E.f* colitogenic activity.

Our data show in conclusion, that the impact of an opportunistic pathogen on inflammation development in a susceptible host can be completely contradictory depending on the microbial environment.

20 INTESTINAL ARGINASE1 EXPRESSION ALTERS THE COMPOSITION OF THE MICROBIOTA AND PERPETUATES COLITIS

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The enzymes arginase 1 (Arg1) and the inducible nitric oxide synthase (iNOS) compete for the common substrate L-arginine. Arg1 converts L-arginine to urea and ornithine, which can be further metabolized to polyamines. Arg1 considerably restricts inflammation, but influences also bacterial growth due to the regulation of polyamine synthesis. iNOS, in contrast, produces citrulline and nitric oxide (NO) which acts anti-microbial. However, nitrate, an oxidative product of NO, can be also used as energy source by distinct bacteria. As patients with inflammatory bowel diseases (IBD) exhibit an enhanced Arg1 expression and activity in the intestinal submucosa, we characterized the role of Arg1-expressing cells on the regulation of intestinal immune homeostasis.

Arg1 was expressed in endothelial and epithelial cells, fibroblasts, innate lymphoid 2 (ILC2s) and myeloid cells in the gut. Unexpectedly, Tie2-Cre x $Arg1^{fl/fl}$ mice that lack Arg1-expression in hematopoietic and endothelial cells developed a significantly less severe colitis than littermate controls upon application of dextran sodium sulfate (DSS). The protection from colitis in Tie2-Cre x $Arg1^{fl/fl}$ mice correlated with an increased alpha- and beta-diversity of the intestinal microbiota, an altered Toll like receptor (TLR) and fatty acid expression profile, reduced endoplasmic reticulum stress and an improved mitochondrial integrity. Furthermore, the endothelial permeability and the inflammatory immune response were significantly reduced in Tie2-Cre x Arg1fl/fl mice compared to littermate controls. Surprisingly, iNOS and NO were not the most prominently affected targets, likely due to an unlimited availability of L-arginine in the gut. Fecal transfers into broad-spectrum antibiotic-treated B6 recipient reconstituted this phenotype suggesting that an altered microbiota in Tie2-Cre x Arg1fl/fl mice decreases the susceptibility of mice to a DSS- induced intestinal damage. Thus, Arg1-expressing myeloid and endothelial cells cause dybiosis, which disrupts the mutual interactions of the microbiota and the host and enhances the severity of colitis due to an enhanced susceptibility to inflammatory pathways and an increased endothelial leakage. How certain bacterial species (that might be missing in IBD patients), altered signaling pathways and intestinal tissue specific factors regulate the expression and/or function of Arg1 and prevent the expansion of a colitogenic intestinal microbiota is part of our ongoing analyses.

21 SPECIES- AND STRAIN-DEPENDENCY OF CANDIDA-BACTERIA COINFECTION DAMAGE OF ENTEROCYTES

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The human gut, as the organ harboring the highest density of bacteria and fungi, is a relevant source of infections. In healthy individuals, gut-residing microbes can be transmitted from the bowel to other niches, for instance the urinary tract. In addition, especially immunocompromised patients are at risk of developing severe systemic infections once microbial agents translocate through mucosal barriers and disseminate into the blood stream (BS).

Until today the mechanisms promoting translocation of the yeast *Candida albicans* into the BS are not fully understood. Yet, recent studies estimate about one third of *Candida* BS infections to be in fact polymicrobial – implying a role for *Candida*-bacteria interactions.

To understand the causality between co-colonization and coinfection, we investigate the interplay of *C. albicans* and typical gut-residing bacteria in an *in vitro* enterocyte model. Most interestingly, our experiments revealed that the mode of interplay depends highly on the bacteria species and in some cases also on the particular strain.

Testing various laboratory strains and clinical isolates of *Proteus mirabilis* coinfecting with *C. albicans*, we consistently observed synergistically increased damage of enterocytes compared to summed-up single-species damage. This synergistic effect was dependent on the presence of *P. mirabilis* hemolysin HpmA. In contrast, *C. albicans* filamentation and candidalysin-mediated cell damage were dispensable for synergistic interactions. Interestingly, less virulent yeasts, *e.g.*, *Saccharomyces cerevisiae* did also promote synergistic host cell damage with *P. mirabilis*. In contrast, a strong strain-dependency was found when comparing numerous *Enterococcus faecalis* strains and isolates for their synergistic potential when combined with *C. albicans*. Finally, a range of *Bacteroides vulgatus* isolates, among them *B. vulgatus* mpk, were identified to be uniformly capable of protecting enterocytes from *Candida* damage.

In summary, our data indicates a yet underestimated level of complexity when describing the composition of microbial communities and puts special focus on cross-kingdom interplay. Experiments aimed to determine the exact modes of action of synergistic damage vs. protection are currently ongoing. In addition to *in vitro* work, we include *in vivo* models using nematodes and mice.

22 STRAIN-LEVEL GUT MICROBIOTA DYNAMICS IN PATIENTS WITH RECURRENT CLOSTRIDIUM DIFFICILE INFECTION FOLLOWING FECAL MICROBIOTA TRANSPLANTATION

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Fecal Microbiota Transplantation (FMT), the infusion of human feces from healthy donors into the gastrointestinal tract of recipient patients, has emerged as a novel treatment approach for infections and inflammation of the intestines. FMT efficacy was shown for recurrent *Clostridium difficile* Infection (rCDI) and attributed to the restoration of normal functionality and composition of the gut microbiota, yet underlying mechanisms have been insufficiently investigated. Here, we analyzed strain-level dynamics of the intestinal microbiota in rCDI patients following FMT treatment by fecal metagenomics. A total of 36 fecal samples from eleven FMT cases were analyzed by whole-genome metagenomic shotgun sequencing. The sample set included multiple rCDI patient samples collected before and up to one year after single allogenic FMT, as well as twelve samples from stool donors, all of which had a family connection to their recipients. Taxonomic profiling of microbial communities on the strain-level was performed with a modified version of Strain Finder, relying on the identification of single nucleotide variants (SNVs) within species-specific marker genes. Preliminary results indicate that strain-level microbiota dynamics after FMT are affected by patient-donor relationships, with evidence for shared bacterial strains in family and household members even before FMT. Furthermore, microbiota engraftment following FMT appears to be dependent on bacterial taxonomy, with cases of donors and rCDI patients carrying either the same or distinct strains before and after FMT, depending on the bacterial species. FMT holds considerable promise as a therapy for rCDI and other microbiota-associated disorders, yet short and long-term effects need to be fully understood to ensure patient safety. Metagenomic strain-level sequence analysis may help disentangle the role of specific members of the microbiota for gut homeostasis and efficacy of FMT.

23 B4GALNT2-EXPRESSION AFFECTS SEVERITY OF INFLAMMATION IN A MOUSE MODEL OF SALMONELLA-INDUCED COLITIS AND INFLUENCES METAL ION METABOLISM IN THE GUT

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Glycans on host mucosal surfaces mediate host-microbe interactions and influence the composition of commensal microbiota. In a murine model of *Salmonella*-induced colitis the lack of intestinal expression of the glycosyltransferase gene *B4galnt2* was associated with reduced gut inflammation. Importantly, this mouse model requires antibiotic treatment to break the colonization resistance to *S*. Typhimurium, which is mediated by host commensal gut microbiota. The effect of *Bgalnt2*-expression on the severity of inflammation negatively correlated with the extent of change in microbiota composition before and after infection with *S*. Typhimurium. Transplantation of feces into previously germ free mice demonstrated that the effect is dependent on the *B4galnt2* genotype-specific microbiota rather than *B4galnt2* expression itself. Shotgun functional metagenomic sequencing of the fecal microbiota revealed that in mice deficient for *B4galnt2* expression, commensal microbiota have a significantly higher abundance of efflux pump genes implicated in bacterial resistance to heavy metals. We therefore hypothesized that the observed difference in severity of inflammation could result from an indirect effect, by which a higher abundance in efflux pump genes within the commensal microbiota is associated with increased residual colonization resistance, e.g. by conferring increased resistance to the streptomycin treatment used in the *S*. Typhimurium colitis model. Interestingly, by mass spectrometry of colon and cecal lumen we found that the lack of *B4galnt2* expression is associated with lower concentrations of metals in the gut content, among them notably, iron, zinc, copper and manganese. Given the diverse roles of metal ions in mediating interactions between hosts and microbes, our findings offer an interesting new perspective on how *B4galnt2* regulates host-microbe interactions.

24 ROLE OF TOLL-LIKE RECEPTOR 11 AND 12 IN SALMONELLA INFECTIONS

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Salmonella enterica serovar Typhimurium is a Gram-negative bacterium that causes gastroenteritis in humans and systemic infection in mice resembling typhoid fever. Recent research has identified Toll-like Receptor 11 (TLR11) to play a pivotal role in activating immune response to *Toxoplasma gondii* and bacterial infection of the urinary tract. TLR11 occurs as a pseudogene in humans, but it is functional in mice and regulates the immune response along with TLR12. UNC93B1 is the chaperone molecule involved in the trafficking of the intracellular Toll-like receptors TLR3, TLR7, TLR9, TLR11, TLR12 and TLR13.
In order to investigate the role of TLR11 in *Salmonella* infection *in vivo*, *tlr11*$^{-/-}$ mice were infected with *S.* Typhimurium using streptomycin pretreated mice model. Higher colonization of bacteria was observed in the colon of *tlr11*$^{-/-}$ *mice* 21 days post infection as compared to the WT mice. To further investigate the role of TLR11 in *Salmonella* infection *in vitro*, human epithelial cells, HT29-MTX were transfected to achieve stable cell lines expressing TLR11 and TLR12. Adhesion and invasion of *S*. Typhimurium was analyzed in these cell lines using Gentamicin killing assay. The infection studies reveal increased invasion of *Salmonella* when TLR12 is expressed. We also performed infection experiments on immortalized bone marrow derived macrophages (BMDM) from wild type and *UNC93B1 3d* mice. Through these set of experiments it was found that *Salmonella* shows a 10 fold higher intracellular replication in BMDM derived from *UNC93B1 3d* mice, 24 hours post infection. Our data shows that Toll-like Receptor 11 and 12 play an important role in case of *Salmonella* infections.

25 DIFFERENCES IN THE FECAL METABOLITE PROFILES OF PROBIOTIC SUPPLEMENTED FORMULA- AND BREAST-FED INFANTS

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The early-life metabolome of the intestinal tract is dynamically influenced by colonization of gut microbiota and diet, i.e. breast milk or formula. The aim of this study was to investigate the effect of bifidobacteria-supplemented formula on the infant fecal metabolome compared to non-supplemented formula and breast milk within the first months of life. Therefore, a trial with 106 healthy neonates receiving infant formula with or without probiotics (*B. bifidum*, *B. breve*, *B. infantis*, *B. longum*) or breast milk was designed and fecal samples were collected over a period of two years at seven time points. Non-targeted metabolomics analysis was performed using reversed phase (RP) and hydrophilic interaction (HILIC) liquid chromatography coupled to high resolution mass spectrometry to cover polar and semi-polar metabolites. Metabolite profiles were clearly distinct between formula- and breast-fed infants. Some of the discriminating metabolites were directly associated with diet, for example human milk oligosaccharides (HMOs), increased levels of unsaturated fatty acids in breast-fed infants and elevated vitamin E glucuronides in formula-fed children. But also, gut microbiota related metabolite classes like bile acids, short chain fatty acids and aromatic amino acid metabolites were affected. For example, the secondary bile acids 7-oxolithocholic, 7-oxodeoxycholic and 3-dehydrocholic acid were increased in formula-fed infants. Furthermore, breast feeding resulted in higher levels of the tyrosine and tryptophan metabolites phenyllactic and indolelactic acid, which can be correlated to the high abundance of bifidobacteria in breast-fed children. Metabolites altered through the addition of bifidobacteria to infant formula were found in the first three months of life. Overall, the differences between breast- and formula-fed infants converged over time, especially seen at the age of 12 and 24 months.

26 Dissecting the role of the intestinal microbiota in an ER stress model of colorectal tumorigenesis and inflammation

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Colorectal cancer (CRC) is a leading cause of cancer-related death in the Western world. Alterations in microbiota composition have been repeatedly linked to CRC onset and progression. In our newly generated mouse model, overexpression of ATF6 results in an inflammation-independent tumor incidence of 100% at 12 weeks of age in homozygous (tg/tg) mice, with tumorigenesis microbiota dependent. This phenotype is further characterised by loss of goblet cells and increased microbial penetration of the mucus layer. Heterozygous (tg/wt) mice never develop tumors. To investigate this further, we sought firstly to sequence tissue-associated bacteria. A small section of proximal colon tissue of tg/tg mice, tg/wt mice and flox controls (fl/fl) was taken at three time points: pre-tumor (5 wk), early-tumor (12 wk) and late-tumor (20 wk) and 16S rRNA profiling was performed. Already at 5 weeks, a significant decrease ($p = 0.015$) in alpha diversity is observed in tg/tg mice. Beta diversity plots reveal all three genotypes are linearly separable. Secondly, we generated a new mouse model crossing tg/wt mice with IL-10 knockout mice to investigate the role of inflammation in tumorigenesis (tg/wt;-/-, fl/fl;-/-). In tg/wt;-/-mice tumor incidence was 62.5% and 60% in 12 and 20 weeks respectively. 16S rRNA sequencing revealed significant differences between tg/wt;-/- and fl/fl;-/- controls. Interestingly, tumor-developing tg/wt;-/- (61.2%) mice showed no significant difference to tg/wt;-/- without tumors (38.8%), the latter of which clustered with fl/fl;-/- controls. Preliminary results suggest that increasing sequencing resolution to tissue level allows for greater distinction between groups, as well as dissecting the role of the microbiota in the heterozygous model.

27 *EcN is resistant towards T4- phage infection and can reduce the T4 phage titer in coincubation*

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Background and Aim
E. coli Nissle 1917 (EcN) is one of the few probiotics that are licensed as a drug. Its genetic stability made it a safe and successful probiotic for the past 100 years. Phage resistance is an important aspect of a probiotic and recently we have observed that EcN is not infected by shiga toxin encoding phages (*stx*-phages) of Enterohaemorrhagic *E. coli* strains (EHEC), lambda phages and could also reduce their phage titer in coincubation (Bury and Soundararajan et al., 2018). We wondered if EcN is also insensitive towards an entire different class of phage such as T4 phage and if so we wanted to elucidate the mechanism(s) behind the phage resistance.
Methods
T4 phage resistance of EcN was tested by phage plaque assays (ppa) and by microscopic examination of *E. coli* with DAPI-stained T4 phages. T4 phage titer was determined by ppa after static incubation (37°C) and sterile filtration (0.22 µm) of supernatant in either LB medium or LB with EcN or *E. coli* MG1655. Transcriptome analysis was performed in the presence and absence of T4 phages. Characterization of the factor(s) responsible for T4 phage titer reduction was conducted with live EcN, EcN supernatant, heat killed EcN, and heat killed and proteinase K (1mg/ml) treated EcN in coincubation with T4 phages. Subsequently phage titer and cfus were determined.
Results
EcN was not infected by the tested T4 phage and microscopic examination of EcN after infection with DAPI-stained T4 phages showed intact EcN cells which did not take up phage DNA. When the phages were incubated with EcN at an MOI of 1000:1 (phages: EcN), EcN reduced the phage titer by ~100-fold after 2 h and 24 h. T4 phage inactivation by EcN was reversed to the initial phage titer between 24 – 48 h of coincubation. However, cfus/ml of EcN were not affected even after 48 h of coincubation. After incubation of EcN in the presence of phages, T4-specific PCR showed the phages to be present predominantly bound to EcN rather than in the sterile filtrate. Interestingly, heat killed as well as heat killed and proteinase K treated EcN could reduce the T4 phage titer by ~10-fold better than live EcN. When these treated EcN samples were characterized by 12% SDS PAGE, there were at least two or more prominent protein bands detected in heat killed and proteinase K treated EcN samples.
Transcriptomic analysis of EcN reveals that 33 genes were upregulated (log2fc>2, padj<0,05) and 123 genes were downregulated (log2fc<-2, padj<0,05) in the presence of T4 phages. The most upregulated gene was EcN_1772: an inner membrane transport protein. Also, noteworthy, 45% of the upregulated genes are coding for either an outer membrane protein, a membrane component or transport related proteins. Most of the metabolism related genes were downregulated in the presence of T4 phages and this is consistent with the observation of reduced EcN growth in the presence of T4 phages at early time points compared to the EcN single culture.
Finally, triculture (EcN, MG1655, T4) experiments clearly showed EcN to interfere with infection of tested *E. coli* K-12 strains.
Conclusions
We conclude that EcN is resistant against T4 phage infection, significantly reduces the T4 phage titer during 24 h coincubation and the responsible factor(s) is heat stable and proteinase K resistant. Furthermore, the transcriptomic response of EcN to T4 phage is different from the one observed for *stx* phages.
Reference: Susanne Bury, Manonmani Soundararajan, Richa Bharti, Rudolf von Bünau, Konrad U. Förstner and Tobias A. Oelschlaeger (2018) The probiotic *Escherichia coli* strain Nissle 1917 combats lambdoid bacteriophages *stx* and λ. Frontiers in Microbiology, in press.

28 Role of host glycosylation in susceptibility to intestinal inflammation

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Gastrointestinal tract expresses substantial amount of glycan structures and glyconconjugates which is an important factor contributing to the composition and physiology of intestinal microbiota. These glycoconjugates were utilized by intestinal microbiota and pathogens for microbial attachment site and as nutrients. Therefore, variation in host glycosylation can mediate susceptibility to intestinal inflammation such as to enteric pathogens. The glycosyltransferase gene *B4galnt2* encodes the beta-1,4-N-acetylgalactosaminyltransferase known to catalyze the last step in the biosynthesis of the Sd(a) and Cad blood group antigens and is expressed in the GI tract of most mammals, including humans. Loss of *B4galnt2* expression is associated with altered intestinal microbiota composition. We show that *B4galnt2* deficient mice had an increased susceptibility to chronic dextran sodium sulfate (DSS) induced colitis. A significantly enhanced pathological score and also increased intestinal inflammatory cytokines was found in *B4galnt2* deficient mice. Furthermore, we also observed that *B4galnt2*-deficient mice showed increased susceptibility to extracellular pathogens such as *C. difficile*. In *C. difficile* infection, *B4galnt2*-deficient mice showed significantly higher bacterial loads and also increased intestinal inflammation. Interestingly, higher loads of opportunistic *Enterococcus faecalis* was also observed in *B4galnt2*-deficient mice. These results show that *B4galnt2* expression modulates susceptibility to intestinal inflammation.

29 THE MICROBIOME OF THE BLADDER IN HEALTHY WOMEN. A LONGITUDINAL PILOT STUDY (MIHOP)

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Background: Recent studies have shown the presence of a specific microbiota of the bladder and investigated its role in health and disease. Nevertheless, there is a lack of studies investigating the urinary microbiota in a longitudinal fashion.

Material and methods: In order to investigate the longitudinal intrapersonal variability of the urinary microbiota in healthy individuals as a future control group, we conducted a prospective non-interventional pilot study with 15 pre-menopausal women. On four visits over the course of six months we collected urine samples obtained by one-time transurethral catheterization, and vaginal and periurethral swabs. One aliquot of each urine sample was subjected to expanded urinary culture. From all other samples, DNA was extracted and 16S rRNA gene amplicon sequencing performed.

Results: We observed intra-individual changes in the microbiota of the bladder over time concerning the beta-diversity as well as the relative abundance of several taxa. However, these variations were only little. The most frequent genera detected by 16S rRNA gene sequencing were *Lactobacillus* and *Bacteroides.* In half the cases all culturally detected genera were also present in 16S rRNA gene sequencing, but often the abundances were below 10%. Periurethral and vaginal swabs showed mostly *Lactobacillus* and *Bifidobacterium*. One individual developed a UTI and was treated with antibiotics. In this case, a consecutive drop in diversity and a culture and 16S detection of *Escherichia-Shigella* as highest abundant genus could be seen.

Conclusion: Preliminary results from our longitudinal cohort study confirm the presence of an individual urinary microbiota with little longitudinal changes over the period of 6 months. Further investigations into the close relatedness with vaginal microbiota are pending.

30 COMPARATIVE AND FUNCTIONAL WHOLE GENOME ANALYSIS OF E. COLI. NISSLE 1917 RE-ISOLATES OBTAINED FROM THE INTESTINE OF DELIBERATELY COLONIZED INDIVIDUALS

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Escherichia coli strain Nissle 1917 (EcN) is one of the most well-studied non-pathogenic, probiotic bacterial strains, that has been successfully used for the prevention and treatment of a range of gastrointestinal disorders. In addition to being used for the treatment of ulcerative colitis (remission phase), constipation and diarrheal diseases, and colonization prophylaxis, EcN is used for enhancing postnatal immune competence in infants. Even though the effects of short-term colonization by EcN have been reported, it has not been analyzed so far to what extent long-term intestinal colonization with this probiotic strain affects the composition of the human microbiota and what the adaptation strategies are used in each host in response to long-term interaction with the individual gut microbiota. In order to assess these events, we performed 16S rDNA-based analysis of stool samples and whole genome analysis of re-isolates from these stool samples of deliberately colonized individuals. Our study showed significant differences in the microbiota composition of infants that were stably colonized by EcN for two years and the placebo group. Additionally, different variations were found within the whole genome of the re-isolates, which could result in phenotypic changes. Our results will contribute to a better understanding of the long-term adaptation of EcN, its impact on the microbiota composition and how this may contribute to the probiotic effect.

31 NEONATAL INFECTIONS LASTINGLY SHAPE ADAPTIVE IMMUNE HOMEOSTASIS AND T CELL PRIMING WITHIN GUT-DRAINING LYMPH NODES

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Peripherally induced Foxp3$^+$ regulatory T cells (pTregs) critically contribute to tolerance towards intestinal commensals and food-born antigens. We could previously show that the high Treg-inducing capacity of mesenteric lymph nodes (mLNs) was retained upon mLN transplantation to the non-tolerogenic, skin-draining site of the popliteal fossa, suggesting a dominant and stably imprinted effect of stromal cells on the generation of pTregs. Furthermore, transplantation of mLNs from neonatal, ten days, four and eight week old mice pinpointed the first ten days after birth as the critical window of opportunity to stably imprint tolerogenic properties into mLN stromal cells. We now aim to dissect whether neonatal infections can affect the tolerogenic imprinting of mLN stromal cells.

To this end, we established a neonatal enteropathogenic *Escherichia coli* (EPEC) infection model, allowing assessing long-lasting immunological alterations subsequent to neonatal infection. Neonatally infected mice rapidly recovered from infection, developed normally and were able to completely eradicate EPEC within twelve weeks post infection (p.i.). Yet, the pathogen was contained to the intestinal tract at three weeks p.i., and profiling of the mLNs revealed an elevated activation and expansion of the stromal and hematopoietic compartment. Remarkably, even twelve weeks p.i., a time point at which the pathogen had been completely eradicated, we still observed a shift in the Treg/Th17 balance favoring RORγt-expressing Th17 cells in the colon accompanied by elevated frequencies of ILC3s. To assess whether *de novo* Treg induction is altered within mLNs of neonatally infected mice, we employed an adoptive transfer model using naïve TCR-transgenic T cells (DO11.10). Upon systemic antigen application, *de novo* Treg induction was significantly enhanced in mLNs of previously infected mice. Surprisingly, Treg induction within the liver-draining lymph nodes (cLNs) was substantially reduced, suggesting permanent, but opposing alterations of the tolerogenic properties of mLNs and cLNs subsequent to neonatal infection.

In the future, we will determine pathogen-specific long-lasting alterations to immune homeostasis by employing additional gastrointestinal neonatal infection models with diverse invasion strategies and tissue tropisms. Transplantations of mLNs and cLNs from previously neonatally infected mice and subsequent assessment of their Treg-inducing capacity and epigenetic modifications will enable us to dissect, which of the intrinsically altered immunomodulatory functions of the LNs can be allocated to the stromal cell compartment.

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