

# **10<sup>th</sup> Seeon Conference**

## Microbiota, Probiotics and Host

## Mikrobiota, Probiotics und Wirt

## 07.-09. JULY 2017

## CONFERENCE CENTER

## MONASTERY SEEON / CHIEMSEE

#### For more information:

#### www.seeon-conference.de





July 7th, 2017

Dear Participant,

On behalf of the German Society of Hygiene and Microbiology (DGHM) and the Organizing Committee, we welcome you to the **10<sup>th</sup> Anniversary of the Conference** "**Microbiota, Probiotics and Host**" at the beautiful Seeon Monastery!

In the past decade, "Microbiome Research" has emerged as *hot topic* in many different scientific fields, which is reflected by an ever increasing number of publications. Alongside with the continuous development of sequencing technologies, first analyses describing microbial composition in different parts of the human body have been successively replaced by comprehensive studies involving large patient cohorts. This led to an avalanche of metagenomic data, linking the microbiota to a variety of inflammatory, atopic, infectious, metabolic, cardiovascular and neoplastic diseases. Although the underlying mechanisms remain largely unclear to date, first clinical trials have shown a therapeutic value of fecal transplantation in selected human patients. On the other hand, research in microbial ecology and bacterial physiology is deepening our insights into the mechanisms of microbe-microbe and microbe-host interactions governing ecosystem assembly, stability, host-signaling and metabolite production. Now, we are on the eve of translating knowledge from basic research into the development of approaches for therapeutic microbiota manipulation: which strategies will finally be effective in improving microbiota function and human health?

Our DGHM section "Microbiota, Probiotics and Host" has established a visible community of talented young and senior scientists across various disciplines, including gastroenterology, nutritional medicine, immunology, infection research, microbial ecology and computational biology. The past activities of our DGHM section 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).

During the last 10 years, the "Seeon Conference" has become a well reputed platform to critically discuss basic mechanisms underlying microbe-microbe and microbe-host cross-talk and share cutting-edge science and technologies, including the development of novel therapeutic approaches for disease intervention. Thank you in advance for your contribution to this meeting. Your willingness to participate and share your expertise is greatly appreciated.

Prof. Dr. Bärbel Stecher	Prof. Dr. Guntram Graßl	Prof. Dr. Thomas Clavel
Max von Pettenkofer-Institut,	Institut für Medizinische	Institut für Medizinische
Lehrstuhl Hygiene und	Mikrobiologie und	Mikrobiologie
Mikrobiologie	Krankenhaushygiene	Forschungsgruppe" Intestinales
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# PROGRAM Friday, July 07th

#### 15<sup>00</sup> - 17<sup>00</sup> Registration 17<sup>00</sup> - 17<sup>15</sup> Welcome: B. Stecher, München

17<sup>15</sup> – 18<sup>00</sup> Keynote Lecture – **Eric Pamer** (Memorial Sloan Kettering Cancer Center, New York, USA): *Microbiota-mediated defense against intestinal infection* Chair: B. Stecher, München

18<sup>15</sup> Dinner

#### MICROBIOME SIGNATURES IN HEALTH AND DISEASE

19<sup>30</sup>– 21<sup>00</sup> Chair: G. Grassl, Hannover

Baumann A, Molecular Nutritional Science, University Vienna, Vienna, Austria Alterations of intestinal barrier and microbiota composition are associated with aging

Metwaly A, Chair of Nutrition and Immunology, Technische Universität München, Freising-Weihenstephan, Germany

Identification of disease-relevant bacterial signatures in gnotobiotic IL-10 deficient mice using faecal samples from IBD patients undergoing hematopoietic stem cell transplantation

Maier L, European Molecular Biology Laboratory, Genome Biology Unit, Heidelberg, Germany

Strong antibacterial effect of non-antibiotic drugs on human gut commensals

Osbelt L, Otto-von-Guericke University Magdeburg, Magdeburg; Helmholtz Centre for Infection Research, Braunschweig, Germany

Distinct microbial signatures are associated with varying susceptibility to Citrobacter rodentium infection in mice

Ecker J, Lehrstuhl für Ernährungsphysiologie, Technische Universität München, Freising-Weihenstephan, Germany *Gut microbiota modulate host fatty acyl chain metabolism* 

Calasan J, Chair of Nutrition and Immunology, ZIEL-Research Center for Nutrition and Food Sciences, Technische Universität München, Freising-Weihenstephan, Germany Role of segmented filamentous bacteria in Crohn's disease-like ileitis

# **PROGRAM Saturday, July 08th**

#### ECOLOGY & EVOLUTION OF MICROBIAL COMMUNITIES

08<sup>30</sup> – 10<sup>00</sup> Chair: J. Baines, Plön

Kabbert J, Institute of Molecular Medicine, RWTH Aachen, Aachen, Germany Diverse binding spectrum of monoclonal IgA to phylogenetically non-related bacteria

Ring C, Department Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam-Rehbrücke, Nuthetal, Germany The role of the commensal gut bacterium Akkermansia muciniphila in inflammatory bowel diseases

Eckstein M, Interdisciplinary Center for Clinical Research, Institute for Molecular Infection Biology, University Würzburg, Würzburg, Germany Mammalian gut colonization by a eukaryotic member of the microbiota

Hanson B, Department of Microbiology and Ecosystem Science, Division of Microbial Ecology, Research Network Chemistry meet Microbiology, University of Vienna, Vienna. Austria

A new source of  $H_2S$  in the gut: Degradation of sulfoquinovose by the complex human intestinal microbiota

Münch P, Computational Biology of Infection Research, Helmholtz Centre for Infection Research, Brunswick; Max von Pettenkofer-Institute for Hygiene and Clinical Microbiology, Ludwig-Maximilian University of Munich, Munich; DZIF, Partner Site Hanover-Brunswick, Brunswick, Germany

EDEN: Evolutionary dynamics within environments

Riva A, Department of Microbiology and Ecosystem Science, Division of Microbial Ecology, Research Network Chemistry Meets Microbiology, University of Vienna, Vienna, Austria

Fine-scale spatial architecture of the murine colon microbiota

#### 10<sup>00</sup> – 10<sup>30</sup> Coffee Break / Poster at the first glance

 $10^{30} - 10^{45}$ Denise Kelly: Investment Advisor for Microbiome Chair: D. Haller, Freising

 $10^{45} - 11^{10}$ Keynote Lecture - André Franke (Christian-Albrechts-University of Kiel, Germany): Inflammatory bowel diseases: From GWAS to systematic host-microbiome association studies Chair: T. Clavel, Aachen

## PROGRAM Saturday, July 08th

- 11<sup>10</sup> 11<sup>35</sup> Keynote Lecture Itzik Mizrahi (Ben-Gurion University of the Negev, Israel): *Plasmidome-centric view of the microbiome* Chair: T. Clavel, Aachen
- 11<sup>35</sup> 12<sup>15</sup> **Podium discussion:** *"Controversies & Consensus: Population-based metagenomics is it real what we see?"* Chair: T. Clavel, Aachen

#### 12<sup>15</sup> – 13<sup>30</sup> Lunch

- 13<sup>30</sup> 14<sup>15</sup> Keynote Lecture Wolf-Dietrich Hardt (ETH Zurich, Switzerland):
   Growing in the gut Lessions from the Salmonella Typhimurium paradigm
   Chair: G. Grassl, Hannover
- 14<sup>15</sup> 17<sup>30</sup> **10<sup>th</sup> Seeon Anniversary event**

#### 18<sup>00</sup> - 19<sup>30</sup> Dinner

19<sup>30</sup> – 21<sup>30</sup> Poster Slam (2 minutes / 2 slides) and Poster Discussion with free beer & wine
 Chair: F. Sommer, Kiel

# PROGRAM Sunday, July 09th

08<sup>30</sup> – 09<sup>15</sup> Keynote Lecture – **Maria Vehreschild** (University Hospital Cologne, Cologne): *Fecal microbiota transfer – a German perspective* Chair: B. Stecher, München

 $09^{15} - 09^{45}$  Coffee Break

#### MECHANISMS OF MICROBE-HOST INTERPLAY

09<sup>45</sup> – 11<sup>15</sup> Chair: M. Hornef, Aachen

Niemiec M, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena; Center of Sepsis Control and Care, Jena, Germany *Synergism of Candida Albicans and Proteus Mirabilis boosts enterocyte damage* 

Ye H, Department of Microbiology and Ecosystem Science, Division of Microbial Ecology, Research Network Chemistry meets Microbiology, University of Vienna, Austria

A new Desulfovibrio species is a potential taurine-degrader in the murine intestinal tract

Bury S, Institute for Molecular Infection Biology, University of Würzburg, Würzburg, Germany

The probiotic E. coli strain Nissle 1917 interferes with the stx-phage infection of E. coli K12 strains by inactivation of stx-phages

Harnack C, Department of Gastroenterology and Hepatology, Charité University Medicine Berlin, Berlin; Department of Molecular Biology, Max Planck Institute for Infection Biology, Berlin, Germany

Epithelial stem cell signalling in colon tissue and microbiota homeostasis

Mandić A, Department of Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Potsdam, Germany

Clostridium ramosum promotes obesity by regulating enterochromaffin cell development and serotonin production

Zou M, Department Experimental Immunology, Helmholtz Centre for Infection Research, Braunschweig, Germany

Microbiota stably imprint inflammation-resistant tolerogenic properties within mesenteric lymph node stromal cells during the neonatal phase

11<sup>15</sup> – 11<sup>30</sup> Awards & Farewell

11<sup>30</sup> Lunch

12<sup>30</sup> Departure

# PROGRAM Friday, July 07th

#### MICROBIOTA-MEDIATED DEFENSE AGAINST INTESTINAL INFECTION

#### Eric G. Pamer

#### Infectious Diseases Service, Memorial Sloan Kettering Cancer Center, USA

Infections caused by antibiotic-resistant bacteria generally begin with colonization of mucosal surfaces, in particular the intestinal epithelium. The intestinal microbiota provides resistance to infection with highly antibiotic-resistant bacteria, including Vancomycin Resistant Enterococcus (VRE) and Clostridium difficile, the major cause of hospitalization-associated diarrhea. Metagenomic sequencing of the murine and human microbiota following treatment with different antibiotics is beginning to identify bacterial taxa that are associated with resistance to VRE and C. difficile infection. We demonstrate that reintroduction of a diverse intestinal microbiota to densely VRE colonized mice eliminates VRE from the intestinal tract. While oxygen-tolerant members of the microbiota are ineffective at eliminating VRE, administration of obligate anaerobic commensal bacteria to mice results in a billion-fold reduction in the density of intestinal VRE colonization. Recent studies have identified specific bacterial species, including Blautia producta and Clostridium bolteae that prevent intestinal colonization with VRE. By treating mice with different antibiotics that result in distinct microbiota changes and lead to varied susceptibility to C. difficile, we correlated loss of specific bacterial taxa with development of infection. Using a workflow involving mouse models, clinical studies, metagenomic analyses and mathematical modeling, we have identified a probiotic candidate that corrects a clinically relevant microbiome deficiency. Our studies indicate that obligate anaerobic bacteria enable clearance of intestinal VRE colonization and may provide novel approaches to prevent the spread of highly antibiotic-resistant bacteria.

#### MICROBIOME SIGNATURES IN HEALTH AND DISEASE

19<sup>30</sup>– 21<sup>00</sup> Chair: G. Grassl, Hannover

**Baumann A**, Molecular Nutritional Science, University Vienna, Vienna, Austria Alterations of intestinal barrier and microbiota composition are associated with aging

**Metwaly A**, Chair of Nutrition and Immunology, Technische Universität München, Freising-Weihenstephan, Germany

Identification of disease-relevant bacterial signatures in gnotobiotic IL-10 deficient mice using faecal samples from IBD patients undergoing hematopoietic stem cell transplantation

**Maier L**, European Molecular Biology Laboratory, Genome Biology Unit, Heidelberg, Germany *Strong antibacterial effect of non-antibiotic drugs on human gut commensals* 

**Osbelt L**, Otto-von-Guericke University Magdeburg, Magdeburg; Helmholtz Centre for Infection Research, Braunschweig, Germany Distinct microbial signatures are associated with varying susceptibility to Citrobacter rodentium infection in mice

**Ecker J**, Lehrstuhl für Ernährungsphysiologie, Technische Universität München, Freising-Weihenstephan, Germany *Gut microbiota modulate host fatty acyl chain metabolism* 

**Calasan J**, Chair of Nutrition and Immunology, ZIEL-Research Center for Nutrition and Food Sciences, Technische Universität München, Freising-Weihenstephan, Germany Role of segmented filamentous bacteria in Crohn's disease-like ileitis

#### ALTERATIONS OF INTESTINAL BARRIER AND MICROBIOTA COMPOSITION ARE ASSOCIATED WITH AGING

<u>A. Baumann</u><sup>1</sup>, A. Brandt<sup>1</sup>, A.J. Engstler<sup>1</sup>, C.J. Jin<sup>2</sup>, A. Nier<sup>1</sup>, C. Frahm<sup>3</sup>, O.W. Witte<sup>3</sup>, A. Camarinha-Silva<sup>4</sup>, I. Bergheim<sup>1,2</sup>

<sup>1</sup>Department of Nutritional Sciences, Molecular Nutritional Science, University Vienna, Vienna, Austria

<sup>2</sup>Institute of Nutritional Sciences, SD Model Systems of Molecular Nutrition, Friedrich Schiller University Jena, Jena, Germany

<sup>3</sup>Hans-Berger Department of Neurology, University Hospital Jena, Jena, Germany <sup>4</sup>Institute of Animal Science, University of Hohenheim, Stuttgart, Germany

Recent studies suggest that aging is associated with alterations in intestinal barrier function and microbiota composition. Indeed, impairments of intestinal barrier function and alterations in microbiota may be responsible for the low-grade inflammation, typically found in elderly. Molecular mechanisms involved in the interaction between gut microbiota, intestinal barrier function and inflammation have not been fully understood. Starting from this background the aim of the present study was to determine at which age changes of intestinal barrier function and intestinal microbiota composition set in and if these alterations are associated with liver degeneration and inflammation. Intestinal microbiota composition, indices of intestinal barrier function including bacterial endotoxin levels, expression of lipopolysaccharide binding protein (LBP) and expression of toll-like receptors (Tlr) 1-10 in liver tissue and subsequent signalling cascades were determined in peripheral blood and liver of young (2-3 months) and old (15, 24, and 30 months) standard-chow-fed C57BL/6J mice. When compared to young animals liver tissue of old animals showed marked signs of inflammation which increased with age and were associated with beginning fibrosis in 24 and 30 months old animals. Hepatic alterations found in old mice were associated with higher endotoxin concentrations in peripheral blood and a gradually increasing expression of LBP as well as TIr-1, TIr-2, TIr-6 and TIr-7 mRNA in liver tissue. Furthermore, old age in mice was associated with a reduced diversity of bacterial families and marked changes in intestinal microbiota composition in upper parts of the small intestine. In conclusion, our data suggest that alterations in intestinal homeostasis being associated with beginning hepatic inflammation are already present in mice aged 15 months and increase with age.

IDENTIFICATION OF DISEASE-RELEVANT BACTERIAL SIGNATURES IN GNOTOBIOTIC IL-10 DEFICIENT MICE USING FAECAL SAMPLES FROM IBD PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION

<u>Amira Metwaly<sup>1</sup></u>, Ludovica F. Buttó<sup>1</sup>, Nadine Waldschmitt<sup>1</sup>, Ilias Lagkouvardos<sup>2</sup>, Ana Maria Corraliza<sup>3</sup>, Aida Mayorgas<sup>3</sup>, Margarita Martinez-Medina<sup>4</sup>, Matthieu Allez<sup>5</sup>, Julian Panes<sup>3</sup>, Azucena Salas<sup>3</sup> and Dirk Haller<sup>1, 2</sup>

<sup>1</sup>Chair of Nutrition and Immunology, Technical University of Munich, Germany

<sup>2</sup>ZIEL-Institute for Food and Health, Technical University of Munich, Germany

<sup>3</sup>Department of Experimental Pathology, Instituto de Investigaciones Biomédicas de Barcelona CSIC, IDIBAPS, CIBERehd Spain

<sup>4</sup>Laboratory of Molecular Microbiology, Biology Department, Universitat de Girona, Girona, Spain <sup>5</sup>APHP, Hôpital Saint Louis, Department of Gastroenterology, INSERM UMRS 1160, Paris Diderot, Sorbonne Paris-Cité University, Paris, France

**Background and aim:** Imbalanced microbial composition has been linked to the pathogenesis of inflammatory bowel diseases (IBD). Hematopoietic stem cell transplantation (HSCT) proved to be successful in inducing remission in a subset of severe, highly refractory Crohn's disease (CD) patients, possibly by erasing immune responses against the gut microbial ecosystem. Gnotobiotic mouse models provided insights into the functional and mechanistic aspects of host-microbe interactions. We used fecal microbiota from CD patients treated with HSCT to colonize an IBD-relevant mouse model. The aim of this study was to assess the functional role of microbiota signatures associated with different disease-states.

**Results and methods:** We performed High-throughput 16S rRNA gene amplicon sequencing on (n=156) fecal samples collected from (n=8) healthy donors and (n=35) HSCT-treated CD patients. Microbiota profiling showed a significantly reduced microbial diversity in patients compared with healthy controls. High level of inter-individual variation in the intestinal microbiota of healthy and diseased donor samples was observed. Germ-free (GF) wild-type (WT) and IL10<sup>-/-</sup> mice (129 Sv/Ev; n=12 mice/group) were colonized with fecal microbiota from CD patients before and after HSCT at different disease states. Selection of CD patients for transplantation into GF mice was based on clinical and endoscopic disease activity; including paired patient samples collected under remission or relapse. While transplanting human microbiota into germ-free mice resulted in a selective transfer of human donor microbiota, humanized mice reflected the dysbiotic features of their respective human donors, indicated by richness and diversity measures. Histopathological evaluation showed moderately to severe inflammation in colon and cecum of the relapse-associated IL10<sup>-/-</sup> mice. In contrast, the remission-associated IL10<sup>-/-</sup> mice remained disease-free. Inflamed and non-inflamed mice showed distinctive microbial signatures.

To validate the phenotype transfer even in the presence of multiple inoculations, we gavaged the mice three times with donor microbiota during the first week of colonization. Remission-associated mice showed higher species richness but still remained disease free, while relapse-associated mice developed enhanced inflammation measured at the level of fecal complement C3 concentrations. Endpoint microbial composition remained similar, regardless of the number of inoculations and F1 generations of mice displayed a stable engraftment of human microbiota.

**Conclusion:** Transfer of patient-derived fecal microbiota can mimic the disease phenotype (remission vs. relapse) in gnotobiotic IL10<sup>-/-</sup> mice. Inflamed and non-inflamed humanized mice show distinctive microbial signatures. We used humanized mice as a tool to identify bacterial signatures associated with disease status in IBD patients treated with HSCT.

## **STRONG ANTIBACTERIAL EFFECT OF NON-**ANTIBIOTIC DRUGS ON HUMAN GUT COMMENSALS

<u>Lisa Maier<sup>1#</sup>,</u> Mihaela Pruteanu<sup>1#</sup>, Michael Kuhn<sup>2#</sup>, Georg Zeller<sup>2</sup>, Anja Telzerow<sup>1</sup>, Ana Rita Brochado<sup>1</sup>, Keith Conrad Fernandez<sup>1</sup>, Hitome Dose<sup>3</sup>, Hirotada Mori<sup>3</sup>, Kiran Raosaheb Patil<sup>2</sup>, Peer Bork<sup>2</sup>, Athanasios Typas<sup>1,2</sup> # contributed equally

<sup>1</sup>European Molecular Biology Laboratory, Genome Biology Unit, Heidelberg, Germany <sup>2</sup>European Molecular Biology Laboratory, Structural and Computational Biology Unit, Heidelberg, Germany

<sup>3</sup>Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Japan

The composition of the human gut microbiota remains fairly stable once established in early life, but can be altered due to disease, lifestyle changes or pharmaceutical uptake. Especially pharmaceuticals have recently emerged as one of the strongest contributing factors to microbiome composition, but data come mostly from single drugs. In this study, we provide a comprehensive view of the effect of drugs on key species of the human gut microbiota by measuring the direct fitness effects of ~1200 marketed drugs on 40 representative strains (covering ~80% of the assignable average abundance of the human gut microbiome at genus level) in a one-by-one screening approach. Surprisingly, 24% of the non-antibiotic drugs affect growth of at least one species of our selection. Considerably more human-targeted drugs interfere with bacterial growth if doses are increased towards recommended administration levels. Interestingly, susceptibility towards antibiotics and non-antibiotic drugs correlates across bacterial species, suggesting common resistance mechanisms. Furthermore, we find that side effect patterns can be used to predict antibacterial activity of non-antibiotic drugs against our selected commensals. In conclusion, our findings offer paths for mitigating microbiota-related side effects or for repurposing non-antibiotic drugs as antibacterials, and challenge our current view on emergence of antibiotic-drug resistance.

### DISTINCT MICROBIAL SIGNATURES ARE ASSOCIATED WITH VARYING SUSCEPTIBILITY TO CITROBACTER RODENTIUM INFECTION IN MICE

Sophie Thiemann<sup>1</sup>, Lisa Osbelt<sup>2</sup>, Till Strowig<sup>1</sup>

<sup>1</sup>*Helmholtz* Centre for Infection Research, Braunschweig, Germany. <sup>2</sup>Otto-von-Guericke University Magdeburg, Magdeburg, Germany.

The gastrointestinal tract is colonized by complex microbial communities collectively called the gut microbiota. These ecosystems have an enormous impact on the host including influencing resistance or susceptibility to bacterial infections. Even though it is widely accepted that varying composition among individuals impacts the outcome of enteric infections in human and mice, it is not well understood which members of these diverse communities contribute to disease severity.

Here we have characterized in mice the impact of microbiota composition on the course of infection with *Citrobacter rodentium*, a mouse model for human enterohemorrhagic *Escherichia coli* (EHEC). We demonstrated that isogenic C57BL/6N mouse lines from different breeding facilities show highly varying disease kinetics after infection with *C. rodentium*. Transfer of the different microbiotas in germ-free mice by fecal transplantation revealed that the varying disease kinetics depend on microbial compositions in the gut. Analysis of the microbial composition using 16S rRNA sequencing revealed a microbial signature, which is associated with reduced pathogenic colonization within the first days of infection. Cohousing experiments of susceptible and resistant mouse lines linked members of the class *Clostridia* within the phylum of Firmicutes to ameliorated pathogen colonization in the early phase of *C. rodentium* infection.

Our findings demonstrate that mice from different breeding facilities feature distinct microbiota compositions and display differences in disease kinetics during *C. rodentium* infection, which are driven by the intestinal microbiota. Further studies will aim to characterize the two uncultured members of the class clostridia that are linked to reduced luminal bacterial burden in cecum and colon at early time points of infection.

### GUT MICROBIOTA MODULATE HOST FATTY ACYL CHAIN METABOLISM

Alida Kindt<sup>2</sup>, Gerhard Liebisch<sup>3</sup>, Thomas Clavel<sup>4</sup>, Gabriele Hörmannsperger<sup>5</sup>, Hongsup Yoon<sup>5</sup>, Dirk Haller<sup>5</sup>, Alexander Sigruener<sup>3</sup>, Sabrina Krautbauer<sup>3</sup>, Dominic Helm<sup>6</sup>, Bernhard Küster<sup>6</sup>, Daniela Kolmeder<sup>1</sup>, Hannelore Daniel<sup>1</sup>, Claudine Seeliger<sup>1</sup>, Jan Krumsiek<sup>2</sup>, <u>Josef Ecker<sup>1</sup></u>

<sup>1</sup>Lehrstuhl für Ernährungsphysiologie, Technische Universität München (TUM), Freising, Germany

<sup>2</sup>Institute of Computational Biology, Helmholtz Zentrum München, Neuherberg, Germany
 <sup>3</sup>Insitute of Clinical Chemistry, Universitätsklinikum Regensburg, Regensburg, Germany
 <sup>4</sup>Institute of Medical Microbiology, Universitätsklinikum Aachen, Aachen Germany
 <sup>5</sup>Lehrstuhl für Ernährung und Immunologie, Technische Universität München (TUM), Freising, Germany

<sup>6</sup>Chair of Proteomics and Bioanalytics, Technische Universität München (TUM), Freising, Germany

The link between the gut microbial ecosystem and host lipid metabolism is highly relevant for host physiology and metabolic diseases. Here, a comprehensive systems view of hepatic processes altered in germfree (GF) mice with focus on lipid metabolism is presented. Multi-omics analyses including transcriptome, proteome, phospho-proteome and lipidome were performed with liver and plasma samples obtained from GF and specific pathogen free (SPF) mice. Through integrated analyses and reconstruction of lipid metabolic pathways we identified that gut microbiota modulate elongation of polyunsaturated fatty acids (PUFA) by fatty acid elongase 5 (ElovI 5) and generation of mono-unsaturated fatty acids by stearoyl-CoA desaturase 1 (Scd 1). This leads to significant alterations in the acyl-chain profile of glycerophospholipids. A composite prediction score calculated from changes observed in fatty acid profiles differentiates both GF and mice treated with antibiotics from controls with high specificity and sensitivity.

In summary, we found that gut microbiota impact the generation of mono- and poly-unsaturated fatty acids leading to altered glycerophospholipid patterns in mice.

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

J. Calasan<sup>1</sup>, N. Waldschmitt<sup>1</sup>, M. Basic<sup>2</sup>, A. Bleich<sup>2</sup>, D. Haller<sup>1</sup>

<sup>1</sup>Technische Universität München, Chair of Nutrition and Immunology, ZIEL – Research Center for Nutrition and Food Sciences, Freising-Weihenstephan, Germany <sup>2</sup>Hannover Medical School, Institute for Laboratory Animal Science, Hannover, Germany

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 was reported in specific pathogen free (SPF) and gnotobiotic environment. SPF mice developed heterogeneous CD-like ileitis phenotype, independently of cage and litter effect. Herein, it has been further shown that severity of ileitis was related to the presence of certain vancomycin- and metronidazole-sensitive commensal bacteria. We used the genetically-predisposed TNFΔARE mouse model of spontaneous chronic CD-like ileitis in different housing conditions to understand the contribution of specific bacteria to ileal inflammation.

TNF $\Delta$ ARE and wildtype littermates were housed in germfree (GF) or gnotobiotic conditions. GF TNF $\Delta$ ARE mice showed no signs of intestinal inflammation.

Differential abundance analysis identified relevant taxa in dysbiotic and disease-unrelated microbial communities. GF TNF $\Delta$ ARE mice were colonized with individual bacterial strains by gavage (*Lactobacillus murinus*, *Alistipes sp., Escherichia coli LF82*) or by co-housing (segmented filamentous bacteria (SFB)). Numbers of colony-forming units grown under anaerobic conditions, total cell counts and qPCR bacterial quantification indicated that all mice were successfully colonised. While colonisation with *Lactobacillus murinus*, *Alistipes sp.* and *Escherichia coli LF82* was not sufficient to trigger intestinal inflammation in ex-GF TNF $\Delta$ ARE mice, SFB strongly induced an inflammatory response suggesting TNF-driven generation of pathogenic Th17 cells. Notably, ileal disease phenotype in SFB-monoassociated TNF $\Delta$ ARE mice extended further to ceacum and colon, which 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. Reduction in Reg3beta as well as Paneth cells' lysozyme expression was also observed along with an increase in granulocyte numbers and MIP2 transcript levels in inflamed TNF $\Delta$ ARE mice.

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 of TNF-driven inflammatory conditions would have substantial clinical implications in the future.

# PROGRAM Saturday, July 08th

#### ECOLOGY & EVOLUTION OF MICROBIAL COMMUNITIES

08<sup>30</sup> – 10<sup>00</sup> Chair: J. Baines, Plön

**Kabbert J**, Institute of Molecular Medicine, RWTH Aachen, Aachen, Germany Diverse binding spectrum of monoclonal IgA to phylogenetically non-related bacteria

**Ring C**, Department Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam-Rehbrücke, Nuthetal, Germany *The role of the commensal gut bacterium Akkermansia muciniphila in inflammatory bowel diseases* 

**Eckstein M**, Interdisciplinary Center for Clinical Research, Institute for Molecular Infection Biology, University Würzburg, Würzburg, Germany *Mammalian gut colonization by a eukaryotic member of the microbiota* 

**Hanson B**, Department of Microbiology and Ecosystem Science, Division of Microbial Ecology, Research Network Chemistry meet Microbiology, University of Vienna, Vienna, Austria

A new source of  $H_2S$  in the gut: Degradation of sulfoquinovose by the complex human intestinal microbiota

**Münch P**, Computational Biology of Infection Research, Helmholtz Centre for Infection Research, Brunswick; Max von Pettenkofer-Institute for Hygiene and Clinical Microbiology, Ludwig-Maximilian University of Munich, Munich; DZIF, Partner Site Hanover-Brunswick, Brunswick, Germany EDEN: Evolutionary dynamics within environments

**Riva A**, Department of Microbiology and Ecosystem Science, Division of Microbial Ecology, Research Network Chemistry Meets Microbiology, University of Vienna, Vienna, Austria

Fine-scale spatial architecture of the murine colon microbiota

#### DIVERSE BINDING SPECTRUM OF MONOCLONAL IGA TO PHYLOGENETICALLY NON-RELATED BACTERIA

Johanna Kabbert<sup>1</sup>, Hedda Wardemann<sup>2</sup> and Oliver Pabst<sup>1</sup>

<sup>1</sup>*RWTH Aachen, Institute of Molecular Medicine, Aachen, Germany* <sup>2</sup>*German Cancer Research Center, Division of B cell Immunology, Heidelberg, Germany* 

IgA accounts for up to 80% of the overall immunoglobulin production per day. In the gut, dimeric IgA is produced by lamina propria (LP) residing plasma cells (PCs) and transported into the gut lumen via the polymeric immunoglobulin receptor as secretory IgA (SIgA). In the intestinal lumen, SIgA binds to microbiota and thereby contributes to intestinal homeostasis. However, the precise mechanisms of how microbe directed IgA is induced and how functionally relevant levels of microbe-directed IgA are obtained remain unknown.

In this study we profiled the binding spectrum of monoclonal IgA antibodies derived from healthy and IBD donors. A total of 169 antibodies derived from healthy donors and 119 from IDB donors were screened by flow cytometry for their binding to bacteria isolated from murine feces. We observed that 11% of IgA from healthy and almost 40% of IgA from IBD donors bound to more than 3% of all bacteria suggesting that single monoclonal antibodies are capable of coating a relevant fraction of intestinal bacteria. To characterize bacteria bound by individual antibodies, coated and non-coated bacteria were sort purified and analyzed by 16S rDNA sequencing. We observed that single monoclonal antibodies bind a diverse spectrum of commensals rather than showing reactivity to single taxa. While antibodies eliciting microbiota reactivity showed frequent somatic mutations, preliminary experiments suggest that germ-line variants lose their microbiota binding capacity. Thus, somatic mutations seem a prerequisite for intestinal IgA to obtain a promiscuous binding to phylogenetically non-related bacteria. We speculate that ongoing somatic hypermutation might select for antibodies with broad binding capacity to different bacterial taxa and thereby generating meaningful levels of IgA targeting bacteria in the intestine.

## THE ROLE OF THE COMMENSAL GUT BACTERIUM AKKERMANSIA MUCINIPHILA IN INFLAMMATORY BOWEL DISEASES

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Akkermansia muciniphila, an abundant and commonly occurring commensal bacterium in the human gut, correlates negatively with inflammatory bowel diseases in humans and improves the metabolic status of diet-induced obese mice. Therefore, *A. muciniphila* is considered as marker for a healthy gut. However, *A. muciniphila* has also been linked to intestinal inflammation. For example, it exacerbates the inflammatory response in the Salmonella enterica Typhimurium induced model. Hence a better understanding of the role of *A. muciniphila* in the gut microbial ecosystem and in particular in the development of intestinal inflammation is indispensable.

To clarify whether the inflammation-promoting effect of *A. muciniphila* is a general feature of this organism, another mouse model, namely the colitis-prone IL-10 deficient mouse, were used. Gnotobiotic mice associated with selected bacterial species were additionally associated with *A. muciniphila* to assess the inflammatory response in dependence of the microbiota and the presence or absence of *A. muciniphila*. Microbiota composition, histology, expression of inflammatory markers, and mucus production were assessed.

*A. muciniphila* mono-associated IL-10 deficient mice showed no signs of intestinal inflammation. Additional association of the mice with the colitogenic *E. coli* UNC led to an inflammation that was similar to that caused by *E. coli* UNC alone. Mice colonized with a simplified human microbiota showed histological signs of inflammation but no increase in expression of inflammatory markers. The addition of *A. muciniphila* did not influence this outcome. Goblet cell numbers and expression of *MUC2* were similar in all groups. In summary, no effect of *A. muciniphila* on intestinal inflammation has been detected in the IL-10 deficient mouse model.

## MAMMALIAN GUT COLONIZATION BY A EUKARYOTIC MEMBER OF THE MICROBIOTA

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The human microbiota comprises members of all three domains of life, *i.e.* bacteria, archaea and eukaryotes. Yet little is known about the biology of the non-bacterial constituents of this microbial community and even less about how they interact with cohabiting microbes. Fungi, in particular, are common residents of the human body and recent research suggests that they play significant roles in shaping at least some of the functions ascribed to the microbiota. Our laboratory is investigating the interplay between the fungus Candida albicans and the bacterium Bacteroides thetaiotaomicron-two of the most prevalent species of each phylum in the human gut. We first imaged the interface between the fungus and the intestine of mice. Because the indigenous mouse microbiota restricts C. albicans settlement, we compared the patterns of colonization in the gut of germ free and antibiotic-treated conventionally raised mice. In contrast to the heterogeneous morphologies found in the latter, we established that in germ free animals the fungus uniformly adopts the round yeast morphology, a key proxy of its commensal state. By screening a collection of C. albicans deletion mutants in gnotobiotic mice, we have identified several genes and cellular functions previously unknown to contribute to in vivo fitness. Similarly, we have constructed a transposon mutant library in *B. thetaiotaomicron* and are employing this collection to uncover genetic determinants that contribute to the in vivo fitness of the bacterium exclusively in the presence of the fungus. We expect that these approaches will provide insights into the mechanisms that gut commensals employ to colonize the mammalian intestine.

## A NEW SOURCE OF $H_2S$ IN THE GUT: DEGRADATION OF SULFOQUINOVOSE BY THE COMPLEX HUMAN INTESTINAL MICROBIOTA

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<sup>1</sup>Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, Research Network Chemistry meets Microbiology, University of Vienna, Austria <sup>2</sup>Department of Biology and Konstanz Research School Chemical Biology, University of Konstanz, Germany

The gut microbiota facilitates the degradation of complex dietary compounds and in the process produces a myriad of metabolites that can influence host physiology. Sulfolipids (such as sulfoquinovosyldiacylglycerol, SQDG) derived from plants or other photosynthetic organisms are among the most abundant organosulfonates in the biosphere. Under anaerobic conditions, the polar sulfonate-containing head group of SQDG, sulfoquinovose (SQ; 6-deoxy-6-sulfoglucose), can be degraded concomitant with production of H<sub>2</sub>S, as has been observed first in a defined co-culture of *E. coli* K-12 and *Desulfovibrio* sp. DHPS1. SQ is initially fermented by *E. coli* to 2,3-dihydroxypropane-1-sulfonate (DHPS), which is further fermented by *Desulfovibrio* spp. to H<sub>2</sub>S and acetate. Because H<sub>2</sub>S is an important 'Janus-faced' metabolite in the gut that exerts either beneficial or detrime

ntal activity on its host, we explored the potential for anaerobic SQ degradation by human fecal microbiota using a microcosm-based approach. Fecal materials from vegetarians were amended with SQ and metabolite analyses verified a rapid degradation of SQ (below detection after 20 h) and later production of  $H_2S$  reaching a peak after 4 days. Additionally, the intermediate DHPS was identified in culture supernatants between 20 and 50 h. These results confirm the presence of microbial populations present in the human gut that are capable of metabolizing SQ via DHPS to  $H_2S$ . To identify the microorganisms involved in SQ degradation, we currently are analyzing 16S rRNA gene and dissimilatory sulfite reductase (*dsrB*) gene amplicon libraries for shifts in community composition, and are reconstructing the genomes of relevant microorganisms from metagenome data. In combination with the isolation of further SQ-degrading microorganisms, these analysis will elucidate the community members carrying out the putative anaerobic co-metabolism of SQ to  $H_2S$  in the human intestinal tract.

## EDEN: EVOLUTIONARY DYNAMICS WITHIN ENVIRONMENTS

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Metagenomics revolutionized the field of microbial ecology, giving access to Gb-sized datasets of microbial communities under natural conditions. This enables fine-grained analyses of the functions of community members, studies of their association with phenotypes and environments, as well as of their microevolution and adaptation to changing environmental conditions. However, phylogenetic methods for studying adaptation and evolutionary dynamics are not able to cope with big data. EDEN is the first software for the rapid detection of protein families and regions under positive selection, as well as their associated biological processes, from meta- and pangenome data. It provides an interactive result visualization for detailed comparative analyses. EDEN is available as a Docker installation under the GPL 3.0 license, allowing its use on common operating systems, at http://www.github.com/hzi-bifo/eden

## FINE-SCALE SPATIAL ARCHITECTURE OF THE MURINE COLON MICROBIOTA

Alessandra Riva<sup>1</sup>, Orest Kuzyk<sup>1</sup>, Alexander Loy<sup>1</sup> and David Berry<sup>1</sup>

<sup>1</sup>Department of Microbiology and Ecosystem Science, Division of Microbial Ecology, Research Network Chemistry Meets Microbiology, University of Vienna, Vienna, Austria

Sequencing technologies have significantly advanced our understanding of the composition and diversity of host-associated microbial communities. However, there is limited knowledge on their spatial organization and co-occurrence. In this study, we performed a diet-shift experiment for one week in wild-type mice with different diets [polysaccharide- and fiber-free (PF), fiber-free (FF), and control (CON) diets] in order to evaluate changes in colon microbiota composition and its spatial organization under conditions of dietary fiber and polysaccharide privation. We collected faecal samples and samples from all colon compartments (proximal, middle and distal) and from luminal and mucosal areas. We studied the spatial organization of the gut microbiota in sectioned colon samples using fluorescence *in situ* hybridization (FISH) and laser capture microdissection (LCM) followed by 16S rRNA gene sequencing and metagenomics.

We found that elimination of microbially-accessible dietary carbohydrates resulted in a reduction of diversity and in changes in microbial biogeography along colon compartments. In particular, the privation of fiber and above all the absence of polysaccharides compromised the microbial diversity from proximal to distal colon. Elimination of dietary polysaccharides reduced mucus thickness and goblet cell numbers, altered the mucus-associated microbiota, and increased the patchiness of microbiota composition throughout the colon. Metagenomics of these patches is being applied to better understand the potential functional consequences of these patterns. This high-resolution approach to capture and examine spatial organization of intestinal microbes has the potential to improve our understanding of the importance of spatial structuring in microbiota function and host–microbiota interactions.

## INFLAMMATORY BOWEL DISEASES: FROM GWAS TO SYSTEMATIC HOST-MICROBIOME ASSOCIATION STUDIES

#### André Franke

Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany

Inflammatory bowel diseases (IBD) with its main sub phenotypes Crohn's disease and ulcerative colitis, are complex, polygenic, chronic and immune-mediated diseases that affect about 2-3 persons out of a 1000 in Western countries. IBD is an archetypical immune-mediated disease, which shares part of its genetic and immunological background with diseases like psoriasis, ankylosing spondylitis, and primary sclerosis cholangitis (PSC). To this end, we have identified over 240 genetic susceptibility loci in the past 10 years through genome-wide association (GWAS) and candidate-gene association studies, which has tremendously changed our view on the etiology of the disease. Complex immunogenetics efforts need to be undertaken to solve this complex prototypic disease. Still, the exact cause of IBD has not been identified and components of the gut microbiome are also likely disease-causing environmental factors for IBD. I will show early results from our microbiome studies in IBD and the related disease PSC (primary sclerosis cholangitis). In the second half of my talk I will show results of our ongoing efforts in host-microbiome association analyses and allude to the methodological challenges of these kind of analyses. In the latest analysis we have identified 4 new genome-wide significant loci with intriguing candidate genes that I will highlight in the presentation.

#### **PLASMIDOME-CENTRIC VIEW OF THE MICROBIOME**

#### Itzhak Mizrahi

The Department of Life Sciences & the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Israel

Plasmids are self-replicating genetic elements often capable of mobilization between different microbial hosts. The intrinsic effect of this movement is horizontal gene transfer, a process considered to be a formative evolutionary force in microbial ecosystems. The close proximity, high number, and vast variety of neighboring cells in the mammalian gut create a favorable environment for horizontal gene transfer via plasmids. A classic example is found in the bovine digestive tract in a compartment termed the rumen, where a highly-dense and complex microbiome resides. The rumen microbiome makes it possible for the animal to digest and convert plant mass into energy. Due to its density and complexity as well as the economical importance of its effect on the host, the microbial community of the rumen serves as a perfect model system to study plasmid mobilization and dispersal in complex gut environments. We have developed a metagenomics toolbox to detect and research plasmids as they occur in nature in such complex environments. This toolbox includes a bioinformatics pipeline to identify and assemble the plasmidome, a term coined to describe the overall assortment of plasmids in an ecosystem. We use this toolbox to address long-standing questions about the plasmidome which, despite their great importance, remain unanswered: What forces govern plasmidome composition and dispersal? What is the plasmidome's functional relevance for the ecosystem? What kinds of interactions exist between the plasmidome and the microbial hosts' genomes? In this lecture, I will discuss some of our recent findings regarding these questions with special emphasis on the ecological impact of plasmids in the rumen as well as in other gut ecosystems and the mechanisms by which they facilitate gene mobility.

#### GROWING IN THE GUT – LESSONS FROM THE SALMONELLA TYPHIMURIUM PARADIGM

#### Wolf-Dietrich Hardt

#### Institute of Microbiology, ETH Zurich, Zurich, Switzerland

The microbiota protects against gut infections by numerous bacterial pathogens. This phenomenon has already been discovered in the 1950's to '70s. However, we are just beginning to decipher the underlying molecular mechanisms, and how this is augmented by innate and adaptive immune responses of the host. We have developed mouse infection models using *Salmonella* Typhimurium as a paradigm. Key principles may also apply to the control of the resident gut microbiota. I will discuss how *S*. Typhimurium can grow up in the face of an intact microbiota, how this leads to disease and how disease boosts the horizontal transfer of plasmids and prophages. This can accelerate the acquisition of new virulence factors and of antibiotic resistance genes. IgA responses by the host can prevent this. By crosslinking growing bacterial cells ("enchained growth"), microbe-specific IgA clumps the pathogen. This prevents disease, accelerates pathogen removal from the gut and retards horizontal DNA transfer. Our data indicate that members of the microbiota may be controlled by equivalent IgA-mediated enchainment

# PROGRAM Sunday, July 09th

#### FECAL MICROBIOTA TRANSFER – A GERMAN PERSPECTIVE

#### Maria J.G.T. Vehreschild

#### Clinical Microbiome Research Group, University Hospital Cologne, Cologne, Germany

The clinical effectiveness of fecal microbiota transfer (FMT) for the treatment of recurrent Clostridium difficile infections (rCDI) has been demonstrated in randomized controlled trials. While North American authorities have enforced regulations that support FMT-based clinical applications and interventional studies alike, the European Medicines Agency has failed to draw up similar standards for the European community. Thus, regulation of FMT in Europe is still being defined at a national level. Therefore, physicians in Europe are likely to encounter difficulties when searching for reliable standards with respect to donor screening, microbiota preparation, and route of administration. At the same time, colleagues considering referral of state-of-the-art microbiota-based studies is stalled by these regulatory hurdles. This lecture will focus on the current regulatory status, real-life application and future of FMT in Germany, pinpointing major shortcomings and pointing out potential solutions.

## **MECHANISMS OF MICROBE-HOST INTERPLAY**

#### 09<sup>45</sup> – 11<sup>15</sup> Chair: M. Hornef, Aachen

**Niemiec M**, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena; Center of Sepsis Control and Care, Jena, Germany *Synergism of Candida Albicans and Proteus Mirabilis boosts enterocyte damage* 

**Ye H**, Department of Microbiology and Ecosystem Science, Division of Microbial Ecology, Research Network Chemistry meets Microbiology, University of Vienna, Austria

A new Desulfovibrio species is a potential taurine-degrader in the murine intestinal tract

**Bury S**, Institute for Molecular Infection Biology, University of Würzburg, Würzburg, Germany

The probiotic E. coli strain Nissle 1917 interferes with the stx-phage infection of E. coli K12 strains by inactivation of stx-phages

**Harnack C**, Department of Gastroenterology and Hepatology, Charité University Medicine Berlin, Berlin; Department of Molecular Biology, Max Planck Institute for Infection Biology, Berlin, Germany

Epithelial stem cell signalling in colon tissue and microbiota homeostasis

**Mandić A**, Department of Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Potsdam, Germany

Clostridium ramosum promotes obesity by regulating enterochromaffin cell development and serotonin production

**Zou M**, Department Experimental Immunology, Helmholtz Centre for Infection Research, Braunschweig, Germany

Microbiota stably imprint inflammation-resistant tolerogenic properties within mesenteric lymph node stromal cells during the neonatal phase

## SYNERGISM OF CANDIDA ALBICANS AND PROTEUS MIRABILIS BOOSTS ENTEROCYTE DAMAGE

#### M. J. Niemiec<sup>1,2</sup>, M. Kapitan<sup>1,2</sup>, I. D. Jacobsen<sup>1,2,3</sup>

<sup>1</sup>Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena, Germany <sup>2</sup>Center of Sepsis Control and Care, Jena, Germany 3 Friedrich Schiller University, Jena, Germany

The human gut, as the organ harboring the highest density of microbes, is a relevant source of life-threatening infections. Numerous opportunistic bacteria and fungi colonize the gut and can disseminate into the bloodstream (BS) upon immunosuppression or impairment of barrier function. While the mechanisms promoting translocation of *Candida albicans* into the BS are not fully understood, recent studies estimate up to 29 % of *Candida* BS infections to be in fact polymicrobial – implying a role for *Candida*-bacteria interactions.

In order to understand the causality between co-colonization and coinfection, we investigate the interplay of *C. albicans* and the gram-negative opportunist *Proteus mirabilis*, an emerging cause of bacterial BS infections.

In the absence of host cells, fungal numbers were reduced during long-term coincubation with *P. mirabilis* while bacterial survival was enhanced, suggesting antagonistic interactions favoring *P. mirabilis*. In contrast, we observed significantly increased damage of enterocytes during *in vitro* coinfections with *C. albicans* and four different *P. mirabilis* strains compared to summed-up single-species damage. This synergistic effect was strongly diminished by deletion of *P. mirabilis* hemolysin. To investigate whether *Candida* filamentation and damage were essential for boosting host cell damage, we tested *C. albicans* mutants with impaired hypha formation or lacking candidalysin as well as non-*C. albicans* yeasts. Surprisingly, neither candidalysin nor filamentation were required for the *Candida-Proteus* synergism and even less virulent yeast species were capable of promoting synergistic host cell damage.

Experiments aimed to determine (i) whether *C. albicans* affects hemolysin production of *P. mirabilis*, (ii) which fungal factors are relevant for this, and (iii) if synergistic virulence occurs in *in vivo* models are currently ongoing.

## A NEW DESULFOVIBRIO SPECIES IS A POTENTIAL TAURINE-DEGRADER IN THE MURINE INTESTINAL TRACT

Benjamin Zwirzitz, <u>Huimin Ye</u>, Buck T. Hanson, Alexander Loy

Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, Research Network Chemistry meets Microbiology, University of Vienna, Austria

Taurine is one of the most abundant amino acid homologs in the human body and Bilophila wadsworthia is the only described microorganism in the human gut harboring the complete taurine degradation pathway. B. wadsworthia metabolizes taurine via sulfoacetaldehyde and sulfite to hydrogen sulfide (H<sub>2</sub>S) as the end product. As a human pathobiont, *B. wadsworthia* is routinely isolated from patients with appendicitis or intestinal inflammation. Remarkably little information is available about the taurine-utilizing bacteria in the intestinal tract of mice. To further reveal novel microorganisms involved in taurine degradation in the gut of laboratory mice, we prepared incubations of the colonic and cecal contents from replicate wild type C57BL/6 mice. Additionally, taurine, formate, and pyruvate were added alone or in different combinations to the incubations to investigate the necessary elements for taurine degradation. Microcosms were subsampled at different time points (0, 4, 8, 12, 24, 48, 54, and 144 h) and the microbial responses to the amendments were monitored by quantification of  $H_2S$ , taurine, and short-chain fatty acids. We found that amendments of taurine and pyruvate together stimulated the production of  $H_2S$  (but not taurine alone) and that this activity was further enhanced when combined with an addition of formate. Furthermore, the decrease of taurine paralleled the increase of  $H_2S$  over time, which provides evidence for the presence and activity of microorganisms harboring a *B. wadsworthia*-like taurine degradation pathway in mice. Based on the dynamics of  $H_2S$  and taurine over time, biomass samples harvested at several time points were selected for 16S rRNA gene and dsrB amplicon sequencing. Analysis of these libraries showed that Deltaoproteobacteria became enriched over time in the treatments receiving taurine, pyruvate, and formate, which was largely due to the increase of a single Desulfovibrio phylotype. In conclusion, these results suggest the presence of a novel taurinedegrading bacterium belonging to the Desulfovibrio genus in the murine intestinal tract.

### THE PROBIOTIC E. COLI STRAIN NISSLE 1917 INTERFERES WITH THE STX-PHAGE INFECTION OF E. COLI K-12 STRAINS BY INACTIVATION OF STX-PHAGES

#### S. Bury<sup>1</sup>, M. Soundararajan<sup>1</sup>, T.A. Oelschlaeger<sup>1</sup>

<sup>1</sup>Institute for Molecular Infection Biology, University of Wuerzburg, Germany

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 stxphages and not only efficiently reduced 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 tri-culture studies of STEC strains with EcN and K-12 strains. EHEC - E. coli K-12 co-culture 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 mono-culture. Simultaneously to the strong reduction of the toxin level the stx-phage titer was up to 1000-fold reduced in the presence of EcN. This phage-neutralizing outcome was equally shown after incubation of EcN with isolated stx-phages. Our results indicate that STEC strains can convert harmless K-12 strains into strong Stx producers. We could 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. The findings from co-culture studies of EcN with stx-phages demonstrated for the first time a phage neutralizing effect of probiotics on phages. These findings encourage us to elucidate the mechanism of the phage reduction by EcN and support the idea of using EcN as a medication in the treatment of human EHEC infections.

## **EPITHELIAL STEM CELL SIGNALLING IN COLON TISSUE AND MICROBIOTA HOMEOSTASIS**

Christine Harnack<sup>1,2</sup>, Bertram Wiedenmann<sup>1</sup>, Thomas F. Meyer<sup>2</sup>, Michael Sigal<sup>1,2</sup>

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<sup>2</sup>Department of Molecular Biology, Max Planck Institute for Infection Biology, Berlin, Germany

Background: The epithelium of the colon consists of differentiated cells that are constantly produced by the colon stem cell compartment. The cells migrate towards the surface and are shed to the lumen resulting in rapid epithelial regeneration that happens under healthy conditions. The signals that control this turnover are not fully understood but it is known that the presence of a healthy microbiota affects epithelial turnover. It is suggested that the turnover is required because surface cells are under constant exposure to potentially toxic gut lumen content including bacteria.

We hypothesize that the epithelial stem cell signaling and turnover are induced by the microbiota and are essential to maintain a barrier between the epithelium and the luminal bacteria.

We have developed technologies to visualize the epithelial stem cells and perform lineage tracing experiments to follow the fate of the stem cell-derived clones. This allows a quantification of turnover dynamics in the crypt. To study how the microbiota affects the stem cell turnover we are generating gnotobiotic stem cell reporter mice and will characterize how bacteria affect stem cell signaling and epithelial turnover.

Moreover we have applied and further developed technologies to visualize the microbiota in the colon using in situ hybridization. We observe that the bacteria are strictly separated from the colonic epithelium under physiological conditions. Our data indicates that the turnover and shedding of cells into the lumen is important to maintain the barrier.

We now are developing genetic systems as a well a pharmacological approaches to manipulate stem cell signaling in vivo and will study how specific pathways affect epithelial turnover dynamics, the microbial composition in the gut as well as the spatial distribution of the bacteria in the colon.

#### CLOSTRIDIUM RAMOSUM PROMOTES OBESITY BY REGULATING ENTEROCHROMAFFIN CELL DEVELOPMENT AND SEROTONIN PRODUCTION

<u>Ana D. Mandić</u><sup>1</sup>, Anni Woting<sup>1</sup>, Tina Jaenicke<sup>1</sup>, Ulrike E. Rolle-Kampczyk<sup>2</sup>, Sven-Bastiaan Haange<sup>2</sup>, Nico Jehmlich<sup>2</sup>, Martin von Bergen<sup>2</sup>, Michael Blaut<sup>1</sup>

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Clostridium ramosum is an anaerobic, spore-forming bacterium that has been linked to obesity in humans and mice [1-4]. To elucidate the mechanism and pathways behind obesogenic effects of C. ramosum we compared germ free (GF) and mice monoassociated with C. ramosum (Cra) fed either high-fat diet (HFD) or low-fat diet (LFD). Consistent with our previous data, Cra mice fed HFD displayed increased body weight compared with Cra LFD mice (13.6%  $\pm$  2.122% versus 2.246%  $\pm$  1.261%, P = 0.0001), epididymal (eWAT), subcutaneous and mesenteric white adipose tissue weight (P = 0.0001). Also we observed increased intestinal and eWAT absorption of fatty acids (Cd 36 - cluster of diffrentation 36, 2-fold, P = 0.002 and Fatp4 - fatty acids transport protein 4, 3-fold, P = 0.0022). Cra mice fed HFD exhibited increased plasma serotonin (5-HT) levels (P = 0.0124) and increased colonic 5-HT production by enterochromaffin (ECs) cells shown by number of double positive 5-HT and ChA cells (Chromogranin A, marker for ECs) (Cra mice 91.2%  $\pm$  2.31 versus GF 71.9%  $\pm$  5.10% of total epithelial cells, respectively, P = 0.0085) and expression of major genes involved in 5-HT synthesis and metabolism (*Tph1* - trypthophan hydroxylase 1, 1-fold, P = 0.0099 and Monoamine oxisdase A 1.5-fold, P = 0.001). Furthermore, upregulation of ChA and the transcription factors Atoh1 (protein atonal homolog 1), Nkx2.2 (homeobox protein Nkx2.2) and Lmx1a (LIM homeobox transcription factor 1 alpha) (2-fold, P = 0.0001) suggest that C. ramosum directly programs epithelial progenitor cells differentiation toward the secretory intestinal epithelial cells and therefore promotes serotonin synthesis. We also observed increased 5-HT production in eWAT measured by ELISA (Cra 16.60 pM ± 2.07 pM versus GF 6.431 pM ± 0.89 pM, P = 0.0061) and deduced from gene expression levels of Tph1 (4-fold, P = 0.0001) and 5-HT receptor HTR2b (1.4-fold, P = 0.0059), as well as reduced expression of genes and enzymes involved in lipolysis and beta oxidation of fatty acids. Surprisingly we also observed reduced lipogenesis in Cra mice fed HFD probably as counter regulation of extensive fat absorption and accumulation. With our ongoing in vitro study we aim to confirm that C. ramosum stimulates host serotonin production and therefore promotes obesity as serotonin produced in the peripheral organs was recently identified as obesogenic factor in mice [5].

<sup>1.</sup> Qin, J., et al., *A metagenome-wide association study of gut microbiota in type 2 diabetes*. Nature, 2012. **490**(7418): p. 55-60.

<sup>2.</sup> Karlsson, F.H., et al., *Gut metagenome in European women with normal, impaired and diabetic glucose control.* Nature, 2013. **498**(7452): p. 99-103.

<sup>3.</sup> Le Chatelier, E., et al., *Richness of human gut microbiome correlates with metabolic markers*. Nature, 2013. **500**(7464): p. 541-6.

<sup>4.</sup> Woting, A., et al., *Clostridium ramosum promotes high-fat diet-induced obesity in gnotobiotic mouse models*. MBio, 2014. **5**(5): p. e01530-14.

<sup>5.</sup> Crane, J.D., et al., *Inhibiting peripheral serotonin synthesis reduces obesity and metabolic dysfunction by promoting brown adipose tissue thermogenesis.* Nat Med, 2015. **21**(2): p. 166-72.

#### MICROBIOTA STABLY IMPRINT INFLAMMATION RESISTANT TOLEROGENIC PROPERTIES WITHIN MESENTERIC LYMPH NODE STROMAL CELLS DURING THE NEONATAL PHASE

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Peripherally induced Foxp3<sup>+</sup> regulatory T cells (Tregs) critically contribute to tolerance towards intestinal commensals and food-born antigens. Our previous work had shown that the high Treg-inducing capacity of mesenteric lymph node (mLN) was retained upon their transplantation into the non-tolerogenic, skindraining site of the popliteal fossa, suggesting a dominant and stably imprinted effect of stromal cells on the generation of peripherally induced Tregs. Furthermore, transplantation of mLN from germ free (GF) and specific-pathogen free (SPF) mice revealed that microbiota is essentially required for the imprinting of tolerogenic properties in mLN stromal cells. The present project aims to identify the time point during ontogeny at which the imprinting process is taking place and to describe the microenvironmental factors that educate mLN stromal cells for their site-specific function. To this end, neonatal, 10 days, four and eight week old mLN were transplanted into the popliteal fossa of recipient mice, and 8-10 weeks later their Treq-inducing capacity was tested. The results clearly demonstrated that the tolerogenic properties of mLN are stably imprinted within the first 10 days after birth, and flow cytometric analysis of mLN stromal cells confirmed high proliferation during these early time points, highlighting the opportunity for commensals to impinge onto epigenetic modifications at this developmental stage. Next, mLN from various donor mice were transplanted to unravel the mechanism of the imprinting process. Transplantation of mLN from RAG2<sup>-/-</sup> mice and short-chain fatty acids (SCFA)-treated GF mice revealed that the imprinting process is independent of IgA and SCFAs. To study the contribution of defined microbial species, mLN from antibiotics-treated SPF mice and GF mice after colonization with defined bacteria were transplanted and analyzed for their Treg-inducing potential. These experiments showed that defined commensals are sufficient to promote the imprinting of tolerogenic properties in mLN and can even result in a superior Treg-inducing capacity. Finally, transplantation of mLN from previously infected mice or mice suffering from chronic colitis revealed that mLN stromal cells stably retain their high Treg-inducing capacity even subsequent to gastrointestinal infections or chronic intestinal inflammation. In conclusion, stromal cells from mLN are shaped early during ontogeny by microbiota during a time frame of high proliferative capacity, resulting in stabilized, inflammation-resistant tolerogenic properties.
# POSTER

#### **1** INTESTINAL MICROBIOTA MODULATES REG3B-DEPENDENT EPITHELIAL HOST RESPONSES

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The intestinal microbiota plays a major role in modulating the interaction between the gastrointestinal (GI) epithelium and the immune system of the host. Many GI tract diseases are assumed to be influenced by microbial dysbiosis. Even though it is known that antibiotics change the microbial composition in the gut, the impact of antibiotic-related microbiota disturbances on epithelial immune parameters is not yet fully uncovered.

In this project we investigated the effects of antibiotic-induced microflora modulation on immunological responses in adult C57BL6 inbred mice. 16S RNA sequencing of feces revealed distinct patterns of microbiota changes after antibiotic treatments, the most prominent being dysbiotic-like changes in rifaximin (RFX)-treated animals. On the epithelial level, mice treated with RFX also showed a downregulation of Reg3b, a lectin with antimicrobial and anti-inflammatory properties. Further, we investigated the relevance of this finding by inducing a DSS colitis and observing that RFX pretreatment and Reg3b deficiency in KO animals show a more severe colitis phenotype.

In order to investigate the pro-homeostatic role of Reg3b, we hypothesized that Reg3b influences the intestinal stem cell compartment. By using small intestinal (SI) organoids as an experimental model, we showed that application of cecal content of RFX-treated animals downregulates stem cell markers, while treatment of organoids with IL-22, PAMPs and even Reg3b upregulate these.

Another aim of this project is explaining how dysbiosis influences Reg3b downregulation. By looking at bacteria reduced after RFX treatment, we spotted Clostridia cluster XIVa, the main producer of the anti-inflammatory metabolite butyrate, and found that fecal butyrate levels are significantly lower in RFX-treated mice compared to non-treated animals. Analysing Reg3b expression in butyrate receptor KOs, we observed a significant Reg3b downregulation. This finally provides a link between microbiome changes and epithelial Reg3b regulation.

In summary, we found that experimental manipulations of gut microbiota induce distinct host responses with Reg3b as an important factor for intestinal homeostasis.

#### **2** ORAL SUPPLEMENTATION OF CITRULLINE IN THE THERAPY OF A DIET-INDUCED NON-ALCOHOLIC STEATOHEPATITIS (NASH): ROLE OF INTESTINAL MICROBIOTA AND BARRIER FUNCTION

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Non-alcoholic liver disease (NAFLD) is by now the most common liver disease worldwide. Beside genetic predisposition and lifestyle factors, changes in intestinal barrier function and microbiota composition are discussed as critical risk factors in the development of NAFLD. Studies suggest that the amino acid citrulline (Cit) may prevent the onset of gut- and liverrelated diseases. Here, we determined if a supplementation of Cit prevents the progression of a diet-induced NASH. Female 6-8 weeks old mice were either pair-fed a standard diet (C) or a Western-style diet (WSD) for 13 weeks to induce a NASH. Starting in week 8, some of the WSD-fed mice were fed Cit (2.5 g/kg body weight) until the end of experimentation. Indices of liver damage and inflammation, markers of toll-like receptor 4 (TIr-4)-dependent signaling pathways were determined. Markers of intestinal barrier function and microbiota composition were analysed. Markers of hepatic inflammation e.g. number of inflammatory foci and TNF $\alpha$ protein concentration, lipid peroxidation and hepatic steatosis were significantly lower in livers of mice treated with Cit when compared WSD-fed animals. While TIr-4 and MyD88 mRNA expression in liver tissue did not differ between WSD-fed mice, bacterial endotoxin levels in portal plasma was significantly lower in WSD+Cit-fed mice when compared to WSD-fed animals. Protein concentration of the tight junction protein occludin and zona occludens 1 (ZO-1) being significantly lower in small intestinal tissue of WSD-fed mice were almost at the level of controls in WSD+Cit fed mice. Analysis of microbial communities in small intestine revealed that microbial community structure showed a statistical difference between C- and WSD-fed groups and between WSD and WSD+Cit with a significantly higher abundance of Blautia, Dietzia and Microbacterium in small intestine of the latter. In conclusion, our data suggest that an oral Cit supplementation may protect mice from the progression of a pre-existing NASH.

#### **3** THE V-TYPE ATPASE AS A CRUCIAL CANDIDATE FOR THE MAINTENANCE OF INTESTINAL HOMEOSTASIS

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Intestinal dendritic cells are the most important interface between a host's immune system and its microbiota. Antigen encounter of immature dendritic cells (DCs) results in a shift of the DC phenotype. This altered phenotype is among others mediated by an altered MHC-II processing which occurs within the lysosome. The regulation of the lysosomal pH is therefore a crucial event for further immunological responses. A key candidate to regulate the lysosomal pH is the V-Type ATPase (VTA) which pumps protons into the lysosome in order to decrease the lysosomal pH. The VTA is in its active form upon assembly of its cytosolic V1 domain and its membraneous V0 domain. Thus, inhibition of the assembly prevents acidification of the lysosome. In dendritic cells the cytosolic V1 domain is inefficiently assembled to the membrane subunits on lysosomes in the immature phenotype resulting in poor lysosomal acidification. We therefore established a method to compare lysosomal pH values between different mouse DC phenotypes. In this context we observed a significant difference in the lysosomal pH comparing semi-mature and fully mature dendritic cells. We could also show that the VTA activity is also directly related to MHC-II surface expression on dendritic cells and thus a crucial factor in further immune responses. The pH alteration is also necessary for several lysosomal enzymes. Among these enzymes are the cathepsins which are involved in antigen degradation, MHC-II processing and degradation of the extracellular matrix. Thus, a tight regulation of the lysosomal pH is necessary in order to retain a physiological level of cathepsin activity. We are currently investigating the biological consequences of the VTA assembly on the activity of the Cathepsins B, K, L and S. We also observed a direct interaction between the VTA and the endogenous molecule Cystatin C. Upon Cystatin C deficiency, dendritic cells show a significantly decreased lysosomal pH, indicating that Cystatin C serves as an endogenous inhibitor of the VTA. We have previously described B. vulgatus mpk as a symbiont to induce a semi-mature phenotype in mouse dendritic cells. We furthermore propose that *B. vulgatus* mpk is able to regulate the V-Type ATPase assembly and thus activity via Cystatin C, resulting in a low amount of MHC-II surface expression, retained physiological cathepsin activity and hence contributes positively to the maintenance of the intestinal homeostasis.

#### **4** EXPERIMENTAL EVOLUTION OF POLYSACCHARIDE-DEGRADING BACTEROIDES THETAIOTAOMICRON, AN ABUNDANT MEMBER OF THE HUMAN GUT

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The intestinal microbiota is important for human health and nutrition by degradation of recalcitrant compounds and synthesis of vitamins among many other host-beneficial activities. The genus *Bacteroides* is an abundant and diverse group that is associated with a healthy digestive tract. Bacteroides species are particularly adapted to utilization of complex dietary and host-derived compounds via an extensive repertoire of polysaccharide utilisation loci. These are evidence of genomic adaptation to survive in response to availability of various nutrient sources. However, the relationship between metabolic flexibility and fine-scale diversification and adaptation to different nutrient-based niches by Bacteroides species has not been extensively studied. In our project, we focus on in vitro evolution of one of the most broadly-studied species of this genus, Bacteroides thetaiotaomicron VPI-5482. The experimental evolution experiment was set up similarly to Lenski's experimental evolution with E.coli albeit under anaerobic conditions. We performed a preliminary testing to identify carbohydrate utilization profile of *B. thetaiotaomicron in vitro* where pure cultures were grown in defined minimal medium (Varel & Bryant, 1974) of single carbohydrates as well as mixtures of them such as amylopectin, pectin, and inulin. We performed a preliminary experimental evolution test for 18 consecutive days (serial transfers) of growing B thetaiotaomicron in defined minimal medium with two mixtures of carbohydrates and glucose as a positive control. This preliminary experiment showed us that much longer times are needed to detect any phenotypic changes such as increased growth rate and biomass. However, we believe that ongoing in vitro experimental evolution experiments with B. thetaiotaomicron in the presence of different polysaccharides will shed light on the importance of dietary polysaccharide complexity and rapid adaptation on microbiota assembly and niche saturation as well as provide essential information for further studies of Bacteroides community assembly both in an in vitro and in vivo environment.

#### **5** IMPACT OF MINIMAL BACTERIAL CONSORTIA ON THE COLITIS DEVELOPMENT AND ITS MODULATION IN AN IL-10 DEFICIENT MOUSE MODEL

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Colonization of germ-free mice with defined bacterial consortia allows standardized analysis of host-microbiota interactions and ensures the reproducibility of studies with microbiota dependent phenotypes such like inflammatory bowel disease (IBD). In our study we analyzed the impact of two defined bacterial consortia. The well-known Altered Schaedler Flora (ASF), consisting of eight bacterial species and the new minimal consortia of 12 bacterial strains, the Oligo-Mouse-Microbiota (OMM), established by Stecher and colleagues. The interleukin-10 deficient (*II-10<sup>-/-</sup>*) mice are used as a model for studying experimental IBD as the disease development and severity of the intestinal pathology depends on mouse strain genetic background and microbiota interactions. Therefore, the aim of the study was to analyze the influence of minimal bacterial communities on the development of colitis in an *II-10<sup>-/-</sup>* mouse model and how this phenotype could be modulated by certain microbiota representatives.

In germ-free mice colonized with minimal bacterial consortia no inflammatory lesions were observed. However, animals which were colonized with defined bacterial communities and subsequently infected with the murine norovirus (MNV) showed an increased intestinal histopathological score. The pathological lesions were predominantly located in proximal colon and characterized by hyperplasia of crypt epithelium and infiltration of inflammatory cells like neutrophilic granulocytes, macrophages, and plasma cells in the intestinal wall. Interestingly, after co-colonization with segmented filamentous bacteria (SFB) the MNV infection was not able to trigger the increase in histopathological score in ASF colonized ex germ-free mice. However, SFB co-colonization showed no impact in animals carrying OMM. Furthermore, mono-colonization of germ-free *II-10<sup>-/-</sup>* mice with SFB or MNV as well as SFB colonization together with MNV did not cause pathological changes in colon.

Altogether, our results showed that MNV infection has detrimental effect and leads to appearance of inflammatory lesions in the intestine of mice carrying ASF or OMM. Furthermore, SFB prevented MNV triggered intestinal inflammation only in ASF. This indicates that the colitogenic effect of MNV depends on the presence of specific bacterial species.

## **6** MICROBIOTA-DEPENDENT SIGNALS LINK **ATF6**-DRIVEN ER**UPR** TO COLONIC TUMORIGENESIS

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Activation of the endoplasmic reticulum unfolded protein response (erUPR) contributes to the pathogenesis of inflammatory bowel diseases and colon cancer. Mechanistic evidence for a causative role of specific UPR arms in inflammation and tumorigenesis is lacking. The activating transcription factor 6 (ATF6) mediates one of three branches involved in sensing and signaling of erUPR. To address the role of ATF6-mediated erUPR signaling in intestinal epithelial cells (IEC), we generated Villin-Credriven IEC-specific transgenic mice overexpressing the activated form of ATF6 (nATF6<sup>IEC</sup>). Homozygous nATF6<sup>IEC tg/tg</sup> mice spontaneously developed colonic adenomas independent of inflammatory processes, with an incidence of 100 % at 12 weeks of age. In contrast, heterozygous nATF6<sup>IEC w/tg</sup> mice reveal fully activated erUPR but fail to spontaneously develop tumors. Loss of mucinfilled goblet cells was associated with increased microbial penetration of the mucus barrier in homozygous nATF6<sup>IEC tg/tg</sup> mice. High-throughput 16S-rRNA gene sequencing of caecal microbiota showed a clear separation of bacterial communities according to the tumor-promoting genotype and reduced bacterial diversity was already developed at a pre-tumor stage in homozygous nATF6<sup>IEC tg/tg</sup> mice. Germ-free housing of nATF6<sup>IEC tg/tg</sup> mice was shown to prevent tumor formation and epithelial hyperproliferation, even in the presence of activated erUPR. Antibiotic treatment induced a shift in microbial composition, but not microbial load, and antagonized hyperproliferation and tumor incidence. Most importantly, the transfer of pre-conditioned microbiota into germ-free recipients reestablished the tumorigenic phenotype in nATF6<sup>IEC tg/tg</sup> mice, clearly demonstrating the causative role of bacterial communities in colonic adenoma formation. Mechanistic insights are currently being investigated with the use of MyD88/TRIF-deficient nATF6<sup>IEC</sup> mice and ex vivo intestinal organoid experiments. We demonstrate a novel role for nATF6 in colonic tumorigenesis and highlight the fact that microbiotaderived signals are required as second hit in an inflammation-independent mechanism.

#### **7** DECIPHERING THE PROTECTIVE EFFECT OF FACULTATIVE ANAEROBIC BACTERIA IN ENTERIC SALMONELLA TYPHIMURIUM INFECTIONS IN OLIGOMM MICE

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The mammalian gut microbiota fulfills many beneficial tasks for the host, such as nutrient degradation, contribution to the development of an intact immune system and protection against enteric infections, 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. Therefore, we used a gnotobiotic mouse model (Oligo-MM<sup>12</sup>) 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 consortium 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 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<sup>12</sup> mice.

## **8** ASSESSMENT OF CPAS-BASED BGISEQ-500 PLATFORM FOR METAGENOMIC SEQUENCING

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**Background:** The widespread application of metagenomic shotgun sequencing in microbiome research relies on the development of high-throughput, cost-effective sequencer. A novel high-throughput sequencing platform, BGISEQ500, has been recently launched by BGI. Here we present the metagenomic shotgun sequencing data from this new platform, and compare its performance with that of the Illumina platform.

**Results:** We evaluated inter-batch, inter-run and inter-platform variations for BGISEQ500. A dataset from 20 healthy individuals was generated, including 8 library replicates and 8 sequencing replicates on BGISEQ500, and 20 pairwise cross-platform comparisons on both BGISEQ500 and Illumina HiSeq2000. 82.45M (96.1% of raw reads) high quality reads per samples with an averaged Phred quality score of 34.7 were obtained by a newly-developed QC method. 77.7% of genes could be mapped to the integrated gene catalog.

High correlations were achieved in both library and sequencing replicate groups at the gene level (Spearman, R > 0.982). Paired test showed that only 2 genes had significantly different abundances between library replicates. However, cross-platform sample pairs showed a relatively low correlation (R = 0.885), and 40342 (0.99%) genes showed discrepancy in their relative abundance on the two platforms.

The ambiguously-sequenced genes were further assessed, and they were found to contain an obvious GC-bias. When these genes were annotated to species, the linear model between relative abundance and GC content showed zero-centralized in BGISEQ500, but in HISEQ2000 data, this value trended to be more positive, suggesting that the differences were more likely to be driven by HISEQ2000.

**Conclusion:** This study provides the first set of performance metrics for BGISEQ 500 and a means to benchmark new platform. The high accuracy and technical reproducibility confirm the applicability of BGISEQ500 for metagenomic study, though caution is still warranted when combining metagenomic data from different platforms.

Keywords: BGISEQ 500, sequencing, metagenomics

#### **9** The IMPACT OF DIETARY FIBERS ON INTESTINAL MICROBIOTA AND HOMEOSTASIS

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Health benefits of dietary fibers are mainly mediated by the intestinal microbiota, e.g. by the production of short-chain fatty acids. This symbiotic relationship between the microbiota and our immune system has a great influence on intestinal homeostasis; dysbiosis plays an important role in the pathogenesis of intestinal as well as extra-intestinal diseases and inflammation.

The aim of this study was to analyse the mechanism by which dietary fibers affect the gut microbiota and intestinal homeostasis. For this purpose we fed conventional and germ-free mice a fiber-free or fiber-containing elemental diet, to distinguish between microbial and diet-mediated effects.

Animals fed fiber-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. Further, we found that dietary fibers dramatically influence the development of the microbiota. Comparing animals of both diets at young age (8 weeks), we found a similar diversity of the cecal microbiota. Interestingly, we observed a massive increase in the microbial diversity between week 8 and 12 exclusively in mice fed fibers. To evaluate whether fibers act directly or via microbial products, we performed global gene array of intestinal epithelial cells and found distinct gene clusters for both groups with the majority of epithelial genes influenced in the presence of microbiota. Taken together, we demonstrate the importance of dietary fibers for intestinal homeostasis in a microbiota-dependent manner.

## **10** CANDIDALYSIN INTERFERES WITH THE GROWTH OF COMMENSAL BACTERIA

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The opportunistic pathogen *Candida albicans* is a common and mostly harmless inhabitant of human mucosal surfaces. Only under predisposing conditions like immunosuppression, damaged barriers, or antibiotic treatment, *C. albicans* can switch to a pathogenic state. Given the importance of a protective bacterial microbiota, it is likely that interactions with other members of the microbiota contribute to its commensal state. The recently discovered peptidetoxin Candidalysin is an important virulence factor of *C. albicans*. Here we investigate the potential activity of Candidalysin against bacteria of the human microbiota.

We screened a total of 35 human-associated bacteria for growth defects in the presence of synthetic Candidalysin and found that the toxin inhibits the growth of distinct bacteria. This effect was confirmed by measuring minimal bactericidal concentrations (MBC) and metabolic activity. We also observed that Candidalysin causes perturbations in membranes of selected bacteria.

We additionally tested derivatives of Candidalysin and found that an N-terminal truncation of Candidalysin considerably improves its antimicrobial activity. These truncated versions show a higher toxicity and are active against more bacterial species than the original Candidalysin. In conclusion, we show that Candidalysin exerts multiple effects against selected bacteria, such as growth inhibition or membrane perturbation and modified versions of the peptide even have increased activity.

#### **11** Loss of NLRP3 leads to greater VARIABILITY OF COLITIS SEVERITY IN THE ACUTE DSS MODEL

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**Background:** Hypofunctional mutations in the NOD-like receptor pyrin domain containing 3 (*NIrp3*) locus have been associated with inflammatory bowel disease. However, conflicting results about both a protective and aggravating nature of NLRP3 in experimental murine colitis have been published.

We hypothesized that the gut microbiota composition of the animals affects the colitis phenotype and demonstrated that loss of *NIrp3* affects the gut microbiota composition under basal conditions and during acute DSS colitis. To further investigate the impact of the gut microbiota, acute DSS colitis was performed in 1, SPF *NIrp3<sup>-/-</sup>* and *NIrp3<sup>+/+</sup>* littermates that had been separated according to genotype at weaning and in 2, *NIrp3<sup>-/-</sup>* and *NIrp3<sup>+/+</sup>* that had been rederived germfree and associated with a limited, defined microbiota (sDMDMm2).

**Methods:** Female *NIrp3<sup>-/-</sup>* and wild-type (WT) littermates (SPF or sDMDMm2 microbiota) underwent dextran sodium sulfate (DSS)-induced acute (7 days 2 % DSS) colitis. Colitis severity was evaluated by body weight, murine endoscopic index of colitis severity (MEICS), colon length, myeloperoxidase activity, mRNA expression of proinflammatory cytokines and histology.

**Results:** SPF and sDMDMm2 *NIrp3<sup>-/-</sup>* mice showed a higher variability in colitis severity in comparison to wildtype littermates, with some animals clearly suffering from a more severe colitis.

**Conclusions:** Loss of *Nlrp3* leads to a higher variability of colitis severity in the context of two different microbiota colonisations. Further investigations will address the differences e.g. in immune cell infiltrate between animals with a more severe disease activity and a milder phenotype.

## **12** ROLE OF STD FIMBRIAE IN VIRULENCE OF SALMONELLA TYPHIMURIUM

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The *Fut2* gene encodes a  $\alpha$ -1,2-fucosyltransferase responsible for the expression of ABO histo-blood group antigens on the gastrointestinal mucosa and bodily secretions. In humans, loss-of-function mutations of FUT2 are known as 'non-secretors'. In the intestine, these individuals have a loss of glycan structures in mucus and on epithelial cells. Fimbriae, filamentous appendages on the bacterial surface, are the most common adhesion system of Salmonella and a major virulence factor. While expression of some types of fimbriae is conserved among different serovars, most Salmonella fimbriae are not produced in vitro. Here, we have investigated the role of stdABCD-encoded fimbriae for S. Typhimurium infection of *Fut2* wildtype and knockout mice. The *std* fimbrial operon encodes  $\pi$ -fimbriae, a clade within the chaperone/usher-dependent fimbrial assembly pathway. It had been shown before that Std fimbriae can bind to terminal fucose residues. Several mutant strains of S. Typhimurium lacking Std fimbriae were created and evaluated for their ability to attach and invade the human epithelial cell lines in vitro. The negative regulator of Std fimbriae, rosE, was inactivated to ensure stable expression in vitro. Using Salmonella enterica serovar Typhimurium infections in mice, we observed significantly lower Salmonella colonization in the colon and cecum of Fut2-deficient mice compared to wildtype controls. Furthermore, decreased histopathological changes were observed in the colon tissue of Fut2-knockout mice. Stronger infiltration of immune cells in Fut2 wildtype mice compared to Fut2-deficient mice was detected by immunofluorescence staining. This increased bacterial colonization as well as stronger inflammation of Fut2 wildtype mice was abolished when mice were infected with a S. Typhimurium strain lacking Std fimbriae. These results show that Std-fucose interaction is important for Salmonella-triggered intestinal inflammation.

#### **13** GASTRIC BYPASS SURGERY ALTERS THE COMMUNITY STRUCTURE AND THE FUNCTIONAL COMPOSITION OF THE INTESTINAL MICROBIOTA

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In a rat model the influence of Roux-en-Y gastric bypass surgery (RYGB) on the microbiota of the ileum, the cecum as well as the colon was investigated and compared to body weight matched animals with sham surgery. To resolve the community structure in regard to taxonomy and enzymatic functionalities 16S rRNA gene sequencing, metaproteomics and targeted metabolomics was performed.

Distribution of taxa and the enzymatic functional capacity of the microbiome were greatly altered after RYGB. We observed greater prevalence of Actinobacteria especially Bifidobacteriales in the ileum, cecum and colon after RYGB with Firmicutes generally seen at lower abundances. For the lower intestinal tract Proteobacteria were also more prevalent in after RYGB than in sham samples. Interestingly, in all investigated gut sections the species *Clostridium perfringens*, a known pathogen, was observed to be at higher abundances after RYGB. On a functional level in the cecum proteins involved in xylan degradation as well as those from the Glycolysis were more pronounced in RYGB. In the colon proteins from pathways involved in amino acid metabolism were observed at altered abundances. For example the histidine degradation pathway was more prevalent in RYGB samples whereas proteins involved in cysteine synthesis were seen at lower levels. Furthermore, metabolomics revealed that total amino acid concentration in the colon was significantly higher in RYGB.

#### **14** CHARACTERISATION OF FLIC FROM ESCHERICHIA COLI ISOLATES FROM HEALTHY AND IBD PATIENTS

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Flagellin (FliC) is a monomeric protein that as a polymer generate the flagella. This protein is essential in the motility of *Escherichia coli* possessing as well immunogenic qualities, being recognized by toll-like receptor 5. FliC can trigger an inflammatory immune response but also homeostasis, as has been proved in our laboratory for the probiotic strain *E. coli* Nissle 1917 (*Ec*N), in which once deleted a discrete amino acidic section on the C-terminus of FliC the protective effect was lost in an induced inflammation animal model.

Notably, in comparison to others *E. coli* and *Salmonella* strains, in average *Ec*N is 95 amino acids longer. Recently we performed analyses showing a scattered pattern of this insert which narrows the area of the deletion we previously assayed in vivo in looking for the specific region that account for protection.

Currently we are preforming sequencing over the *fliC* gene from healthy controls and IBD patients, preliminary results point out that this two groups present a different profile of FliC in *E coli* at the genomic level. The healthy controls present at both flanks of the gene a conserved region, while the IBD patients have a conserved profile in the overall *fliC* length.

At this moment, we have successfully cloned and obtained *Ec*N FliC with a high degree of purity. With this method, we pretend to purify ultra-pure *Ec*N FliC chimeric mutants to test our hypothesis that a healthy patient presents some FliC motifs similar to *Ec*N FliC. In the same direction and to gain insights into the function of the different FliC motifs we are in the process of obtaining the crystallographic structure of the protein.

#### **15** The role of host-protozoan INTERACTIONS IN NLRP6 DEFICIENCY ASSOCIATED DYSBIOSIS

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The mammalian gut is host to a wide consortium of microbes from diverse kingdoms including viruses, prokaryotes, archaea and eukaryotes. For a long time commensal protozoan have been underappreciated. Recent studies demonstrated that protozoan alter the gut microbiota and shape the intestinal immune landscape. Yet, the impact of these species on the host in general and, in particular, on the immune system is still not fully understood. The imbalanced microbiota of NIrp6 inflammasome deficient mice has been demonstrated to affect susceptibility to diverse diseases including metabolic syndrome and intestinal inflammation, but research has largely focussed on bacterial components of this community.

Here, we show by microscopy and molecular methods that this dysbiotic community contains a protozoan that we identify as *Tritrichomonas musculis* (*T. mu*), which has recently been suggested to modulate host immunity through inflammasome-mediated activation of IL-18. We are currently exploring the role of different inflammasome sensors as well as microbiota alterations after *T. mu* colonization in *T. mu* induced immune remodelling to eventually advance our understanding of host-protozoan interactions. Therefore, we colonized protozoan-less mice that differ in their composition of bacterial microbiota with purified *T.mu* and are currently analysing changes in their immune state as well as microbiota composition along the gastrointestinal tract. We are also planning targeted and untargeted metabolome analysis to investigate *T.mu* affected metabolites. In our preliminary analysis we observed in line with previous findings a strong induction of IFN<sub>γ</sub> and IL-17 in CD4+ T cells after *T.mu* infection in WT mice. However, to our surprise also Caspase-1-deficient mice, which are impaired in IL-18 production, displayed a similar increase in IFN<sub>γ</sub> and IL-17 production in CD4+ T cells.

Future experiment will clarify the role of specific host factors in shaping the intestinal microbiota and the mucosal immune system as well as their respective contribution to intestinal inflammation.

#### **16** Exploring the role of individual commensals in enteric Salmonella enterica serovar Typhimurium infection

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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.

## **17** ROLE OF COLIBACTIN IN COLON CARCINOGENESIS

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Colorectal cancer is the third most frequent cancer 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 a colon organoid model derived from primary epithelial cells of murine and human colon. 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. We are in the process of performing RNA sequencing to determine genes that are regulated in response to infection. Further, we will establish a mouse model to characterize the sites of colonization and potential interaction with stem cells at the base of the colon crypts. Our data aims to provide a better understanding of how colibactin could contribute to malignant transformations and facilitate cancer progression.

## **18** INTERACTIONS BETWEEN CANDIDA ALBICANS AND ENTEROCOCCI

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*Candida albicans* is the most common cause of life-threatening nosocomial fungal infections generally caused by endogenous strains colonizing mucosal surfaces. Both, as a commensal and pathogen, *C. albicans* interacts not only with the host but also with other members of the microbial flora. One genus of bacteria often coisolated with *C. albicans* is *Enterococcus*<sup>1</sup>. The two most common species associated with life-threating infections are *E. faecalis* and *E. faecium*.

To investigate fungal-bacterial interactions between *C. albicans* and enterococci, we developed cocultivation and coinfection assays with human enterocytes. Interestingly, the quality of interactions was not only species- but also highly strain-dependent, ranging from antagonistic to synergistic effects, and was influenced by the presence of host cells. In the absence of host cells, *E. faecalis* strains showed enhanced growth in presence of *C. albicans* or impaired fungal long-term survival. In contrast, coinfection of enterocytes commonly led to increased damage if *C. albicans* was combined with *E. faecalis*, whereas most tested *E. faecium* strains reduced overall enterocyte damage. Importantly, these synergistic and antagonistic effects were additionally influenced by oxygen availability, a factor that highly varies in the host. Synergistic damage was enhanced by a higher dose of viable *C. albicans* but not *E. faecalis* cells. Interestingly, this synergism does not require filamentation and can also be observed for *Saccharomyces cerevisiae* as well as *Candida glabrata*. We will further investigate this fungal-enterococcal relationships and mechanisms responsible for host cell damage, as well as look into host factors that might contribute to damage reduction or enhancement.

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<sup>&</sup>lt;sup>2</sup> Graham CE, Cruz MR, Garsin DA, Lorenz MC. *Enterococcus faecalis* bacteriocin EntV inhibits hyphal morphogenesis, biofilm formation, and virulence of *Candida albicans*. Proc Natl Acad Sci U S A 2017, 114(17): 4507-4512.

#### **19** Loss of Caseinolytic protease P (CLPP) IMPAIRS MITOCHONDRIAL FUNCTION AND ANTAGONIZES INTESTINAL EPITHELIAL STEMNESS

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Impaired mitochondrial proteostasis, associated with mitochondrial dysfunction has been implicated in various pathologies including cancer and inflammatory bowel disease (IBD). We have recently shown that mitochondrial unfolded protein response (MT-UPR) is activated upon knockout of the mitochondrial chaperone *Hsp60* and that mitochondrial function controls intestinal epithelial stemness. To further characterize the impact of deregulated mitochondrial proteostasis on intestinal epithelial cell (IEC) homeostasis, we generated a conditional knockout mouse model for the mitochondrial matrix protease *ClpP*.

Mice lacking *ClpP* in IEC showed a reduction in plasma citrulline levels, indicative of reduced mitochondrial function, despite no obvious changes in intestinal morphology. *Ex vivo* knockout of *ClpP* in intestinal organoids resulted in circular crypt morphology associated with diminished expression of intestinal epithelial stem cell (ISC) markers *Lgr5* and *Olfm4*. qPCR analysis confirmed activation of MT-UPR signalling and alterations in mitochondrial dynamics. In addition, genes involved in mitochondrial and cytoplasmic oxidative stress responses, *Sod2*, *Catalase*, *Ho-1*, and *Hif1a* were induced, indicating an enhanced ROS production. Expression levels of *HtrA2*, a protease located in the mitochondrial intermembrane space were elevated upon *ClpP* loss, suggesting compensatory mechanisms to sustain mitochondrial proteostasis. In parallel to data obtained in mice with an IEC-specific and ISC-specific *Hsp60*-deletion, disturbances in mitochondrial proteostasis and subsequent MT-UPR activation led to changes in IEC subpopulations, suggesting specific types of IEC to be more sensitive to impaired mitochondrial function.

Our results highlight the importance of mitochondrial function on intestinal epithelial stemness and homeostasis. Therefore, mitochondrial proteostasis might represent a cellular checkpoint at the edge of intestinal tissue homeostasis and repair/healing processes in the context of diseases.

## **20** CHARACTERIZATION OF THE THREE SAT GENES SECRETED BY THE PROBIOTIC ESCHERICHIA COLI STRAIN NISSLE 1917 (ECN)

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The secreted autotransporter toxin (Sat) relates to the Serine Protease Autotransporters of *Enterobacteriaceae* (SPATE) family. It was originally described as a virulence factor in pathogenic *E. coli* strains such as uropathogenic *E. coli* (UPEC) [1], avian pathogenic *E. coli* (APEC) [2], enteroaggregative *E. coli* (EAEC) [3] and others. Members of the SPATE family show a broad range of different functions (adhesion, protease, lipase, etc.) [4,5] in the extracellular space. The SPATEs, including Sat have three different characteristic domains: a N-terminal signal sequence, a passenger domain and a C-terminal autotransporter domain. The last one mediates the transport through the outer membrane [1,6]. It could be shown that EcN harbors at least three genes for different secreted serine autotransporter proteases which belong to the SPATE family.

We asked the questions are these genes expressed in EcN at all and if so do they show protease activity and why EcN, a probiotic strain, harbors these genes, which are typically described in context of pathogenicity? Furthermore are they responsible for particular properties of EcN related to its probiotic potential, like inhibition of shiga toxin expression in EHEC strains [7], inactivation of (*stx*) phages or reduction of the invasion rate of *Salmonella typhimurium* into human epithelial cells [8].

To approach these questions we construct single, double and triple *sat*-deletion-mutants of EcN. These mutants will be tested for protease activity, for inhibition of shiga toxin expression, phage inactivation and *Salmonella typhimurium* invasion. The results of our investigation will help to understand the role of the *sat* genes in EcN.

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#### **21** Identification of the flagellin (FLIC)-REGIONS OF THE E. COLI STRAIN NISSLE 1917 RESPONSIBLE FOR THE INTERACTION WITH TLR5 AND MUCIN-2

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In the last 100 years *E. coli* Nissle 1917 (EcN) has been used in the medicine for the treatment of various gastrointestinal disorders. Although it is one of the most investigated probiotic bacteria, little is known about the mechanisms behind its probiotic nature. One property our lab is interested in is the interaction of FliC with mucin-2. It was shown that the mucus component gluconate is necessary for efficient FliC binding. However, neither domain D3 nor D2 of FliC are involved in interaction with mucin-2. The deletion of a 90-amino acids spanning portion of the N-terminal part of domain D1 resulted in lack of binding to mucin-2. Even deletion of just the 8 amino acids necessary for interaction with TLR5 abolished linkage to mucin-2. Chimeras consisting of FlaA (flagellin of *Campylobacter jejuni*) which were reconstituted with specific regions of *Salmonella enteritidis* (similar to the FliC of EcN) and internal FliC (EcN) deletion mutants will be used to determine TLR5 and mucin-2 interaction. This will be achieved by employing mucin-2 ELISAs and a pair of isogenic TLR5 positive and negative cell lines (HEK-Blue<sup>TM</sup> hTLR5 Cells and HEK-Blue<sup>TM</sup> Null1 Cells).

#### **22** How can external stimuli influence Genome diversification in Mouse Gut-Associated Bacteroides vulgatus?

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In previous work we have shown that *B. vulgatus* strain mpk harbors a large and diverse set of mobile genetic elements compared with other sequenced *Bacteroides* strains. We therefore proposed that they might be utilized for genome evolution. Because we found evidence of a number of different horizontal gene transfer events and a genome landscape that must have been extensively altered by different mobilization events. We suggested that the high genome plasticity and the introduced genome instabilities of *B. vulgatus* mpk arising from the various mobilization events might play an important role, not only in its adaptation to the challenging intestinal environment in general, but also in its ability to interact with the gut microbiota.

We conducted further experiments to prove that suggestion. Our strain was challenged with different stimuli under different conditions to find out which external factors are able to influence genome evolution. After that we sequenced the respective strains and compared them to the reference sequence and identified the variants. We want to find out which genes and gene functions were affected. Furthermore we seek to answer the question if the mobilization happens coincidently or if it follows a particular pattern, regarding variations in specific regions on the chromosome or in distinct gene categories.

#### **23** The IMPACT OF A 3-DIMENSIONAL PORCINE EXTRACELLULAR MATRIX ON TRANSCRIPTIONAL IMMUNE REGULATION BY BUTYRATE PRETREATED CACO2-CELLS AFTER INFLAMMATORY STIMULUS AND COMPARISON TO A 2D IN-VITRO-MODEL

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Gastrointestinal epithelial cells, which also function as signal transducers as part of immune responses, stand at the beginning of a complex regulatory network, which is modified by bacterial metabolites. Despite recent advances in identifying the impact of specific metabolites on single cell types, it is difficult to distinguish their individual contribution within the whole system. In particular, the role of G-proteincoupled metabolite sensing receptors (GPRs) has to be further investigated. In-vitro models allow the precise control of culture conditions, but have so far been almost entirely limited to cell monolayers grown on plastic surfaces. Therefore, these models do not take into account that cell function highly depends on cell differentiation which, in turn, can be strongly affected by the extracellular microenvironment. Conducting human in-vivo assays and obtaining human tissue samples is challenging, and mouse models do not always seem to appropriately mimic the human situation due to species-specific differences in the expression and function of certain GPRs. To overcome these limitations, we made use of a "humanized" intestinal 3D in-vitro model based on a 3-dimensional porcine extracellular matrix seeded with human adeno-carcinoma cells (CACO2). The expression of selected GPRs, solute carries and chemokines/cytokines was analyzed by qPCR in this intestinal 3D in-vitro model and compared to a conventional 2D CACO2 cell culture. First qPCR analyses revealed different, model-specific transcript levels of the butyrate sensing receptor GPR109A as well as differential expression of certain chemokines and cytokines (such as CCL-20, CXCL-8 and TNFα) after TNFα stimulation. Interestingly, these model-specific differences were even more pronounced when the inflammatory TNFα stimulus was combined with a 24h-pretreatment with 5 mM Sodium-Butyrate.

In the future, further experiments with the use of other metabolites or with co-cultures of enterocytes with other immune-modulating cells (like innate immune cells or fibroblasts), and comparison of the results to human in vivo data will be performed. This will clarify whether the applied intestinal 3D model can act as a reliable model to mimic in-vivo-processes in humans and could thereby offer new prospects to investigate the impact of bacterial metabolites on immune regulation and metabolism.

#### **24** ANALYSIS OF FECAL FATTY ACID PROFILES BY GAS CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY (GC-MS)

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The gut microbiota represents a complex ecosystem, its composition and diversity depends on various factors including diet, environment, health and disease. Our preliminary data strongly indicate that gut microbiota influence host lipid metabolism.

Here we present a rapid and sensitive GC-MS method for quantification of fecal fatty acid profiles. Fatty acids methylation was carried out by transesterification with acetyl-chloride and methanol. Fatty acid methyl esters (FAMEs) were separated with a short and polar cyanocolumn permitting separation of double bond isomers (including cis/trans isomer) as well as branched chain iso and anteiso-FAMEs. Additionally, hydroxyl-FAMEs were included to cover typically fatty acids derived from bacteria. The method was applied for human and mouse fecal samples and is currently validated.

This GC-MS method complements a powerful mass spectrometric tool box (including direct tandem mass spectrometric and liquid chromatography coupled methods) to study the influence of gut microbiota on host lipid metabolism as well as lipid profiles of the microbiome.

#### **25** INTESTINAL MICROBIOTA COUNTER-REGULATES INFLAMMATORY IMMUNE RESPONSES BY THE INDUCTION OF REGULATORY **B** CELLS

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The key feature of B cells is the regulation of the adaptive immune response by the production of antibodies, resulting in an optimal CD4<sup>+</sup> T-cell activation. Additionally, B cells modulate the innate immune system via presentation of antigens and the secretion of immune-modulating cytokines. Furthermore, a specific subset of B cells (Bregs) exhibit immunosuppressive functions and can also negatively regulate the immune response in mouse models of autoimmune diseases. Thereby, the intestinal microbiota plays a critical role for the induction of different B cell phenotypes either by direct or indirect interaction. In previous experiments we could demonstrate that the symbiotic gut commensal *Bacteroides vulgatus* mpk induces tolerant and tolerogenic bone marrow derived dendritic cells (BMDCs) with anti-inflammatory properties *in vitro* and *in vivo*. On the contrary, the pathobiontic *Escherichia coli* mpk induces mature BMDCs expressing high quantities of T cell activation markers and secreting pro-inflammatory cytokines.

In order to clarify the influence of the intestinal microbiota composition on B cell-mediated immune responses and the role of Bregs in supporting immune homoeostasis, we analyse the immune system activating capacities of this two completely sequenced gut commensal strains (*B. vulgatus* mpk and *E. coli* mpk).

On the one hand, we could already show that the stimulation of isolated naïve B cells with symbiotic *B. vulgatus* leads to a reduced B cell proliferation *in vitro* and a diverse differentiation of B cell subsets, particularly regarding the development of Bregs. This effect is depended on the specific antigen recognition via *pattern recognition receptors*, since it is abolished in Toll-like receptor deficient mice. On the other hand, the treatment of cultured B cells with *E. coli* leads not only to an increased production of pro-inflammatory proteins but also to an elevated level of anti-inflammatory and immune regulatory molecules.

## **26** INFLAMMASOME ACTIVATION BY E. COLI NISSLE 1917

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Inflammasomes are cytosolic multi-protein complexes which cause a pro-inflammatory immune response when activated. Different sensor proteins belonging to the NLR and AIM family respond to a variety of intra- and extracellular stimuli by forming inflammasome complexes which activate caspase-1, IL-1 $\beta$  and IL-18. A dysfunctional activation can lead to diseases such as inflammatory bowel disease. Our studies focus on the activation of the inflammasome by the probiotic Escherichia coli Nissle 1917 (EcN). In a mouse model of DSSinduced colitis we could show that a treatment with EcN not only protects the mice from colonic inflammation, it also lowers the secretion of the pro-inflammatory cytokine IL-1ß by colonic mucosal cells, a common marker for inflammasome activity. We suppose that EcN- type Flagellin (FliC), which makes up the filament of the bacterial flagella, is the protective factor responsible for this observation since it was shown to be structurally different from FliC of other bacterial species such as S. Typhimurium. Furthermore, in vitro experiments showed a lower IL-1β secretion by mouse TLR2<sup>-/-</sup>TLR4<sup>-/-</sup> BMDCs when stimulated with EcN compared to TLR2<sup>-</sup> <sup>/-</sup>TLR4<sup>-/-</sup> BMDCs stimulated with an EcN-mutant expressing FliC of the pathobiont *E.coli* mpk. This clearly shows an involvement of EcN-type FliC in decreased inflammasome activation and inflammatory response. FliC can either activate NLRP3 inflammasomes via TLR signalling or NAIP6/NLRC4 inflammasomes by intracellular binding. Future experiments will elucidate the sensor protein involved in activation by EcN-type FliC and how EcN can lower or prevent inflammation by influencing inflammasome activation.

## **27** PURITY MAKES THE DIFFERENCE – STRATEGY OF USING LPS IN GALLERIA MELLONELLA

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The lepidopteran greater wax moth *Galleria mellonella (G.m.)* is a new and promising *in vivo* model organism in the field of host-microbiota interaction. *Galleria mellonella* larvae are suitable for several questions in innate immunity research including local or systemic infection. Low costs, easy handling and no conflicts with animal protection law or ethic guidelines raise the community of scientific users according to increasing numbers of publication. This organism is an auspicious candidate to reduce scientific used amounts of rodents in infection biology and contributes to the 3R strategy (Reduction, Refinement, Replacement) of animal experiments.

Lipopolysaccharides (LPS) of Gram negative bacteria are one of the most potent components for the activation of the host innate immune system and therefore LPS recognition is crucial for the host organism to clear infections of invading bacterial pathogens. LPS is also a common used stimulator of *Galleria mellonella* immune system and often used as prior stimulus in survival studies.

In our experiments we demonstrate high differences in using "standard" and "ultrapure" LPS of *Escherichia coli* in *Galleria mellonella*. To investigate whether varied compositions of LPS including ultrapure and standard LPS (contaminated with TLR2 ligands i.e. peptidoglycans according to manufactures description) influence the activation of innate immune system we stimulated *G.m.* larvae via hemolymph injection. The immune status after injection was analysed via several assays. For instance significant differences in mRNA expression levels of six antimicrobial peptides four hours after injection were detectable. Further an injection of high doses of standard LPS for more than 4 days leads to significant reduces survival rates while ultrapure does not. Therefore experiments with ultrapure LPS mixed with TLR2 ligands (synthetic and biological origin) to generate lethality were performed.

The induction of immune processes leading to death are probable not inducible with an ultrapure ligand. Thus these data suggest that induction of lethality is a multicomponent event. Furthermore the application of LPS as prior stimulus in standard quality and the effect on following survival studies should be scrutinized.

#### **28** Identifying and sorting host compound foragers from the gut microbiota by heavy water-based activity labelling and raman microspectroscopy

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The secreted mucus layer that separates the mammalian intestinal epithelium from the lumen provides a habitat and serves as a nutrient source for a subset of gut bacteria. The ability to degrade and utilize mucin-derived glycans has been shown to confer a competitive advantage to many gut organisms in vivo, in particular when complex diet-derived compounds become scarce. Due to the complexity of mucin and the diversity of the O-glycan carbohydrate chains that it consists of, our knowledge about the molecular strategies employed by gut bacteria to utilize mucin glycans still remains incomplete. To study the capacity of the mouse colon community to forage on mucin and to metabolize monosaccharides originating from O-glycans, we used a stable isotope probing approach that employs heavy water (D<sub>2</sub>O)based activity labelling and Raman microspectroscopy. With this approach we could observe that a significant percentage of the microbial community was stimulated by the addition of each of the O-glycan monosaccharide constituents (i.e., sialic acid, fucose, N-acetylglucosamine, N-acetylgalactosamine and galactose), or by mucin itself. Stimulation of the community in response to the galactose amendment was the most prominent, in agreement with the observation that galactose is abundant in many dietderived compounds and therefore a broader set of organisms are expected to utilize it. By Raman-based cell sorting of active (D<sub>2</sub>O-labeled) cells with optical tweezers and subsequent multiple displacement amplification and whole genome sequencing, near-complete genomes of mouse gut microbes that can forage on N-acetylglucosamine were recovered. Genes predicted to encode the necessary enzymes for complete or nearly complete N-acetylglucosamine catabolism were identified in all of the cell-sorted genomes or on the genomes of the next closest relatives, including N-acetylglucosaminidases that allow these organisms to scavenge the N-acetylglucosamine monosaccharide from the mucin O-glycans. This approach successfully enabled the establishment of a link between an organism's function in the context of a complex microbial community and its genomic content, and will further help in dissecting the identities and functions of key-players in the mucin-associated niche.

## **29** IDENTIFICATION OF HUMAN GUT BACTERIA UTILIZING DIETARY SULFONATES

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The impact of diet on the gut microbiota composition received increasing attention in the last decades. A recent mouse study highlighted the fact that changes in the gut microbiota induced by a high-fat diet rich in saturated fatty acids may cause gut inflammation in colitis-prone IL-10-deficient mice. It was demonstrated that this high-fat diet resulted in a higher secretion of bile acids proportions conjugated with taurine. The colitogenic bacterium Bilophila wadsworthia is capable of utilizing the sulfite  $(SO_3^{2})$  moiety of taurine as electron acceptor resulting in sulfide  $(S^{2})$  formation, leading to an enhanced growth this bacterium. Interestingly, diet may also be a major source of sulfonated compounds including taurine and sulfoquinovosyl diacylglycerol (SQDG). The sulfolipid SQDG is present in chloroplasts of leafy vegetables, e.g. in spinach or salad. Post-ingestion, SQDG is metabolized via sulfoquinovose (SQ), but the exact mechanism of degradation is unknown. In a previous study it has been shown that SQ is degraded by Escherichia coli to form the final product 2,3-dihydroxypropane-1-sulfonate (DHPS). Based on this, we hypothesize that dietary intake of sulfonated compounds and its sulfonated degradation products may serve as a SO<sub>3</sub><sup>2</sup>-source for *B. wadsworthia* and related organism. Furthermore, that the availability of sulfonated compounds would stimulate the growth of colitogenic bacteria such as B. wadsworthia.

To investigate the capacity of the human gut microbiota converting taurine and SQ, fecal slurries were incubated with the individual sulfonates under strict anoxic conditions. Mixed bacterial cultures utilizing the  $SO_3^{2^-}$  moiety of taurine were then identified by measuring the formation of S<sup>2-</sup> by the methylene blue method.

Results show that bacteria of the human gut microbiota may convert taurine (20 mM) to S<sup>2-</sup> during the enrichment in a gas flushed growth medium (H<sub>2</sub>/CO<sub>2</sub> or N<sub>2</sub>/CO<sub>2</sub>, 80/20 %) containing formate or lactate (40 mM each) as electron donors. SQ may be utilized by human gut bacteria in an enrichment mineral salt medium containing SQ (4 mM) flushed with a gas mixture of N<sub>2</sub>/CO<sub>2</sub> (80/20 %). Formation of DHPS and other degradation products, as well as the characteristics of isolated gut bacteria and their immunoregulatory properties, will be further analyzed in ongoing studies.

#### **30** INFLUENCE OF B4GALNT2 EXPRESSION ON ANTIBIOTIC RESISTANCE OF COMMENSAL GUT MICROBIOTA

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Glycans on host mucosal surfaces play important roles in host-microbe interactions. We previously demonstrated that the lack of intestinal expression of the glycosyltransferase gene B4gaInt2 results in reduced inflammation in a murine model of Salmonella-induced colitis. Importantly, the mouse model of colitis we employed requires antibiotic treatment to break the colonization resistance to S. Typhimurium, which is mediated by host commensal gut microbiota. Interestingly, the effect of BgaInt2-expression on the severity of inflammation in our experiment negatively correlated with the extent of change in microbiota composition before and after infection with S. Typhimurium, and transfer of feces into previously germ free mice demonstrated that the effect is dependent on the *B4gaInt2* genotype-specific microbiota. In follow-up experiments we here subjected the same fecal microbiota samples to shotgun functional metagenomic sequencing, whereby intriguingly, animals deficient for B4gaInt2 expression in the gut show a significantly higher abundance of efflux pump genes. This suggests that differences in S. Typhimurium susceptibility associated with the presence/absence of B4gaInt2 in the gut may be mediated by an indirect effect on colonization resistance, whereby residual colonization resistance of the commensal microbiota is facilitated by a higher standing level of efflux pump abundance. Although preliminary, this would present a case in which a single host genetic factor could significantly influence the outcome of antibiotic treatment on the endogenous microbiota. We here present an experimental approach to examine the effects of B4gaInt2-expression on microbiota composition and its impact on the efficacy of different classes of antibiotics.

## **31** ATG16L1 DEFICIENCY IN MYELOID CELLS TRIGGERS MICROBIOTA-DEPENDENT AUTOINFLAMMATION BY UNCONTROLLED NON-CANONICAL NF-KB SIGNALING

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Defects in the autophagic machinery are associated with a poor outcome in sepsis and chronic inflammatory disorders in humans. The molecular mechanisms by which defective autophagy modulates immune responses are poorly understood. In mice with specific deletion of Atg16l1 in myeloid cells (Atg16l1LysM) we observed an IL1R-independent, spontaneous autoinflammatory syndrome characterized by hepatosplenomegaly and hyper-activated CD11b+ myeloid cells and expansion of myeloid-derived suppressor cells. Atg16l1LysM mice were highly sensitive to endotoxin shock in vivo. Atg16l1-deficient bone marrow-derived macrophages (BMDMs) showed an earlier, increased and sustained activation and phosphorylation of NF-kB after LPS stimulation. We show that non-canonical NF-kB activation via p62SQSTM1 and the atypical protein kinase C PKCZ is required for the hyper-inflammatory cellular phenotype. Antibiotic treatment, but not longterm IL1R blockade attenuated the immune phenotype in vivo, indicating that the spontaneous inflammation is driven, at least partially, by the commensal microbiota. We here demonstrate that loss of ATG16L1 in myeloid cells impairs temporal control of TLR4 signaling via dysregulated adaptophagy. The study provides essential insights into the role of autophagy in controlling inflammation and suggests the autophagic removal of p62/PKCζ and its impact on non-canonical NF-kB signaling as a potential therapeutic target for inflammatory diseases.

#### **32** TRACING HEAVY WATER IN METAPROTEOMICS ENABLES GLOBAL DETECTION OF METABOLIC ACTIVITY

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In the last decade, stable isotope probing (SIP) rised in popularity as an efficient tool to identify active organisms in complex consortia. Commonly, a substrate or nutrient is labeled with the heavy stable isotope of 13C or 15N to investigate the substrate specific or nutrient atomic flow, respectively, within a microbial community. However, these model compounds lead to detection of only those bacteria that can metabolize this specific substrate, but in all consortia that grow on a mixture of substrates this selection is too limited for detecting all metabolic active organisms. On top of that the addition of a surplus of a labelled substrate can potentially affect the biological balance within a consortium. In order to overcome thes shortcomings of subtrate based stable isotope probing we developed labeling with heavy water, either as D<sub>2</sub>O or as H<sub>2</sub>(18)O for metaprotemics. This approach can serve as a substrate independent strategy to capture the entirety of microbial activity of all ongoing processes. The coupling to metaproteomics enables the quantitative determination of metabolic phyla in microbial communities. Heavy water can be incorporated into the biomass by different biochemical pathways such as the TCA cycle, where its central intermediates,  $\alpha$ -ketoglutarate and oxaloacetate, are also amino acid precursors, or the β-oxidation of fatty acids. The amount of possible incorporation depends on the amount of supplied heavy water, the labeling type, either deuterium or 18-oxygen, and on other hydrogen or oxygen sources besides water in the medium.

In this study, we demonstrate the concentration-dependent incorporation of deuterium and 18-oxygen from heavy water in the pure culture E.coli K12 and a defined microbial community enriched from a healthy human feces sample. In summary, metaproteomic based stable isotope probing by heavy water provides the time- and concentration-dependent baseline of the metabolic activity of many genera in moderate to complex microbial communities.

#### **33** ROLE OF TOLL-LIKE RECEPTOR 11 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 intracellular Toll-like receptors like TLR3, TLR7, TLR9, TLR11, TLR12 and TLR13 thereby initiating an innate immune response, both in humans and mice.

In order to investigate the role of TLR11 in Salmonella infection in vivo, tlr11<sup>-/-</sup> mice were infected with S. Typhimurium using streptomycin pretreated mice model. Higher colonization of bacteria was observed in case of *tlr11<sup>-/-</sup> mice* as compared to the wild type mice in spleen in an acute infection indicating systemic dissemination of the bacteria. 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. The expression levels of these two genes were studied by qRT-PCR. Adhesion and invasion of S. Typhimurium was analyzed in these cell lines using Gentamicin killing assay. The infection studies reveal variation in adhesion and invasion pattern of Salmonella in these cell lines. Similar infection assays were performed on immortalized bone marrow derived macrophages (BMDM) from wild type, *tlr9<sup>-/-</sup>*, *tlr7<sup>-/-</sup>* and 3d mice. 3d mice contain a single point mutation (H412R) in the UNC93B1 altering it's binding to TLRs. Through these set of experiments it was found that Salmonella shows a 10 fold higher intracellular replication in BMDM derived from 3d mice, 24 hours post infection. Also this effect is independent of the expression of flagellin. Currently, H&E sections are being analyzed for differences in the histology of tissue sections obtained from *tlr11<sup>-/-</sup> mice* as compared to the wild type mice. Our data shows that Toll-like Receptor 11 and UNC93B1 mediated signalling pathways are important in case of Salmonella infections.

#### **34** IMPACT OF BIFIDOBACTERIA-SUPPLEMENTED FORMULA AND BREAST FEEDING ON INFANT FECAL METABOLITE PROFILE

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Probiotic intervention has shown to be effective in preventing neonatal diseases like infantile colic. However, the impact of early life intervention with probiotics on intestinal metabolites and bacterial communities in healthy children is unclear. This study investigates the effect of bifidobacteria-supplemented formula on the infant gut microbiota assembly and fecal metabolome compared to non-supplemented formula and breast milk within the first year of life. Therefore, a randomized, double-blinded, placebo-controlled intervention trial with 106 healthy neonates receiving infant formula with or without bifidobacteria (B. bifidum, B. breve, B. infantis, B. longum) or breast milk was designed and fecal samples collected over a period of two years were analyzed by high-resolution mass spectrometry. Infant formula significantly altered the fecal metabolite profile at early age. Particularly gut microbiota related metabolite classes like bile acids and short chain fatty acids were affected. Formula feeding resulted in higher levels of glycine conjugated bile acids. Furthermore, increased levels of lactic and pyruvic acid were found in breast-fed infants, whereas propionic, butyric, valeric and isovaleric acid were higher in the formula-fed infants, independent of probiotic intervention. Correlation between metabolites and 16S rRNA amplicon sequencing data revealed several positive correlations contributing to feeding-specific separation, for example between lactic acid, as well as indole lactic acid and the lactic acid producing species Bifidobacterium, Streptococcus and Lactobacillus. The differences between breast- and formula-fed infants converged over time, especially seen at the age of 12 and 24 months. We found several metabolites which were altered in early life through the addition of bifidobacteria to infant formula, however these changes did not sustain after the intervention.

#### **35** CASPASE-1 ACTIVATION IN CX3CR1<sup>+</sup> CELLS IN LIMITS TRANSFER OF SALMONELLA TO SYSTEMIC SITES AFTER ORAL INFECTION

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The microbiota provides colonization resistance against enteric pathogens and disturbances of normal microbiota function, e.g. through antibiotics, increases susceptibility to infection. Specifically, oral *Salmonella enterica* serovar Typhimurium (*S*. Tm.) infection after streptomycin-induced reduction of colonization resistance causes gastroenteritis and bacterial dissemination to systemic sites. Diverse immune pathways contribute to an immune defence against *S*. Tm., including the inflammasome, a platform that controls Caspase-1 activity and thereby pyroptosis and secretion of several proinflammatory cytokines. Notably, Caspase-1 is expressed in many cell types, but the relative contribution of Caspase-1 in these cells to different phases of Salmonella infection and immune defence is not known.

Here, we characterized oral *S*. Tm. infection in  $Casp1^{-/-}$  mice, lacking Caspase-1 in all body cells, as well as in mice with cell-type specific deletions of Caspase-1, which allow assessing the contribution of caspase-1 activity in different immune cells *in vivo*. We specifically studied a *S*. Tm. strain ( $\Delta spi1$ ) unable to cause severe intestinal inflammation to focus on the role of Caspase-1 during systemic dissemination.

We identified that compared with isobiotic WT mice recently generated  $Casp1^{-/-}$  mice show a higher Salmonella load in the cecum, liver and MLNs, but not in the small intestine or spleen after oral infection. Notably, no difference in weight loss was observed after infection. Strikingly, mice lacking Caspase-1 in Cx3cr1-expressing cells ( $Casp1^{\Delta CX3CR1}$ ) displayed higher *S*. Tm loads in the liver and mesenteric lymph nodes (mLNs) similar to Casp1-/- mice, but in contrast not in the cecum. This suggests that Cx3cr1-expressing cells, which predominantly include myeloid cells, contribute to spreading of *S*. Tm, which is in WT mice counteracted by Caspase-1.

In summary, using experimental models with defined microbiota composition and genetics we demonstrated that  $Casp1^{-/-}$  and  $Casp1^{\Delta CX3CR1}$  display a similarly enhanced spread of Salmonella into gut-draining tissues such as mLNs and liver. Differences in systemic spread between these mouse lines highlight that other so far unknown Caspase-1 expressing cell types are also involved in the immune mediated restriction of systemic Salmonella infection.
# **36 DUOX2 -** REGULATOR OF INTESTINAL HOST-MICROBIOTA INTERACTIONS AND PHYSIOLOGY

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The intestinal microbiota contributes to host physiology but also poses an infection danger. Reactive oxygen species (ROS) have antibiotic properties and therefore eukaryotes employ ROS as protective component of innate immunity. NADPH oxidases are the main ROS producing enzymes and within the intestine Duox2 its most highly expressed member. Intestinal epithelial cells (IEC) express DUOX2 and pathogenic infection but also the normal microbiota elevate Duox2 expression. To investigate the role of DUOX2 for physiology of the metaorganism, we generated DUOX2-∆IEC mice, which lack DUOX2 specifically in IECs. Under basal unchallenged conditions DUOX2-AIEC mice did not display any obvious phenotype. However, loss of DUOX2 in IECs altered composition of the mucosal microbiota, for example enriching for the anti-inflammatory commensal Akkermansia muciniphila or depleting several Bacteroidetes taxa associated with energy extraction. Surprisingly, DUOX2-AIEC mice did not display an altered susceptibility towards inflammatory challenge using the acute DSS colitis model. However, under dietary challenge using a high-fat-highsugar-feeding regime DUOX2-AIEC mice showed a worsened susceptibility towards diet induced obesity including increased weight gain and an impaired glucose metabolism. We currently extend our analyses by testing whether these differential physiological responses depend on the altered microbiota of DUOX2-AIEC mice. Our data highlight the importance of mucosal ROS for priming beneficial host-microbiota interactions and host physiology.

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Keywords: ROS / DUOX2 / microbiota / inflammation / metabolism

# **37** COMPARATIVE TRANSCRIPTOME OF E. COLI NISSLE 1917 IN VARIOUS CONDITIONS

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**Background:** During First World War, Professor Alfred Nissle isolated the probiotic strain *E. coli* Nissle 1917 (EcN) from the feces of a soldier who did not develop infectious diarrhea, which was a widespread disease. Though EcN is a successful probiotic for the past 100 years, the mechanisms behind its probiotic nature are poorly understood. Previous studies have shown that EcN can reduce the Shiga toxin (Stx) level and growth of enterohemorrhagic *E. coli* (EHEC) strains in coculture. We believe *E. coli* is very economical and factors that affect pathogenic bacteria are produced only when necessary. Hence, we aim to identify some of these factors responsible for the inhibitory effect of EcN using transcriptome analysis.

**Methods:** Transcriptome of EcN in single culture was compared at different culturing conditions such as fermenter and LB-overnight grown. Due to the efficient antagonistic activity of EcN, we are also interested in analyzing the transcriptome of EcN in coculture with the EHEC strain EDL933 and the nonpathogenic *E. coli* strain MG1655 in comparison with EcN monoculture. The coculture experiments were performed in a transwell system. To identify the time, at which EcN starts to influence the Stx level of EDL933, samples were taken, at various time points of coincubation for Stx determination by ELISA. For the transcriptome analysis, RNA was isolated from EcN incubated with just medium or together with either EDL933 or MG1655 after 3 h, 5 h, 7 h and 8 h of coincubation and sent for sequencing and subsequent transcriptome analysis.

**Results:** Analysis of changes in gene expression of the fermenter culture revealed a strong upregulation of the different iron uptake systems and curli fimbrial determinant when compared to LB-overnight culture. The time point assay performed in transwell system showed a 50% reduction in Stx level already after 5 hours of coincubation. Preliminary data analysis suggests that several genes were differentially expressed in EcN incubated with EDL933 and interestingly these genes were regulated differently only in the presence of EDL933 and not in presence of MG1655.

**Conclusion:** Transcriptome analysis of EcN in fermenter culture identified upregulated genes which might be important for its probiotic action in the host. And, it was clearly observed that, even if EcN is separated by a membrane not permeable for bacteria, it efficiently reduces the Stx level of EDL933 strain. Further, transcriptome analysis is in progress and the impact of the differentially regulated genes will be discussed.

# **38** WEAK AGONISTIC LIPOPOLYSACCHARIDE OF A SYMBIOTIC COMMENSAL RESTORES INTESTINAL IMMUNE HOMEOSTASIS

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*Bacteroides vulgatus* mpk is a symbiotic gut commensal from the mouse intestine. It is known that administration of this bacterium protects from colonic inflammation in various mouse models for experimental colitis. However, *B. vulgatus* mpk is even able to restore gut homeostasis in an already inflamed intestine. This effect is mainly mediated by the unique structure of its lipopolysaccharide (LPS) which disclosed a hypoacylated and monophosphorylated lipid A species and a unique galactofuranose containing core oligosaccharide structure which does not result in an antagonistic, but rather in a weak agonistic activity. These weak agonistic properties are responsible for its active inflammation silencing characteristics and convert intestinal dendritic cells into a tolerant and tolerogenic phenotype that mediates the intestinal homeostasis-restoring properties of *B. vulgatus* mpk LPS. Hence, this specific LPS might be a novel and effective therapeutic agent for the treatment of intestinal inflammatory disorders like Inflammatory Bowel Diseases (IBD). Thus we promote symbiotic *B. vulgatus* mpk LPS as a potential alternative therapeutic approach for the treatment of IBD. Furthermore, insights gained from the structural analysis of *B. vulgatus* mpk LPS might help to chemically design novel potent inflammation-silencing drugs.

# **39** DISTINCT MICROBIAL COMMUNITIES TRIGGER COLITIS DEVELOPMENT VIA INNATE OR ADAPTIVE IMMUNE CELLS

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Inflammatory bowel disease is a group of heterogeneous diseases characterized by chronic and relapsing mucosal inflammation. Alterations in microbiota composition have been proposed to contribute to disease development, but no uniform signatures have been identified yet (*Gevers et al., Cell Host Microbe 2014*).

Here, we compare the ability of a diverse set of microbial communities to exacerbate intestinal inflammation after chemical damage to the intestinal barrier. Besides the dysbiotic community (DysM) from *NIrp6<sup>-/-</sup>* mice (*Elinav et al, Cell 2011*), interestingly also certain but not all specific pathogen free communities demonstrated the abilities to cause severe intestinal inflammation in immunocompetent mice. While the colitogenic communities displayed differences in the relative abundance of specific bacterial families, they all shared similarly decreased ratio of Firmicutes to Bacteroides compared to communities causing only mild disease. Strikingly, mice displayed different inflammatory responses depending on their intestinal microbiota composition, either characterized by infiltration of neutrophils or the presence of proinflammatory CD4+ T cells. By utilizing gene-deficient mice and antibody-mediated depletion of T cell subsets we demonstrated that the DysM but not another colitogenic community depends on CD4+ T cells to exacerbate DSS colitis severity.

Our data identifies that specific interactions between colitogenic communities and host immune pathways drive colitis development via distinct mechanisms. This highlights the potential of screening the microbiota to stratify patients and to develop microbiota-centered interventions for personalized IBD therapy.

# **40** SUGAR ROCKS! THE IMPORTANCE OF TERMINAL GLYCANS FOR ENTERIC INFECTIONS

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Glycans play important roles in host-microbe interactions. Host-derived glycans expressed on and secreted from mucosal surfaces can shape the intestinal microbiota composition. Interestingly, glycans can be targeted as host receptors by invading pathogens, but can also be presented as defensive decoys by the host to prevent infection. Here, we examined Salmonella enterica serovar Typhimurium adhesion to and invasion into the CHO Lec1 cell line usina gentamicin killing assav. In Lec1 cells а mutation in the Nacetylglucosaminyltransferase I (GnT1) gene leads to synthesis of N-glycans exclusively terminated by mannose residues. The infection studies revealed that S. Typhimurium adhered and invaded Lec1 cells more efficiently than wildtype CHO cells. To determine whether the exposed mannose residues are responsible for the better adherence of S. Typhimurium to Lec1 cells, competition experiments with exogenous mannose were performed. The results showed that the attachment of S. Typhimurium to Lec1 was inhibited. Furthermore, S. Typhimurium FimH mutant, which lacks the adhesive subunit of type 1 fimbriae mediating mannose-sensitive binding to host cells, showed lower adhesion in Lec1 cells in comparison to S. Typhimurium wildtype. In addition, other Salmonella serovars such as S. Paratyphi A and S. Enteritidis as well as C. rodentium also adhered better to Lec1 cells compared to wildtype CHO cells. Thus, our data demonstrate that bacteria better adhered into Lec1 cells because of mannose-terminating accumulation in the Lec1 cell surface.

# **41** POSTNATAL DEVELOPMENT OF THE MURINE GUT MICROBIOTA

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The enteric microbiota represents a dense and highly dynamic microbial community consisting mainly of bacteria but also viruses, phages and archaea. It exerts a major influence on many aspects of the host's organism including structural and functional aspects of the immune system, tissue maturation and remodeling as well as metabolism. Emerging epidemiological and experimental evidence suggests that alterations of the enteric microbiota are linked to highly prevalent human diseases such as the susceptibility to infection, as well as several non-communicable inflammatory diseases. In the adult intestine, the microbiota displays a dense bacterial community with relatively stable composition. In contrast, neonates are born essentially sterile with the establishment of the microbiota starting immediately after birth. Since the most dramatic changes in the density and composition of the microbiota are observed during the postnatal period and early childhood, this developmental period might critically influence the ultimate composition of the enteric microbiota and the life-long maintenance of host-microbial homeostasis.

Therefore, we conducted a systematic analysis of the time kinetic of murine bacterial colonization during the immediate postnatal period (day 1, 3, 7, 14, 21 and 28 after birth). Particular attention was paid to the longitudinal course of the colonization of both the small and large intestine in mice. Our analysis included 16S rDNA V4 sequencing, the use of bacterial group specific PCR primers at various time points and anatomical sites after birth.

We observed a rapid colonization of the neonate intestine, decrease in richness (choa1) early after birth, and increase in richness combined with a major shift in composition during weaning. The post-weaning microbiota was closely related to the maternal adult microbiota. The microbiota composition was found to be highly individual directly after birth, but shifted towards a more homogenous pattern within one week. Small intestine and colon harbored a comparable microbiota composition during the pre-weaning period. Our results are consistent with the existence of selective host mechanisms that shape the initial, largely environment-dependent colonization pattern and ensure the development of a beneficial mature microbiota composition.

# **42** BILE ACID METABOLISM IN METFORMIN TREATED DIABETIC MICE

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Bile acids (BAs) are major components of bile that are synthesized from cholesterol in the liver and an important role in the digestion of dietary lipids. They are antimicrobial and have different signaling functions for example in their own regulation of biosynthesis. Moreover, they are key metabolites of co-microbial metabolism and undergo several modifications by bacterial metabolism such as deconjugation, dehydroxylation and epimerization, resulting in secondary BAs. Studies have already shown that metformin changes fecal BA content by decreasing BA reabsorption in the ileum (1). Therefore, we attempt to study the effect of metformin on BA metabolism. Diabetic mice were treated with metformin (300mg/kg body weight) in a single or sub-chronic manner for 1 day (1D) or 14 days (14D), respectively. Metformin (1D and 14D) reduced significantly blood glucose in diabetic mice as already shown by Neschen et al. (2). Metformin had a significant effect on cecal BA profiles, especially when analyzed in acute treated mice. We observed a threefold increase of total BA amount in treated mice. In contrast, 14D treated mice had a reduced total BA pool, which was independent of metformin treatment, compared to healthy controls. In detail, metformin increased significantly primary BAs such as cholic acid and aTMCA and reduced allocholic acid in single treated diabetic mice, when corrected for total BA amount. In sub-chronic group, the effect was less prominent, and BA profile was similar to diabetic control mice, except  $\beta$ MCA which was increased in treated mice. The difference in bile acid profiles is likely due to different sampling times of 1D and 14D metformin treated mice. Additionally, healthy non-diabetic mice have increased concentration of secondary bile acids including  $\omega$ MCA and  $\omega$ TMCA.

<sup>(1)</sup> Scarpello, J. H., E. Hodgson and H. C. Howlett (1998). "Effect of metformin on bile salt circulation and intestinal motility in type 2 diabetes mellitus." Diabet Med 15(8): 651-656.

<sup>(2)</sup> Neschen, S., M. Scheerer, A. Seelig, P. Huypens, J. Schultheiss, M. Wu, W. Wurst, B. Rathkolb, K. Suhre, E. Wolf, J. Beckers and M. Hrabe de Angelis (2015). "Metformin supports the antidiabetic effect of a sodium glucose cotransporter 2 inhibitor by suppressing endogenous glucose production in diabetic mice." Diabetes 64(1): 284-290.

### **43** RAPID GENETIC DIVERSIFICATION OF BACTEROIDES THETAIOTAOMICRON IN THE MURINE GUT

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The human genome encodes the ability to degrade a limited diversity of the many polysaccharides present in a normal diet. Members of the gut microbiota therefore play a critical role in breaking down these recalcitrant compounds to provide additional energy for the body. Members of the genus Bacteroides, one of the most abundant bacterial taxa in the gut, encode a large repertoire of polysaccharide utilization genes and therefore are key to the digestion of complex dietary compounds. It is still unclear, however, how members of the microbiota adapt to successfully colonize and occupy niches in the gut. In this study, we investigated the role of rapid genetic diversification in Bacteroides thetaiotaomicron VPI-5482 colonizing the germ-free murine intestine over a four week period. Shotgun genomic resequencing was performed at regular intervals on fecal pellets to evaluate the extent of genomic diversification during this period. Analysis of genetic polymorphisms revealed extensive accumulation of single nucleotide polymorphisms as well as structural variations. In addition, functional classification of polymorphisms, showed an overrepresentation of certain COG categories. Experimental evolution is therefore a powerful approach to identify novel colonization factors and to unravel the interplay of ecology and evolution in the assembly and function of the gut microbiota.

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