12th Seeon Conference

Microbiota, Probiotics and Host

Mikrobiota, Probiotics und Wirt

JUNE 28TH- 30TH, 2019

CONFERENCE CENTER

MONASTERY SEEON / CHIEMSEE

For more information:

www.seeon-conference.de

June 28th, 2019

Dear Participant,

We warmly welcome you at Seeon Monastery for the 12th Seeon Conference "Microbiota, Probiotics and Host" 2019.

This meeting is annually organized by the German Society of Hygiene and Microbiology **(DGHM) section "Microbiota, Probiotics and Host"** and co-funded by **the Priority Program SPP1656** of the German Research Foundation (DFG).

Since the first event in 2008, the "Seeon Conference" has become a forum to integrate various disciplines in basic and clinical sciences unified by the aim to understand the human microbiome. The past activities have made an important contribution to the formation of the **DFG Priority Programme "MICROBIOTA – a Microbial Ecosystem at the Edge between Immune Homeostasis and Inflammation"** (SPP 1656) which funded over 30 research groups from 2013 until now.

Microbiome research in Germany gained ever more momentum: established in 2015, the **Collaborative Research Center CRC1182 "Metaorganisms"** in Kiel, northern Germany, studies how resident microbes influence fitness of their plant and animal hosts and ultimately form a metaorganism. In 2018, the Tübingen **Cluster of Excellence CMFI - Controlling Microbes to Fight Infections** was granted joining researchers from the fields of molecular, bioinformatics and clinical disciplines to find new targeted agents which will have a positive effect on the microbiome. The most recent research consortium is **CRC1371 "Microbiome Signatures"** (TU Munich), which aims to understand the functional relevance of microbiome signatures and to determine their precise contribution in a disease-specific manner.

Starting in 2018, this year already the **2nd Summer School on "Microbiome in Health and Disease"** took place just before this conference. We aim to establish this Summer School a permanent institution in the future to train and promote young scientists across various disciplines, including gastroenterology, nutritional medicine, immunology, infection research, microbial ecology and computational biology in the area of basic and applied microbiome research.

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

Prof. Bärbel Stecher-Letsch and Prof. Thomas Clavel

Prof. Dr. Bärbel Stecher-Letsch LMU Munich Max von Pettenkofer Insititute Pettenkoferstrasse 9a 80336 München Tel.: +49-(0)89-2180-72948 stecher@mvp.uni-muenchen.de	Prof. Dr. Thomas Clavel Institute of Medical Microbiology University Hospital of RWTH Aachen Pauwelsstraße 30 52074 Aachen Tel.: +49-(0)241-80-85523 tclavel@ukaachen.de

SPONSORS

Many thanks to our sponsors!
Our meeting wouldn´t be possible without them:

Ardeypharm GmbH

Yakult Deutschland GmbH

SymbioPharm GmbH

Laves Arzneimittel GmbH

PROGRAM Friday, June 28th

15^{00} - 17^{00} Registration
17^{00} - 17^{15} Welcoming: Bärbel Stecher-Letsch, LMU Munich, Max von Pettenkofer Institute

---

17^{15} – 18^{00} Keynote Lecture - **Francois Leulier** (Instit. de Génomique Fonctionnelle de Lyon, Université de Lyon, France):
Host-Microbiota Mutualism upon Chronic Undernutrition: Lessons from Gnotobiotic Animal Models

---

18^{15} Dinner

MICROBIOME, INFECTION AND ANTIMICROBIALS

19^{30}– 21^{00} Chair: Tom Clavel, Uniklinik RWTH Aachen

Ehrhardt Katrin, Institute of Medical Microbiology and Hospital Epidemiology and German Center for Infection Research (DZIF), Partner Site Hannover, Hannover Medical School, Hannover, Germany
Chronic Salmonella Typhimurium Infection Induces Matrix Metalloproteinase 10 Expression which Dampens the Host Inflammatory Response

Klimek Hanna, Section of Microbial Genome Plasticity, Institute of Hygiene, University of Münster, Münster, Germany
Communication at the Epithelial Interface: The Transcriptional Response of Epithelial Cells to Probiotic and Extraintestinal Pathogenic Escherichia Coli

Krüger Wibke, Microbial Immunology, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena, Germany
Gut Microbiota-mediated Colonisation Resistance Against Candida Albicans

Maier Lisa, Interfaculty Institute of Microbiology & Infection Medicine, University of Tübingen, Tübingen, Germany
Determining the Target Spectrum of Antibiotics on Human Gut Commensals

Wagner Stefanie, Institute of Molecular Pathogenesis, Federal Research Institute for Animal Health, Jena, Germany
Effects of a New Antiinfective Substance on the Intestinal Microbiome of Pigs

Zhou Mangge, Experimental Immunology, Helmholtz Centre for Infection Research, Braunschweig, Germany
Long-lasting Consequences of Neonatal Infections on the Intestinal Immune System

PROGRAM Saturday, June 29th

08^{30} – 09^{15} Keynote Lecture - **Amanda Ramer-Tait** (Food Science and Technology, University of Nebraska-Lincoln, USA):
Microbial Control of Metabolic Health

09^{15} - 09^{45} Coffee Break / **Poster at the first glance**

GNOTOBIOTIC MODELS

09^{45} – 11^{15} Chair: Bärbel Stecher-Letsch, LMU Munich, Max von Pettenkofer Institute

Amend Lena, Helmholtz Centre for Infection Research, Braunschweig, Germany
The Ecological Niche of Prevotella spp. in Mouse Intestine

Bolsega Silvia, Institute for Laboratory Animal Science and Central Animal Facility, Hannover Medical School, Hannover, Germany
Colonization of Segmented Filamentous Bacteria Depends on the Intestinal Microbiota Composition

Budzinski Lisa, Department of Microbiota & Inflammation, German Rheumatism Research Center (DRFZ), a Leibniz Institute, Berlin, Germany
Multi-parameter Flow Cytometry of Human Gut Microbiota

Fokt Hanna, Max Planck Institute for Evolutionary Biology, Plön, Germany; Institute for Experimental Medicine, Kiel University, Kiel, Germany
The Evolution of the Genus Bacteroides in the House Mouse Species Complex

Harris Danielle, Zoological Institute, Kiel University, Kiel, Germany; Max Planck Institute for Evolutionary Biology, Plön, Germany
Microbiota and Behaviour: From Complex Extract to Bioactive Molecule

Streidl Theresa, Functional Microbiome Resarch Group, Institute of Medical Microbiology, RWTH University Hospital, Aachen, Germany
A New Gnotobiotic Mouse Model to Study the Impact of Secondary Bile Acids Production on Host Physiology

---

11^{15} – 12^{00} Keynote Lecture - **Emma Slack** (Food Immunology, ETH Zurich, Switzerland):
Using Vaccines to Manipulate Intestinal Bacteria

12^{00} - 13^{45} Lunch

MICROBIOME, IMMUNOREGULATION AND DISEASE MODELS

13^{45} – 15^{15} Chair: Till Strowig, Helmholtz-Zentrum f. Infektionsforschung, Brunswick

Baumeister Theresa, Innere Medizin II, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany
Farnesoid-X-Receptor Expression Has a Protective Function and Emerges as Potential Target to Prevent Progression from Barrett Esophagus to Adenocarcinoma

Fischer Florence, Institute of Medical Microbiology, University Marburg, Marburg, Germany
The Impact of Dietary Fibre on Intestinal Microbiota and Homeostasis

Kreft Luisa, Center of Allergy and Environment (ZAUM), Helmholtz Center Munich and Technical University of Munich, Munich, Germany
Revealing a Microbial Impact on Intestinal Immune Tolerance by Using Gnotobiotic Mice

Reitmeier Sandra, Institute for Food & Health (ZIEL), Technical University of Munich, Freising, Germany
Arrhythmic Microbiota Improves Diagnostic Profiling of Type-2-Diabetes in a Prospective Cohort

Stein-Thoeringer Christoph, Innere Medizin II, Klinikum rechts der Isar, Technical University Munich, Munich, Germany
Lactose Drives Enterococcus Expansion to Promote Alloreactive T-Cell Mediated Intestinal Inflammation

Wylensek David, Functional Microbiome Research Group, RWTH University Hospital, Aachen, Germany
Causative Role of Secondary Bile Acid-Producing Gut Bacteria in Colorectal Cancer

---

15^{15} – 15^{45} Coffee Break

15^{45} – 16^{30} Keynote Lecture - **Nicola Segata** (Comput. Metagenomics Lab, CIBIO - University of Trento, Italy):
Toward Uncovering the Hidden Diversity of the Human Microbiome

---

16^{30} – 17^{30} SPP 1656 Member Meeting
17^{30} – 18^{00} DGHM Fachgruppenmeeting 05

17^{30} – 19^{00} Dinner

19^{00} – 21^{30} **Poster Slam** (2 minutes / 2 slides) **and Poster Discussion**

PROGRAM Sunday, June 30th

08^{30} – 09^{15} Keynote Lecture - **Frank Maixner** (Institut für Mumienforschung, Eurac Research, Bozen, Italy):
Metagenomic Analysis of Mummified Human Remains

09^{15} – 09^{45} Coffee Break

09^{45} – 10^{00} **Poster Award**

OMICS ANALYSIS OF THE MICROBIOME

10^{00} – 11^{30} Chair: Guntram Grassl, Hannover Medical School

Belheouane Meriem, Institute for Experimental Medicine, Kiel University and Max Planck Institute for Evolutionary Biology, Plön, Germany
The Microbiome as a Prognostic Indicator in Anorexia Nervosa Patients – Preliminary Results of a Longitudinal Study

Burkhardt Wiebke, Department of Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Germany
Dietary Sulfonates Do Not Promote Bacteria-Induced Gut Inflammation

Ertl Verena, Institute of Clinical Chemistry and Laboratory Medicine, University Hospital of Regensburg, Regensburg, Germany
Development and Application of a High Resolution Mass Spectrometry Method to Identify and Quantify Faecal Lipid Species

Fricker Alena, Department of Microbiome Research and Applied Bioinformatics, University Hohenheim, Stuttgart, Germany
High/Low Sterol Conversion Is Associated with Different Microbiota and Metabolite Responses to Ketogenic Diet

Metwaly Amira, Chair of Nutrition and Immunology, Technical University of Munich, Freising, Germany
Integrated Microbiota and Metabolite Profiles Identified Functional Signatures in Crohn's Disease with a Link to Sulfate Metabolism

Rausch Philipp, Laboratory of Genomics and Molecular Biomedicine, University of Copenhagen, Copenhagen, Denmark
Dietary Protein Sources Determine Obesity Development – a Systems Biological Perspective

---

12^{00} "Lunch-to-go" and Departure

PROGRAM
Friday,
June 28th

HOST-MICROBIOTA MUTUALISM UPON CHRONIC UNDERNUTRITION: LESSONS FROM GNOTOBIOTIC ANIMAL MODELS

François Leulier

Institute de Génomique Fonctionnelle de Lyon, Université de Lyon, France

Metazoans establish reciprocal interactions with their commensal bacterial communities. Despite recent progress, a clear view of the physiological benefits associated with host/microbiota relationship remains elusive. Hence the molecular mechanisms through which the microbiota exerts its beneficial influences are still largely undefined. In this line, we aim at deciphering the molecular dialogue governing the mutualistic interaction between intestinal bacteria and their host. To this end, we are using a genetically tractable gnotobiotic animal model: *Drosophila melanogaster*, which is mono-associated to one of its natural dominant commensal, *Lactobacillus plantarum*. We are developing multiscale functional approaches to identify the mechanisms that underlie their mutualistic relationship, which results in the promotion of host juvenile growth upon chronic undernutrition. Our approaches aim at identifying both the bacterial and host genetic networks required to sustain their mutualistic relationship. In addition we are now translating our discoveries to mouse gnotobiotic and conventional models by studying the impact of selected strains of *L.plantarum* on mice juvenile growth. I will present our latest results using Drosophila and Mouse models.

Selected publications:

Martino, M.E., Joncour, P., Leenay, R., Gervais, H., Shah, M., Hughes, S., Gillet, B., Beisel, C., and Leulier, F. Bacterial Adaptation to the Host's Diet Is a Key Evolutionary Force Shaping *Drosophila-Lactobacillus* Symbiosis. Cell Host Microbe *24*. Published online June 28, 2018. 10.1016/j.chom.2018.06.001.

Storelli G, Strigini M, Grenier T, Bozonnet L, Schwarzer M, Daniel C, Matos R, Leulier F. Drosophila Perpetuates Nutritional Mutualism by Promoting the Fitness of Its Intestinal Symbiont Lactobacillus plantarum. Cell Metab. 2017 Dec 26. pii:S1550-4131(17)30679-4. doi: 10.1016/j.cmet.2017.11.011.

Matos RC, Schwarzer M, Gervais H, Courtin P, Joncour P, Gillet B, Ma D, Bulteau AL, Martino ME, Hughes S, Chapot-Chartier MP, Leulier F. D-Alanylation of teichoic acids contributes to Lactobacillus plantarum-mediated Drosophila growth during chronic undernutrition. Nat Microbiol. 2017 Dec;2(12):1635-1647. doi:10.1038/s41564-017-0038-x. Epub 2017 Oct 9.

Schwarzer M, Makki K, Storelli G, Machuca-Gayet I, Srutkova D, Hermanova P, Martino ME, Balmand S, Hudcovic T, Heddi A, Rieusset J, Kozakova H, Vidal H, Leulier F. Lactobacillus plantarum strain maintains growth of infant mice during chronic undernutrition. Science. 2016 Feb 19;351(6275):854-7. doi:10.1126/science.aad8588.

Erkosar B, Storelli G, Mitchell M, Bozonnet L, Bozonnet N, Leulier F.
Pathogen Virulence Impedes Mutualist-Mediated Enhancement of Host Juvenile Growth via Inhibition of Protein Digestion. Cell Host Microbe. 2015 Oct 14;18(4):445-55. doi: 10.1016/j.chom.2015.09.001. Epub 2015 Oct 1.

Storelli G, Defaye A, Erkosar B, Hols P, Royet J, Leulier F. Lactobacillus plantarum promotes Drosophila systemic growth by modulating hormonal signals through TOR-dependent nutrient sensing. Cell Metab. 2011 Sep 7;14(3):403-14. doi:10.1016/j.cmet.2011.07.012.

MICROBIOME, INFECTION AND ANTIMICROBIALS

19^{30}– 21^{00} Chair: Tom Clavel, Uniklinik RWTH Aachen

Ehrhardt Katrin, Institute of Medical Microbiology and Hospital Epidemiology and German Center for Infection Research (DZIF), Partner Site Hannover, Hannover Medical School, Hannover, Germany
Chronic Salmonella Typhimurium Infection Induces Matrix Metalloproteinase 10 Expression which Dampens the Host Inflammatory Response

Klimek Hanna, Section of Microbial Genome Plasticity, Institute of Hygiene, University of Münster, Münster, Germany
Communication at the Epithelial Interface: The Transcriptional Response of Epithelial Cells to Probiotic and Extraintestinal Pathogenic Escherichia Coli

Krüger Wibke, Microbial Immunology, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena, Germany
Gut Microbiota-mediated Colonisation Resistance Against Candida Albicans

Maier Lisa, Interfaculty Institute of Microbiology & Infection Medicine, University of Tübingen, Tübingen, Germany
Determining the Target Spectrum of Antibiotics on Human Gut Commensals

Wagner Stefanie, Institute of Molecular Pathogenesis, Federal Research Institute for Animal Health, Jena, Germany
Effects of a New Antiinfective Substance on the Intestinal Microbiome of Pigs

Zhou Mangge, Experimental Immunology, Helmholtz Centre for Infection Research, Braunschweig, Germany
Long-lasting Consequences of Neonatal Infections on the Intestinal Immune System

CHRONIC *SALMONELLA TYPHIMURIUM* INFECTION INDUCES MATRIX METALLOPROTEINASE 10 EXPRESSION WHICH DAMPENS THE HOST INFLAMMATORY RESPONSE

K. Ehrhardt[1], AL. Becker[1], P. Braubach2, GA. Graßl[1]

[1]Institute of Medical Microbiology and Hospital Epidemiology and German Center for Infection Research (DZIF), Partner Site Hannover, Hannover Medical School, Hannover, Germany
[2]Institute for Pathology, Hannover Medical School, Hannover, Germany

Salmonella cause a variety of diseases ranging from self-limiting enterocolitis to severe systemic infections. Dependent on the serovar, 2-5% of immunocompetent individuals become chronic carries thus representing a reservoir for transmission. The factors contributing to chronic *Salmonella* infection are incompletely understood. Chronic infection of mice with *Salmonella* enterica serovar Typhimurium leads to persistent bacterial shedding, intestinal transmural inflammation, infiltration of immune cells, and fibrosis. Microarray analyses revealed differential regulation of proteases and protease inhibitors in persistently infected mice. Proteases and inhibitors demonstrated site and cell-type specific expression patterns. Among the highest upregulated was the matrix metalloproteinase (MMP) family. Upon in vitro infection a high upregulation of *Mmp10* mRNA was detected in primary bone marrow-derived macrophages (BMDM). Infection of BMDM from MMP-10 deficient mice induced an increased proinflammatory response as observed by higher levels of MCP-1, IFN-beta, IFN-gamma, and nitrosative stress in comparison to infected wild type BMDM. *Salmonella* can survive better when MMP-10 is absent in long-term infected cells (3 days), while there was no difference at early points post-infection (6 hours and 1 day). Furthermore, filamentous growth of *Salmonella* was strongly increased in *Mmp10* deficient BMDM 3 days post infection. Filamentous growth can be caused by intracellular stress like nitrosative stress and might represent a survival strategy. In what manner the increased inflammatory response and survival of *Salmonella* in macrophages as observed in vitro affect acute and chronic *Salmonella* infection of mice is currently investigated.

In conclusion, our results show that chronic *Salmonella* infection regulates proteases and protease inhibitors in vivo and in vitro and that MMP-10 plays a role in restricting *Salmonella* survival and dampening the host inflammatory response to infection in vitro.

COMMUNICATION AT THE EPITHELIAL INTERFACE: THE TRANSCRIPTIONAL RESPONSE OF EPITHELIAL CELLS TO PROBIOTIC AND EXTRAINTESTINAL PATHOGENIC *ESCHERICHIA COLI*

H. Klimek[1], C. Cichon[2], J. Putze[1], U. Dobrindt[1]

[1]*Section of Microbial Genome Plasticity, Institute of Hygiene, University of Münster, Münster, Germany*
[2]*Institute of Infectiology, Center for Molecular Biology of Inflammation, University of Münster, Münster, Germany*

Probiotic *E. coli* strain Nissle 1917, uropathogenic *E. coli* (UPEC) strain CFT073 and asymptomatic bacteriuria (ABU) *E. coli* isolate 83972 are clonal and their genomes exhibit an average nucleotide identity (ANI) of > 99.8 %. While gut isolate Nissle 1917 (EcN) has a remissive effect on ulcerative colitis and ABU isolate 83972 is used to treat recurrent urinary tract infections (UTIs), UPEC CFT073 causes symptomatic UTI. To study the molecular mechanisms responsible for EcN's well-studied probiotic traits, such as strengthening epithelial barriers and countervailing the colonisation of pathogenic *E. coli*, we use a colonic cell culture model with an adherent mucus layer. Epithelial cells in the colon are covered by a mucus layer, which shields the cells from direct contact with bacteria. We culture HT29-MTX-E12 cells in a transwell system under continuous shaking, to create a semi-wet interface. This triggers the secretion and formation of an adherent mucus layer; the cells polarize, form a three-dimensional architecture and functional tight junctions. The aim was to use a model that better represents the morphology and function of the gut epithelium *in vivo* than cell monolayers do. We also infected renal A-498 cells to examine effects of the three clonal *E. coli* isolates with different virulence phenotypes on epithelial cells of the urinary tract. This way, we opted to find differential transcriptional responses of the intestinal and renal epithelial cells upon bacterial adhesion or contact with bacterial culture supernatants. We also compared the cytotoxic effects of the tested *E. coli* strains on the host cells. Whether considered pathogenic or probiotic in distinct niches, all tested *E. coli* strains induced the expression of crucial pro-inflammatory cytokines in the renal and gut epithelial cell lines. Bacterial cytotoxicity was less pronounced if epithelial cells were protected by a mucus layer. We believe that differential host cell response is an important factor that determines bacterial pathogenicity or probiotic traits.

GUT MICROBIOTA-MEDIATED COLONISATION RESISTANCE AGAINST *CANDIDA ALBICANS*

W. Krüger[1], S. Vielreicher[1], I. D. Jacobsen[1,2]

[1]*Microbial Immunology, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena, Germany*
[2]*Institute of Microbiology, Friedrich Schiller University Jena, Germany*

The human body provides multiple niches for a variety of microorganisms, consisting of bacteria, fungi, archaea, and viruses. The members of the microbiota interact in a complex network and have a significant impact on health and disease. One function of the microbiota is to confer colonisation resistance to control the number of facultative pathogens. A commensal of the human gut with pathogenic potential is the fungus *Candida albicans*. Intestinal dysbiosis due to antibiotic treatment can lead to enhanced fungal proliferation, and is a major risk factor for both mucosal and systemic candidiasis. Over the past decades, the incidence of systemic candidiasis in intensive care units increased and became a major complication. Understanding which members of the microbiota control *C. albicans* in the gut might provide possibilities for prevention of the disease.

The aim of the project is to identify genetically identical mouse colonies with distinct microbiomes to study the impact of the intestinal microbiota on colonisation resistance and the pathogenicity of *C. albicans*.

Therefore, faeces were collected from commercial and academic facilities. Bacterial and fungal composition of the intestinal microbiota of 15 breeding colonies of C57BL/6 mice were analysed by 16S and ITS sequencing. Quantitative analysis of microbial burden was performed by qPCR of gDNA.

Analysis revealed differences in fungal and bacterial burden between the facilities. Furthermore, colonies varied in α- and β-diversity (both bacteria and fungi), Bacteroidetes:Firmicutes ratio, Ascomycota:Basidiomycota ratio, and composition at the family to genus level. This included variances in the colonisation level with *Lactobacillus* spp. and *Bacteroides* spp., bacteria described to mediate colonisation resistance against *Candida*. Compared to bacteria, the fungal composition at the species level was more variable within colonies.

This confirms previously reported variations of the bacterial microbiota, and is, to our knowledge, the first study on mycobiome variations in german mouse colonies.

DETERMINING THE TARGET SPECTRUM OF ANTIBIOTICS ON HUMAN GUT COMMENSALS

L. Maier[1,7#], CV. Goemans[1#], J. Wirbel[2], M. Pruteanu[1,6], M. Kuhn[2], T. Banerjee[1], E. Cacace[1], EE. Anderson[1], U. Löber[3], S. Forslund[3], KR. Patil[2], P. Bork[2,3,4,5], G. Zeller[2] and A. Typas[1,2]

[1]European Molecular Biology Laboratory, Genome Biology Unit, Heidelberg, Germany
[2]European Molecular Biology Laboratory, Structural and Computational Biology Unit, Heidelberg, Germany
[3]Max-Delbrück-Centre for Molecular Medicine, Berlin, Germany
[4]Molecular Medicine Partnership Unit, Heidelberg, Germany
[5]Department of Bioinformatics, Biocenter, University of Würzburg, Würzburg, Germany
[6]current address: Institute for Biology, Humboldt University Berlin, Berlin, Germany
[7]current address: Interfaculty Institute of Microbiology & Infection Medicine Tübingen, Tübingen, Germany

Antibiotics are meant to inhibit human bacterial pathogens, but they also harm a wide range of commensals. So far, systematic studies of their target spectrum on gut microbes are still missing. Here, we provide a comprehensive view of the effect of 144 antibiotics from all major classes on 38 representative species of the healthy human gut microbiome. Interestingly, we observed antibiotic class-dependent effects. For β-lactams, even closely related species often differ in susceptibility profiles. Quinolones showed strong drug-generation dependent effects with newer derivatives inhibiting more commensals. Macrolides and tetracyclines showed a broad effect on the commensals tested. Further analysis revealed that both macrolides and tetracyclines, two prototypical bacteriostatic antibiotics, actually exert bactericidal activity on many key gut microbes. This shows that the divide into bactericidal and bacteriostatic antibiotics does not hold true for gut commensals and potentially explains the strong effects of these antibiotic classes on the gut microbiome of animals and patients.

In order to explore ways to mitigate the strong effects of macrolides and tetracyclines on gut commensals, we screened for antagonistic compounds, which protect abundant species from antibiotics. Some of the identified antagonists could even protect additional commensal species, without interfering with the antibiotic effect against common pathogens.

Altogether, this study provides new insights on the target spectrum of antibiotics on gut commensals, uncovers bactericidal effects of textbook bacteriostatic antibiotics and opens avenues to revert the collateral damage caused by antibiotics on human gut commensals.

EFFECTS OF A NEW ANTIINFECTIVE SUBSTANCE ON THE INTESTINAL MICROBIOME OF PIGS

S. Wagner[1], M. Brauer[2,3], J. Herrmann[4], R. Müller[4], K. Riedel[2,3], T. Fuchs[1]

[1]*Institute of Molecular Pathogenesis, Federal Research Institute for Animal Health, Jena, Germany*
[2]*Institute of Microbiology, University of Greifswald, Greifswald, Germany*
[3]*Institute of Marine Biotechnology, Greifswald, Germany*
[4]*Helmholtz Institute for Pharmaceutical Research Saarland, Department Microbial Natural Products, Saarbrucken, Germany*

An incessant threat for human health is arising from antibiotic-resistant pathogens. This problem is further afflicted with a drastic lack of new antiinfective drugs. The validation of new antibiotic substances may reveal unsuited substrates that are not only effective against the pathogenic bacteria, but also have an impact on the commensal gut microbiome. An unspecific efficacy leads to detrimental side effects like diarrhoea or allergic reactions, and increases the patient's susceptibility for secondary infections. To study the effect of antibiotic therapies *in vivo*, pigs can function as suitable model organisms, since they have a gut architecture and microbiome similar to human.

For this reason, our study focuses on a newly developed antiinfective substance and its impact on the gut microbiome in swine as a large animal model. Our cooperation partner, the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), synthesized the macrolide chlorotonil, a substance that showed a specific inhibitory effect on Gram-positive bacteria during previous *in vitro* experiments. The current study investigates the systemic effects of the macrolide in comparison with the conventionally used antibiotic tylosin. In an *in vivo* experiment, we fed piglets either chlorotonil or tylosin and monitored their general condition for several days. Additionally, we analysed the composition of their intestinal microbiome via sequencing of the bacterial 16S rRNA genes.

The newly developed substance caused a specific inhibition of only a few species in the piglet's gut microbiome with little impact on the commensal flora. Consistently, piglets showed no side effects after administration. Together with our cooperation partners, the HIPS and the University of Greifswald, we currently analyse the impact of chlorotonil on the microbiome in more detail. We will apply different multi-omics approaches to investigate the effects on transcriptional level and protein synthesis, and thus also the functionality of the piglet's gut microbiome.

LONG-LASTING CONSEQUENCES OF NEONATAL INFECTIONS ON THE INTESTINAL IMMUNE SYSTEM

M. Zou, J. Pezoldt, C. Wiechers, L. Hao, A. Dupont, M. Beckstette, E. Vafadarnejad, T. Strowig, M. Hornef, AE. Saliba, P. Dersch, J. Huehn

Introduction: The neonatal window of opportunity was first revealed by epidemiological studies highlighting correlations between postnatal environmental exposures and susceptibility to diverse diseases. Our own studies also had identified the first ten days after birth as a critical neonatal window of opportunity for the stromal cells of gut-draining mesenteric lymph nodes (mLN) to be stably imprinted by microbiota with the capacity to foster an efficient *de novo* induction of regulatory T cells (Treg), which are crucial for the establishment of intestinal tolerance.

Objective: We aimed to study the long-lasting consequences of transient infections with gastrointestinal pathogens during the neonatal period on the intestinal immune system.

Methods: Neonatal BALB/c mice were infected intragastrically with enteropathogenic *Escherichia coli* (EPEC) and *Yersinia pseudotuberculosis* (*cnfY* knock-out strain YP147). Different time points post infection (p.i.), the intestinal immune cell composition was analyzed by flow cytometry and the Treg-inducing properties of the gut-draining LN were investigated by adoptive transfer of naïve ovalbumin-specific CD4+ T cells from DO11.10 mice followed by systemic T cell priming. LN from previously infected mice were transplanted to dissect the impact of neonatal infections on the LN stromal cell compartment. Low-input RNAseq and 16S rDNA analyses were performed to unravel infection-induced global changes on transcriptomes of stromal cell subsets and microbiota composition, respectively.

Results: Twelve weeks p.i. with EPEC or YP147 a clear shift in the Treg/Th17 balance favoring RORγt-expressing Th17 cells accompanied by elevated frequencies of ILC3s could be observed in the colon, suggesting a long-lasting impact of the neonatal infections on the innate and adaptive mucosal immune system. In addition, the *de novo* Treg induction capacity was substantially impaired in liver-draining celiac lymph nodes (celLN), while surprisingly mLN was not negatively affected by the neonatal infections. These findings were supported by LN transplantation experiments, which demonstrated a strong, long-lasting negative effect of neonatal infections on the tolerogenic properties of celLN stromal cells. Low-input RNAseq allowed the identification of up/down-regulated genes in celLN fibroblastic stromal cells (FSC) as a consequence of the neonatal infections, and neonatally infected mice also showed an altered microbiota composition lasting until adulthood.

Conclusion: Together, our data suggest that transient gastrointestinal infections during the neonatal window can have long-lasting consequences that result in a disturbed microbiota composition, an altered intestinal immune cell homeostasis and impaired functional properties of the liver-draining LN, finally affecting the *de novo* induction of Tregs as key players of peripheral tolerance.

PROGRAM
Saturday, June 29th

MICROBIAL CONTROL OF METABOLIC HEALTH

Amanda Ramer-Tait

Department of Food Science and Technology, University of Nebraska – Lincoln, Lincoln, United States of America

Dietary modulation of the gut microbiota represents an exciting opportunity to improve health. Prebiotic fibers have generated much interest because they induce both compositional and functional alterations in the gut microbiota, which are often hypothesized to mediate the metabolic benefits observed during human trials. However, the findings that support this hypothesis are based primarily on observed correlations between microbiota changes and the physiological effects of dietary fibers—the precise causal mechanisms are unknown. I will present results from two projects aimed at assigning cause-and-effect relationships when studying the metabolic effects of a prebiotic. Capitalizing on comparisons of germ-free and conventionalized mice fed high-fat diets, we have shown that the improvements in insulin sensitivity induced by the dietary fiber resistant starch can occur independently of the gut microbiota. Although the exact microbiome-independent mechanism underlying this benefit is unknown, resistant starch consumption reduced gene expression of adipose tissue macrophage markers and altered cecal concentrations of several taurine-conjugated bile acids. These changes were strongly correlated with plasma insulin levels, suggesting that resistant starches may influence bile acid signaling and adipose tissue immune modulation to bring about metabolic benefits without a contribution from the microbiota. In stark contrast, we have demonstrated that the metabolic improvements observed during feeding of the prebiotic galactooligosaccharide (GOS) are strictly microbiome-dependent. Experiments in gnotobiotic mice allowed us to identify the GOS-responsive organisms responsible for the metabolic improvements to the host; however, their effects differed markedly in mice harboring different gut microbiomes. These results demonstrate that the taxonomic architecture of the microbiota and/or the presence of select species within complex microbiomes can either enable or antagonize GOS-responsive organisms. When considered together, these findings highlight the vast differences in the importance of the gut microbiota in mediating the metabolic benefits of dietary fibers. If the microbiome is important, then the effects are influenced by inter-individual differences in gut microbiota configurations, which suggests that prebiotic applications could benefit from personalized approaches.

GNOTOBIOTIC MODELS

09^{45} – 11^{15} Chair: Bärbel Stecher-Letsch, LMU Munich, Max von Pettenkofer Institute

Amend Lena, Helmholtz Centre for Infection Research, Braunschweig, Germany
The Ecological Niche of Prevotella spp. in Mouse Intestine

Bolsega Silvia, Institute for Laboratory Animal Science and Central Animal Facility, Hannover Medical School, Hannover, Germany
Colonization of Segmented Filamentous Bacteria Depends on the Intestinal Microbiota Composition

Budzinski Lisa, Department of Microbiota & Inflammation, German Rheumatism Research Center (DRFZ), a Leibniz Institute, Berlin, Germany
Multi-parameter Flow Cytometry of Human Gut Microbiota

Fokt Hanna, Max Planck Institute for Evolutionary Biology, Plön, Germany; Institute for Experimental Medicine, Kiel University, Kiel, Germany
The Evolution of the Genus Bacteroides in the House Mouse Species Complex

Harris Danielle, Zoological Institute, Kiel University, Kiel, Germany; Max Planck Institute for Evolutionary Biology, Plön, Germany
Microbiota and Behaviour: From Complex Extract to Bioactive Molecule

Streidl Theresa, Functional Microbiome Resarch Group, Institute of Medical Microbiology, RWTH University Hospital, Aachen, Germany
A New Gnotobiotic Mouse Model to Study the Impact of Secondary Bile Acids Production on Host Physiology

THE ECOLOGICAL NICHE OF *PREVOTELLA* SPP. IN MOUSE INTESTINE

L. Amend[1], A. Iljazovic[1], EJC. Galvez[1], T. Strowig[1]

[1]*Helmholtz Centre for Infection Research, Braunschweig, Germany*

The human gut microbiome contributes to the host's health and alterations in these communities have been associated to the development of various diseases. The specific impact of numerous taxa on the human health status is still unknown. *Prevotella*, a genus of anaerobic Gram-negative bacteria belonging to the phylum Bacteroides, is a prevalent colonizer in the human intestine. However, its influence on the host remains largely unclear. Whereas *Prevotella* has been associated with beneficial health properties such as improvement of glucose metabolism and the consumption of plant-rich diet, its intestinal colonization has also been linked to multiple diseases, including rheumatoid arthritis, inflammatory bowel diseases and diabetes. Detailed characterizations of *Prevotella* spp. are complicated due to high species and strain diversity, their unknown niche as well as the lack of publicly available intestinal isolates.

Here, we describe the isolation of three uncharacterized *Prevotella* species from mouse intestine and their genomic diversity as well as specific functional properties. Co-colonization experiments revealed high interspecies competition, resulting with one strain (*Prevotella intestinalis*) outcompeting the others. Genomic and transcriptomic analysis detected a high variability between the three new species and identified the largest repertoire of enzymes involved in the utilization of carbohydrates (Polysaccharide utilization loci) in *P. intestinalis*. Diet intervention experiments revealed that complex carbohydrates were essential as substrates for microbiota domination by *P. intestinalis*. Moreover, we were able to show modulation of the abundance of *P. intestinalis* by using specific complex carbohydrates. Taken together, our observations contribute to the understanding about the metabolic niche and the role of *Prevotella* spp. in the intestine and support our knowledge about the shaping of the microbial composition by dietary patterns.

COLONIZATION OF SEGMENTED FILAMENTOUS BACTERIA DEPENDS ON THE INTESTINAL MICROBIOTA COMPOSITION

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

[1]*Institute for Laboratory Animal Science and Central Animal Facility, Hannover Medical School, Hannover, Germany*
[2]*Max von Pettenkofer Institute of Hygiene and Medical Microbiology, Faculty of Medicine, LMU, Munich, Germany*

The lumen of intestine is habitat to a complex microbial community that is engaged in host`s metabolic and immunologic processes as well as in intestinal barrier defense. The indigenous bacteria have been shown to protect the gut from bacterial overgrowth and invading microbes. These microbial interactions, also known as colonization resistance, can prevent enteric infection resulting in intestinal inflammation. Due to high complexity of microbiome it is difficult to analyze microbe-microbe and host-microbe interactions. Therefore, in this study we used gnotobiotic mice colonized with defined minimal microbiota to explore in more detail the effect of added bacteria to an existing consortium. Germ-free (GF) and gnotobiotic *Il10*-deficient (*Il10*$^{-/-}$) mice associated with the Altered Schaedler Flora (ASF) or Oligo Mouse Microbiota (OMM^{12}) were co-colonized with segmented filamentous bacteria (SFB). SFB are commensals that potently stimulate host´s mucosal immune system and were associated with both beneficial and adverse effects on the host physiology. Seven weeks after SFB inoculation we analyzed the SFB colonization rate in feces of GF, ASF and OMM^{12} associated *Il10*$^{-/-}$ mice. SFB presence was confirmed only in ex GF and ASF colonized mice, but not in mice carrying OMM^{12}. To verify these results, fluorescence *in situ* hybridization with a SFB-specific probe was performed. However, positive staining was not detected in OMM^{12} colonized mice. Furthermore, to determine whether SFB cannot colonize OMM^{12} associated mice or are lost over the course of experiment, SFB colonization kinetic was performed. The results revealed that SFB are not able to colonize in the presence of OMM^{12} consortium. To analyze whether the adaptive immunity in OMM^{12} associated mice plays a role in SFB colonization, B6-*Rag2*$^{-/-}$ mice were colonized with OMM^{12} and SFB showing that the adaptive immunity is not responsible for preventing SFB colonization. Interestingly, SFB presence was detected in *Il10*$^{-/-}$ mice, when the order of colonization was reversed suggesting that SFB can coexist with OMM^{12} consortium when they colonize first.

Altogether, our results showed that SFB colonization depends on the microbiota composition. This study also indicates that SFB can compete with OMM^{12} members when they colonize first and that established OMM^{12} consortium provides colonization resistance to SFB.

MULTI-PARAMETER FLOW CYTOMETRY OF HUMAN GUT MICROBIOTA

L. Budzinski[1], R. Maier[1], T. Sempert[1], T. Momtaz[1], A. Beller[1], P. Durek[1], GA. Heinz[2], T. Kaiser[3], A. Shah[1], V. von Goetze[1], K. Heiking[1], U. Hoffmann[1], MF. Mashreghi[2], A. Radbruch[4], HD. Chang[1]

[1]Department Microbiota & Inflammation, German Rheumatism Research Centre (DRFZ), a Leibniz Institute, Berlin, Germany
[2]Department Therapeutic Gene Regulation, German Rheumatism Research Centre (DRFZ), a Leibniz Institute, Berlin, Germany
[3]Flow Cytometry Core Facility, German Rheumatism Research Centre (DRFZ), a Leibniz Institute, Berlin, Germany
[4]Department Cell Biology, German Rheumatism Research Centre (DRFZ), a Leibniz Institute, Berlin, Germany

The human gut microbiota impacts human health. Composition and dynamics can change drastically in the context of inflammatory diseases, cancer or neurodegenerative diseases. However, it remains unclear which changes are causative for the disease or whether it could be regulated by the individual immune response. The investigation of the complex interactions and influencing factors between bacteria and the individual human requires analysis tools capable of elucidating multiple parameters at the same time. We are applying flow cytometry for the characterization of the human gut microbiota to rapidly access complexity and dynamics of bacterial populations. We aim to establish a multi-parameter panel that allows for a phenotypical discrimination of bacterial populations in order to define their crosstalk with the human immune system in greater detail. We have evaluated coating by different immunoglobulin isotypes, quantitative DNA staining and light scatter for the flow cytometric assessment of human stool samples of different donors singly and in combination, and determined the specificity of these stainings. From such flow cytometric data and concomitant 16S rDNA sequencing we can highlight the individual differences of bacteria-host interaction in certain donors or elucidate commonalities between multiple individuals. The correlation of these data with clinical data of the given donor can pave the way towards personalized microbiota-based medicine.

THE EVOLUTION OF THE GENUS *BACTEROIDES* IN THE HOUSE MOUSE SPECIES COMPLEX

H. Fokt[1,2], S. Doms[1,2], D. Prasse[3], RA. Schmitz-Streit[3], JF. Baines [1,2]

[1]*Max Planck Institute for Evolutionary Biology, Plön, Germany*
[2]*Institute for Experimental Medicine, Kiel University, Kiel, Germany*
[3]*Institute of General Microbiology, Kiel University, Kiel, Germany*

Understanding the forces that shape variation in host-associated bacterial communities within- and between host species is key to understanding the evolution and maintenance of metaorganisms. While a multitude of studies have investigated the influence of genetic and environmental factors at the level of community profiling, little information on the underlying functional genomic basis exists. In this study, we used a combined approach investigating the Eastern *Mus musculus musculus* and the Western *Mus musculus domesticus* subspecies on several levels. This includes (i) a survey of gut microbial variation across the geographic range of the two subspecies, (ii) QTL mapping in a cross involving a unique panel of hybrids of the two subspecies, and (iii) cultivation and bacterial genome sequencing of microbial traits identified in both (i) and (ii). Accordingly, indicator species analysis reveals taxa belonging to *Bacteroides* and *Lactobacillus* as potentially important mouse subspecies-specific traits. These indicator taxa were also identified by the QTL mapping in 320 second generation hybrid intercrossed mice. *Bacteroides* taxa display the closest similarity to both *B. acidifaciens* and *B. uniformis*, suggesting that species-/strain-level variation exists with respect to host subspecies. We are currently analyzing the genomes of 156 *B. acidifaciens* isolates with the aims to identify putative strain-level variations with respect to host subspecies, characterize the observed associations with the host genome at the bacterial genomic level, and identify candidates for functional validation.

MICROBIOTA AND BEHAVIOUR: FROM COMPLEX EXTRACT TO BIOACTIVE MOLECULE

DMM. Harris[1,2], AM. Murillo[1,2], A. Klimovich[1], J. Uhl[3], B. Kanawati[3], P. Schmitt-Kopplin[3], TCG. Bosch[1]

[1]*Zoological Institute, Christian-Albrechts-Universität zu Kiel, Kiel, Germany*
[2]*Max Planck Institute for Evolutionary Biology, Plön, Germany*
[3]*Institute for Ecological Chemistry, BioGeoChemistry, and Analytics, Helmoltz Zentrum München, Germany*

Host microbiota affect host health in a multitude of ways, although the biochemical means through which these interactions occur is not always clear. Previous work from our lab has demonstrated that the frequency of nervous system-dependent spontaneous body contractions of the freshwater polyp *Hydra vulgaris* are influenced by its conserved bacterial community, but the molecular mechanisms at play remain obscured. To address this, we employ an untargeted mass spectrometry-based approach. Medium conditioned with the *Hydra* microbial community nearly restores the contraction frequency of germ-free *Hydra* to levels of conventionally-raised *Hydra*, implying that the biochemical agent(s) influencing contraction frequency are secreted by the microbiota and are present in these extracts. To identify the chemicals in these complex extracts that have contraction-mediating bioactivity, we use a comparative approach, looking for microbial signals in conventionally-raised *Hydra* polyps that are not present in the germ-free polyps, and that are also present in the bioactive extracts. Because the bioactive extract was produced by microbes cultured in vitro, we further reasoned that the microbe(s) responsible for the bioactivity may produce the compound when monocultured in vitro. Therefore, we also investigate the metabolomes of the five main colonizers of *Hydra* (in monoculture and in co-culture). In sum, our approach uses *Hydra* as a model to decode the molecular conversation that occurs between resident bacteria and their host.

A NEW GNOTOBIOTIC MOUSE MODEL TO STUDY THE IMPACT OF SECONDARY BILE ACIDS PRODUCTION ON HOST PHYSIOLOGY

T. Streidl[1], I. Kratochvil[2], R. Segura Muñoz[3], C. Eberl[4], U. Rolle-Kampczyk[2], K. Schubert [2], K.M. Schneider[5], C. Trautwein[5], B. Stecher[4,6], M. von Bergen[2,7], A. Ramer-Tait[3], T. Clavel[1,8]

[1]*Functional Microbiome Research Group, Institute of Medical Microbiology, University Hospital of RWTH Aachen, Germany*
[2]*Department of Molecular Systems Biology, Helmholtz-Centre for Environmental Research – UFZ, Leipzig, Germany*
[3]*Department of Food Science & Technology, University of Nebraska-Lincoln, NE, United States of America*
[4]*Max von Pettenkofer Institute of Hygiene and Medical Microbiology, Ludwig-Maximilians-University of Munich, Germany*
[5]*Department of Internal Medicine III, University Hospital of RWTH Aachen, Germany*
[6]*German Center for Infection Research (DZIF); Partner Site Munich, Germany*
[7]*Institute of Biochemistry, Leipzig University, Leipzig, Germany*
[8]*ZIEL Institute for Food and Health, Technical University of Munich, Freising, Germany*

Extibacter muris is a newly discovered bacterial species within the mouse gut microbiota capable of metabolizing cholic acid (CA) to deoxycholic acid (DCA) via 7α-dehydroxylation. Bile acids are known to influence metabolic and inflammatory responses, but functional studies using bile acid-metabolizing gut bacteria are lacking. The goal of the present study was to assess the impact of *E. muris* colonization on host physiology, with particular focus on the gut-liver axis.

Male C3H mice were colonized from birth on with the minimal microbiota Oligo-MM[12] with or w/o addition of *E. muris.* Over a period of 8 weeks, the mice were fed either control diets with low fat content (LF; 10% kcal from fat) or experimental diets with high fat content (HF; 48% kcal from fat). In addition, two fat sources (lard or palm oil) were used for each diet category, allowing to study the influence of dietary fat compositions.

All mice were successfully colonized with *E. muris*, the relative abundance of which was higher in mice fed HF diets (1.4-fold increase on average, p-value < 0.0001). The presence of *E. muris* was associated with increased relative abundances of *Akkermansia muciniphila* whilst the occurrence of *Bacteroides caecimuris*, *Turicimonas muris*, and *Flavonifractor plautii* was decreased, independent of diet. As expected, DCA was detected only in the plasma of *E. muris* colonized mice (80 ± 40 nmol/l). *E. muris* colonization did not impact body weight, white adipose tissue mass, and blood levels of cholesterol, insulin, leptin, IL-6, and TNF. Concentrations of liver enzymes (ALAT and AST) were normal and histological investigation of both liver and colon tissues did not reveal significant alterations. These findings indicate that *E. muris* colonization has no pathological consequences for the host. According to proteomic analysis, pathways involved in gluconeogenesis, fatty acid oxidation and detoxification were altered in the liver of *E. muris*-colonized mice. To further characterize the impact of *E. muris* on the host, liver metabolomes, bile acid signalling, as well as proliferation and apoptosis in intestinal tissues are currently being analysed.

USING VACCINES TO MANIPULATE INTESTINAL BACTERIA

M. Diard[3,2], E. Bakkeren[3], D. Hoces[1,3], V. Lentsch[1], F. Böhi[3], K. Schumann-Moor[3], J. Adamcik[1], L. Piccoli[4], A. Lanzavecchia[4], B. Stadtmueller[5], M. van der Woude[6], P. Viollier[7], L. Falquet[8], D. Wuethrich[9], F. Bonfiglio[9], A. Egli[9], G. Zandomeneghi[10], R. Mazzenge[1], O. Holst[11], B. Meier[10], M. Aebi[3], WD. Hardt[3], Emma Slack[1,3]

[1]*Institute for Food Nutrition and Health, ETH Zurich, Switzerland*
[2]*Biozentrum, University of Basel, Switzerland*
[3]*Institute for Microbiology, ETH Zürich, Switzerland*
[4]*Institute for Biomedical Research, Bellinzona, Switzerland*
[5]*School of Cellular and Molecular Biology, University of Illinois, USA*
[6]*Department of Biology, University of York, UK*
[7]*Microbiology and Molecular Medicine, University of Geneva, Switzerland*
[8]*Department of Biology, University of Fribourg, Switzerland*
[9]*Department of Biomedicine, University of Basel, Switzerland*
[10]*Laboratory of Physical Chemistry, ETH Zurich, Switzerland*
[11]*Research Centre Borstal, Germany*

Secretory IgA ("SIgA") is the only component of the adaptive immune system present in the gut lumen, i.e. in the same physical space as the intestinal microbiota. Whilst the specificity of endogenous SIgA for the microbiota remains a hotly debated topic, it is clear that oral vaccines can induce high-affinity, T-cell dependent SIgA responses specific for vaccine-antigens. These can protect the intestine, predominantly by driving enchained growth and agglutination of bacteria deep in the gut lumen. Large bacterial clumps generated cannot interact with the epithelium and are rapidly cleared in the fecal stream. However, the majority of protective SIgA in fact recognizes bacterial surface glycans such as the O-antigens of lipopolysaccharide. As these are highly repetitive and enzymatically generated, single point mutations are sufficient to generate huge changes in glycan structure, and thus vaccine escape. Given the very large population size of most gut bacterial species, within-host evolution is rapid and inevitable. This has long been seen as the Achilles heel of vaccines targeting bacterial surfaces, but in fact the very inevitability of this evolution can be turned to our advantage: Oral vaccines can be specifically designed to direct bacterial evolution in the gut lumen. We have recently demonstrated this concept to force the evolution of attenuation in *Salmonella* Typhimurium. "Evolutionary trap" vaccines therefore have the potential to generate an overlooked form of non-sterilizing herd immunity with major implications in clearing livestock reservoirs of zoonotic and animal pathogens. We could also begin to imagine more subtle applications of this technique for directed evolution of bacteria in the gut lumen.

MICROBIOME, IMMUNOREGULATION AND DISEASE MODELS

13^{45} – 15^{15} Chair: Till Strowig, Helmholtz-Zentrum f. Infektionsforschung, Brunswick

Baumeister Theresa, Innere Medizin II, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany
Farnesoid-X-Receptor Expression Has a Protective Function and Emerges as Potential Target to Prevent Progression from Barrett Esophagus to Adenocarcinoma

Fischer Florence, Institute of Medical Microbiology, University Marburg, Marburg, Germany
The Impact of Dietary Fibre on Intestinal Microbiota and Homeostasis

Kreft Luisa, Center of Allergy and Environment (ZAUM), Helmholtz Center Munich and Technical University of Munich, Munich, Germany
Revealing a Microbial Impact on Intestinal Immune Tolerance by Using Gnotobiotic Mice

Reitmeier Sandra, Institute for Food & Health (ZIEL), Technical University of Munich, Freising, Germany
Arrhythmic Microbiota Improves Diagnostic Profiling of Type-2-Diabetes in a Prospective Cohort

Stein-Thoeringer Christoph, Innere Medizin II, Klinikum rechts der Isar, Technical University Munich, Munich, Germany
Lactose Drives Enterococcus Expansion to Promote Alloreactive T-Cell Mediated Intestinal Inflammation

Wylensek David, Functional Microbiome Research Group, RWTH University Hospital, Aachen, Germany
Causative Role of Secondary Bile Acid-Producing Gut Bacteria in Colorectal Cancer

FARNESOID-X-RECEPTOR EXPRESSION HAS A PROTECTIVE FUNCTION AND EMERGES AS POTENTIAL TARGET TO PREVENT PROGRESSION FROM BARRETT ESOPHAGUS TO ADENOCARCINOMA

T. Baumeister, S. Islam Sayin,J. Ingermann, A. Nuber, HY. Fang, N. Stevens Münch, A. Metwaly, D. Haller, RM. Schmid, TC. Wang, M. Quante

The main precursor condition of Esophageal Adenocarcinoma (EAC) is Barrett´s Esophagus (BE). BE is mainly attributed to inflammation due to chronic reflux of gastric and bile acid. Risk of progression to EAC correlates with obesity and a western-style diet, which further affects the gut microbiome, including bile acid metabolizing bacteria. Western style diet in humans, resembled by high fat diet in mice leads to altered bile acid metabolites in the blood, which correlate with accelerated malignant progression in humans and mice. Gene expression analyses demonstrated that the nuclear bile acid receptor FXR, a key regulator in bile acid-, metabolic- and inflammatory signaling, was upregulated in BE but again downregulated in EAC in humans and mice. In an established genetic BE mouse model (L2-IL-1β), germline knockout of FXR accelerated malignant progression of BE. FXR, on metaplastic BE cells seems to be an important player to regulate the inflammatory effects of bile acid exposure and the stem cell character as well as differentiation potential of progenitor cells at the gastro-esophageal junction, suggesting a protective function of FXR. In Organoids, treatment with Obeticholic acid (OCA), a selective FXR agonist, had protective effects compared to treatment with the secondary bile acid Deoxycholic acid (DCA). In L2-IL-1β mice fed HFD, OCA decreased proliferation in the metaplastic tissue, suggesting it to suppress malignant progression. In concordance, while OCA treatment was capable of reducing certain bile acid subsets in the serum of L2-IL-1β HFD mice, other bile acid subsets were upregulated in L2-IL-1β FXR knockout mice. Combined analyses of already collected data from targeted serum bile acid metabolomics together with 16s- microbiome sequencing of mice and human patients will give further insight in understanding the systemic effects of dietary intake and resulting microbiome shifts on the FXR – bile acid axis and following on disease progression from BE to EAC. Thus, we provide evidence for a novel mechanism how bile acids accelerate esophageal carcinogenesis and how FXR activation could be used for preventive or therapeutic approaches in BE and EAC patients.

1. Quante, Michael, et al. "Bile acid and inflammation activate gastric cardia stem cells in a mouse model of Barrett-like metaplasia." *Cancer cell* 21.1 (2012): 36-51.

THE IMPACT OF DIETARY FIBRE ON INTESTINAL MICROBIOTA AND HOMEOSTASIS

F. Fischer[1], A. Višekruna[1], A. Walker[2], M. Klein[3], K. Neuhaus[4], B. Stecher[5], M. Basic[6], U. Steinhoff[1]

[1]Institute of Medical Microbiology, University Marburg, Germany
[2]Research Unit Analytical Biogeochemistry, Helmholtz Centre Munich, Germany
[3]Institute of Immunology, University Mainz, Germany
[4]ZIEL - Institute for Food & Health, Core Facility Microbiome/NGS, TU Munich, Germany
[5]Max von Pettenkofer Institute of Hygiene and Medical Microbiology, University Munich, Germany
[6]Institute of Laboratory Animal Science, Hannover Medical School, Germany

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

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

Animals on cellulose-free diet gained body weight comparable to controls and showed no signs of impaired fitness. However, they were highly susceptible to DSS induced intestinal inflammation, characterized by enhanced expression of pro-inflammatory cytokines and a leaky epithelial gut barrier. 16S rRNA amplicon analysis of the intestinal microbiota revealed, that cellulose dramatically influences the development (diversity) of the intestinal microbiota. At age of eight weeks, a similar diversity of the cecal microbiota was seen, independently of the diet. Between week eight and twelve, a massive increase in the microbial diversity was exclusively found in mice that were fed a diet containing cellulose. In the absence of dietary cellulose some bacterial genera were almost entirely lost.

To further investigate the impact of the altered microbial ecosystem, we used the Oligo-MM12 mouse model, which harbours twelve representative members of the murine intestinal microbiota. We focused on an anaerobic species, Alistipes finegoldii, which is strictly dependent on dietary cellulose. This bacterium was able to stably colonize the Oligo-MM12 microbiota. Interestingly, DSS-treated Oligo-MM12 mice associated with A. finegoldii were protected from weight loss and inflammation in comparison to Oligo-MM12 mice.

In conclusion, our findings show that dietary cellulose impacts on intestinal homeostasis and increases the microbial community. We identified A. finegoldii, a cellulose-dependent bacterium of the microbiota, which contributes to intestinal health. We currently investigate the mechanism of this protective effects.

REVEALING A MICROBIAL IMPACT ON INTESTINAL IMMUNE TOLERANCE BY USING GNOTOBIOTIC MICE

L. Kreft[1], C. Meng[2], B. Bächler[2], K. Kleigrewe[1], M. Basic[3], B. Stecher[4], A. Bleich[3], C. Ohnmacht[1]

[1]Helmholtz Center Munich and Technical University of Munich, ZAUM, Germany
[2]Technical University of Munich, BayBioMS, Germany
[3]Hannover Medical School, Institute for Laboratory Animal Science and Central Animal Facility, Germany
[4]Ludwig-Maximilians-University Munich, Max von Pettenkofer-Institute for Hygiene and Medical Microbiology, Germany

The risen prevalence of allergies has been attributed to changing lifestyle factors affecting intestinal microbiota composition. Microbial dysbiosis - particularly early in life - has been associated with atopic diseases later in life. Mice housed under germ-free (GF) conditions show an impaired intestinal immune system. In particular, GF or antibiotic-treated mice have drastically reduced frequencies of peripherally-induced regulatory T Cells (pTreg). Re-colonization of GF mice re-establishes such inducible Treg populations, which are defined by the co-expression of Foxp3 and RORγt. We hypothesize that these cells are key regulators in intestinal immune tolerance. The microbial factors driving this Treg induction remain poorly understood. By using mice with reduced microbial complexities: ASF (Altered Schaedler flora, 8 strains), Oligo-MM (Oligo-Mouse Microbiota, 12 strains) and SPF (specific pathogen free, complex microbiota), we aim to decipher which microbial factors and host-microbiome relationships drive this pTreg induction important for intestinal tolerance. Intestinal samples are compared by using Metatranscriptomics/-genomics and –bolomics approaches to identify microbiota-derived parameters. Results are combined with the analysis of intestinal immune cell characteristics to identify mechanisms of pTreg induction. Preliminary results show that RORγt+ Tregs are reduced in the small intestine (SI) of ASF- and OligoMM-colonized mice compared to SPF mice. The abundance of RORγt+ Tregs thereby negatively correlates with microbial complexity. This phenotype is confirmed in the spleen indicating a systemic effect of a reduced microbiota on pTreg abundance. In contrast, GATA3+ T Helper 2 cells are increased particularly in the SI of ASF- and OligoMM-colonized mice, inversely correlating with microbial complexity and indicating that these mice could be more susceptible to type 2 immune disorders. Thus, identifying the microbial parameters from these defined consortia impacting on immune cell balance may reveal key mechanisms of increased susceptibilities to allergies due to microbial dysbiosis.

ARRHYTHMIC MICROBIOTA IMPROVES DIAGNOSTIC PROFILING OF TYPE-2-DIABETES IN A PROSPECTIVE COHORT

S. Reitmeier[1], T. Clavel[1,2], S. Kießling[1,6], K. Neuhaus[1], M. Troll[4,5], H. Grallert[4,5], A. Peters[4], D. Haller[1,6]

[1]*Technische Universität München, ZIEL - Institute for Food & Health, Germany*
[2]*Functional Microbiome Research Group, Institute for Medical Microbiology, RWTH Aachen, Germany*
[4]*Research Unit of Molecular Epidemiology, Institute of Epidemiology, Helmholtz Zentrum München Research Center for Environmental Health, Neuherberg, Germany*
[5]German Center for Diabetes Research (DZD), Neuherberg, Germany
[6]*Technische Universität München, Chair of Nutrition and Immunology, Freising-Weihenstephan, Germany*

Background. Population-based studies have identified regional variations in individual compositions of the fecal microbiota, limiting the relevance of disease-associated signatures from currently available cross-sectional cohorts. KORA is a regionally confined and prospectively followed population study with a focus on metabolic health. In an interval of 5-years, microbiota profiling was performed on consecutive stool samples using high-throughput sequencing.

Methods. Bacterial profiling of two sampling time points (t_1=1,976 and t_2=701 samples) was performed by amplicon sequencing of the V3/V4 and V1/V2 regions of 16S rRNA genes. To assess shifts in the microbiota linked to metabolic conditions, individuals were stratified based on body mass index (BMI), impaired glucose tolerance (HbA1c, fasting glucose and OGTT) to discriminate between healthy, prediabetic and Type-2 diabetes (T2D) conditions. Diurnal rhythms of the gut microbiome were analysed to identify disease associated bacterial signatures.

Results. We obtained a total of 13,352 (V3-V4) and 14,379 (V1-V2) high-quality sequences per sample representing 370±76 OTUs and 255±55 OTUs, respectively, with a shannon diversity of 4.71±0.37 and 3.99±0.37. Permutational multivariate analysis identified 46 subject-related variables that significantly explained nine percent of the variations in gut microbiota profiles. Besides triglyceride concentration in blood and body weight, rural versus urban habitats strongly influenced compositional profiles (explaining 0.9% variations). Data was fitted to a cosinus curve to determine rhythmicity in bacterial abundance over a 24-h frequency based on sampling time point. T2D cases became arrhythmic in alpha diversity as well as in the two dominant phyla *Firmicutes* and *Bacteroides*. In total 10% of the families are rhythmic in no-T2D and only 2% are rhythmic in T2D. OTUs which are losing rhythmicity in T2D are selected to generate a random forest model for a diagnostic profiling in T2D. Arrhythmic OTUs improves the T2D classification model in comparison to a simple BMI and age-based model (AUC=0.91; AUC=0.96). The model with the selected features – *Oscillibcater*, *Odoriobacter*, *Feacalibacterium* and *Clostridium* – classifies T2D with an accuracy of 80.54% and is validated in an independent cohort (AUC=0.82). For the prospective data the model classified T2D at baseline with an accuracy of 78.75% (AUC =0.82). Currently the selected features can not properly predict metabolic health based on incident T2D cases (AUC=0.67).

Conclusion. The microbial ecosystem in the human gut is characterized by a large variation influenced by environmental factors associated with geographic regions, lifestyle, and health conditions. Taking arrhythmic bacterial signatures into account improves the diagnostic profiling and underlines the important of diurnal rhythm in the gut microbiome.

LACTOSE DRIVES *ENTEROCOCCUS* EXPANSION TO PROMOTE ALLOREACTIVE T-CELL MEDIATED INTESTINAL INFLAMMATION

CK. Stein-Thoeringer[1,3], KB. Nichols[1,3], A. Lazrak[1,3], M. Docampo[1,3], AE. Slingerland[1,3], JB. Slingerland[1,3], G. Armijo[1,3], ALC. Gomes[1,3], Y. Shono[1,3], A. Staffas[1,3], M. Burgos da Silva[1,3], S. Devlin[4], KA. Markey[1,2,3], D. Bajic[5], R. Pinedo[20], A. Tsakmaklis[6,19,21], ER. Littmann[1], A. Pastore[1], Y. Taur[7], S. Monette[8], ME. Arcila[9], AJ. Pickard[10], M. Maloy[2], RJ. Wright[1], LA. Amoretti[1], E. Fontana[1], D. Weber[11], AD. Sung[12], D. Hashimoto[13], C. Scheid[6], JB. Xavier[14], JA. Messina[15], K. Romero[16], M. Lew[12], A. Bush[12], L. Bohannon[12], K. Hayasaka[17], K. Hasegawa[13], MJGT. Vehreschild[6,19,21], JR. Cross[10], DM. Ponce[2,3], MA. Perales[2,3], SA. Giralt[2,3], RR. Jenq[18], T. Teshima[13,17], E. Holler[11], NJ. Chao[12], EG. Pamer[1,3,7], JU. Peled[1,2,3], MRM van den Brink[1,2,3]

[1]Department of Immunology, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY
[2]Adult Bone Marrow Transplantation Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY
[3]Weill Cornell Medical College, NY
[4]Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY
[5]Department of Internal Medicine II, Technical University of Munich, Germany
[6]Internal Medicine I, University Hospital Cologne, Germany
[7]Infectious Disease Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY
[8]Laboratory of Comparative Pathology, Memorial Sloan Kettering Cancer Center, The Rockefeller University, Weill Cornell Medicine, NY
[9]Diagnostic Molecular Pathology Laboratory, Memorial Sloan Kettering Cancer Center, NY
[10]Donald B. and Catherine C. Marron Cancer Metabolism Center, Memorial Sloan Kettering Cancer Center, New York, NY
[11]Internal Medicine III, University Clinic Regensburg, Regensburg, Germany
[12]Division of Hematologic Malignancies and Cellular Therapy, Department of Medicine, Duke University Medical Center, Durham, NC
[13]Department of Hematology, Hokkaido University, Faculty of Medicine, Sapporo, Japan
[14]Computational and Systems Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY
[15]Division of Infectious Diseases, Department of Medicine, Duke University, Durham, NC
[16]Office of Clinical Research, Duke University School of Medicine, Durham, NC
[17]Division of Laboratory and Transfusion Medicine, Hokkaido University Hospital, Sapporo, Japan
[18]Department of Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston
[19]Department of Internal Medicine, Infectious Diseases, Goethe University Frankfurt, Germany
[20]Gnotobiotic Facility, Memorial Sloan Kettering Cancer Center, New York, NY
[21]German Center for Infection Research, Partner site Bonn-Cologne, Cologne, Germany

Disruption of intestinal microbial communities, characterized by expansions of facultative pathogens, may underlie many human illnesses. However, the mechanisms that promote this dysbiosis and its adverse consequences are poorly understood. We describe a high incidence of *E. faecium* expansion in patients from 4 different stem cell transplant centers across the globe who receive allogeneic hematopoietic-cell-transplantation (allo-HCT). This expansion was associated with graft-versus-host disease (GVHD) and mortality. Enterococci also expand in mouse intestines after allo-HCT, and exacerbate disease severity in gnotobiotic models. Enterococci expansion *in vitro* was dependent on lactose, and dietary lactose depletion in mice attenuated its outgrowth and reduced GVHD severity. Allo-HCT patients bearing a lactose-non-absorber genotype were less capable to clear post-antibiotic *Enterococcus* domination. We identified a mechanism in which lactose as a common nutrient can stimulate the expansion of an opportunistic bacterium to exacerbate inflammation.

CAUSATIVE ROLE OF SECONDARY BILE ACIDS-PRODUCING GUT BACTERIA IN COLORECTAL CANCER

D. Wylensek[1], TCA. Hitch[1], A. Afrizal[1], T. Riedel[2,3], B. Abt[2,2], K. Neuhaus[4], K. Flisikowski[5], L. Navarro Suarez[6], T. Looft[7], A. Neumann[8], I. Lagkouvardos, G. Suen[8], D. Haller[4], S. Rohn[6], K. Kristiansen[9], E. Allen-Vercoe[10], J. Overmann[2,3], A. Schnieke[5], T. Clavel[1,4*]

[1]*Functional Microbiome Research Group, RWTH University Hospital, Aachen, Germany*
[2]*Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany*
[3]*German Center for Infection Research (DZIF), Partner site Hannover/Braunschweig, Germany*
[4]*ZIEL - Institute for Food & Health, Technical University of Munich, Freising, Germany*
[5]*Chair of Livestock Biotechnology, Technical University of Munich, Freising, Germany*
[6]*Institute of Food Chemistry, University Hamburg, Hamburg Germany*
[7]*National Animal Disease Center, United State Department of Agriculture, Ames, IA, USA*
[8]*Department of Bacteriology, University of Wisconsin, Madison, WI, USA*
[9]*Laboratory of Genomics and Molecular Biomedicine, Department of Biology, University of Copenhagen, Denmark*
[10]*Department of Molecular and Cellular Biology, University of Guelph, Canada*

Gut microbiota signatures have been associated with colorectal cancer (CRC) and secondary bile acid production by gut bacteria has been implicated in CRC development. Previous epidemiological and interventional studies have pointed at the association between CRC and increased dietary intake of fat and red meat as well as increased fecal levels of secondary bile acids. However, functional proof of these associations is missing. In the present study, we aimed at dissecting the causative role of secondary bile acid production in CRC.

Transgenic (*APC*$^{1311+/-}$)[1] pigs were fed either a control diet or an isocaloric diet enriched in red meat and lard (RML) for three months. Pigs fed the RML diet were characterized by increased number and size of polyps after dietary intervention, as assessed by colonoscopy. This was accompanied by specific shifts in fecal microbiota profiles (high-throughput 16S rRNA gene amplicon sequencing) and changes in fecal metabolomes (NMR profiling). Fecal metagenomes and mucosal transcriptomes are currently being generated. In line with our hypothesis, fecal concentrations of the secondary bile acids deoxycholic acid and 12-keto lithocholic acid were increased in RML pigs (ca. 6- and 2-fold, respectively; $p < 0.001$). In order to perform targeted colonization studies using secondary bile-acid producing strains, we followed strategies used in our previous work on the mouse gut microbiota[2] and established a comprehensive collection of pig intestinal bacteria that will be made publicly available. This collection contains 112 species spanning 37 families across 9 phyla, including 39 strains proposed to represent novel taxa, especially one member of a novel family within the *Bacteroidales*. Of these isolates, 42 were shown to deconjugate primary bile acids and one strain of the species *Clostridium scindens* was able to convert the primary bile acid cholic acid via dehydroxylation and dehydrogenation. Microbiota transfer experiments and colonization studies using minimal consortia of strains in gnotobiotes are ongoing to mechanistically study the relevance of secondary bile acid production in CRC.

[1]Flisikowska *et al.* (2012) A porcine model of familial adenomatous polyposis. Gastroenterol 143:1173.
[2]Lagkouvardos *et al.* (2016) The Mouse Intestinal Bacterial Collection (miBC) provides host-specific insight into cultured diversity and functional potential of the gut microbiota. Nat Microbiol 1:16131.

TOWARDS UNCOVERING THE HIDDEN DIVERSITY OF THE HUMAN MICROBIOME

Nicola Segata

Computational Metagenomics Lab, Center for Integrative Biology (CIBIO), University of Trento, Povo, Italy

Shotgun metagenomics has uncovered a substantial amount of diversity in the human microbiome, but a large fraction of the sequences in a metagenome remains uncharacterized. In my talk I will show how the combination of multiple reference-based and assembly-based computational approaches applied on several thousand of diverse human metagenomes can lead to the discovery of hundreds of previously unknown species and strain-level variants of known species. I will describe the catalog of 154,723 microbial genomes that we reconstructed from 9,428 metagenomes spanning body sites, ages, countries, and lifestyles. These genomes were recapitulated into 4,930 species-level genome bins (SGBs), 77% without genomes in public repositories. Some novel candidate species are very prevalent, expand underrepresented phyla, and are enriched in non-Westernized populations. The new catalog permits deeper microbiome analyses and increase the average mappability of metagenomic reads from 67.76% to 87.51% in the gut (median 94.26%) and 65.14% to 82.34% in the mouth, and the newly recovered diversity range from new bacterial families to novel subspecies of already studied microbes. I will also discuss what are the challenges in performing comparative microbial genomics on the resulting vast catalog of human-associated microbial strains.

PROGRAM
Sunday,
June 30th

METAGENOMIC ANALYSIS OF MUMMIFIED HUMAN REMAINS

Frank Maixner[1], A. Tett[2], K. Huang[2], F. Boulund[3], K. Thorell[3], K. Reinhard[4], L. Engstrand[3], O. Rota-Stabelli[5], T. Rattei[6], N. Segata[5], A. Zink[1]

[1]*Institute for Mummy Studies, EURAC research, Viale Druso 1, 39100 Bolzano, Italy.*
[2]*Centre for Integrative Biology, University of Trento, Trento, Italy.*
[3]*Center for Translational Microbiome Research, CTMR, Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, Nobels Väg 16, 171 77 Stockholm, Sweden.*
[4]*School of Natural Resources, University of Nebraska-Lincoln, Lincoln, NE, 68502.*
[5]*Department of Sustainable Agro-Ecosystems and Bioresources, Fondazione Edmund Mach, San Michele all'Adige, Italy.*
[6]*CUBE - Division of Computational Systems Biology, Department of Microbiology and Ecosystem Science, University of Vienna, Althanstr. 14, 1090 Vienna, Austria.*

The application of metagenomic analysis on dated ancient human specimens opens a window into the past that enables scientists to address unique evolutionary research questions. Reconstructed ancient pathogen genomes provide new insights into the evolutionary history of bacterial-mediated diseases. First metagenomic analysis of mummified human remains were done on gastrointestinal (GI) tract biopsies of the Iceman, a 5300-year-old European Copper Age mummy. By using metagenomic diagnostics and targeted genome capture, we determined the presence of *Helicobacter pylori* and reconstructed its complete genome. Our study provided, beside the indication for a possible disease manifestation in the mummy, interesting new details on the origin of the stomach pathogen in Europe. The application of technological and conceptual advances in the Iceman study has paved the way for future studies on *H. pylori* in different ancient human remains. Currently, we are analyzing coprolite material and GI content of precious historical mummies from the American, African and Asian continents. Most mummified GI tract contents show the presence of various intestinal microbiome members, possibly allowing future reconstruction of parts of the original GI tract microbiome of the Iceman and of coprolite material from all over the world. By comparing ancient and modern human gut microbiomes we may get first evidences for changes in gut microbial diversity within the last millennia.

OMICS ANALYSIS OF THE MICROBIOME

10^{00} – 11^{30} Chair: Guntram Grassl, Hannover Medical School

Belheouane Meriem, Institute for Experimental Medicine, Kiel University and Max Planck Institute for Evolutionary Biology, Plön, Germany
The Microbiome as a Prognostic Indicator in Anorexia Nervosa Patients – Preliminary Results of a Longitudinal Study

Burkhardt Wiebke, Department of Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Germany
Dietary Sulfonates Do Not Promote Bacteria-Induced Gut Inflammation

Ertl Verena, Institute of Clinical Chemistry and Laboratory Medicine, University Hospital of Regensburg, Regensburg, Germany
Development and Application of a High Resolution Mass Spectrometry Method to Identify and Quantify Faecal Lipid Species

Fricker Alena, Department of Microbiome Research and Applied Bioinformatics, University Hohenheim, Stuttgart, Germany
High/Low Sterol Conversion Is Associated with Different Microbiota and Metabolite Responses to Ketogenic Diet

Metwaly Amira, Chair of Nutrition and Immunology, Technical University of Munich, Freising, Germany
Integrated Microbiota and Metabolite Profiles Identified Functional Signatures in Crohn's Disease with a Link to Sulfate Metabolism

Rausch Philipp, Laboratory of Genomics and Molecular Biomedicine, University of Copenhagen, Copenhagen, Denmark
Dietary Protein Sources Determine Obesity Development – a Systems Biological Perspective

THE MICROBIOME AS A PROGNOSTIC INDICATOR IN ANOREXIA NERVOSA PATIENTS – PRELIMINARY RESULTS OF A LONGITUDINAL STUDY

M. Belheouane[1], J. Seitz[2], N. Schulz[2], A. Dempfle[3], JF. Baines[1], B. Herpertz-Dahlmann[2]

[1]Institute for Experimental Medicine, Kiel University and Max Planck Institute for Evolutionary Biology, Plön, Germany.
[2]Department of Child and Adolescent Psychiatry, Psychotherapy and Psychosomatics, University Hospital, RWTH University Aachen, Aachen, Germany,
[3]Institute of Medical Informatics and Statistics, Kiel University, Kiel, Germany

Anorexia nervosa (AN) is one of the most common chronic illnesses in adolescence. Evidence is now growing that AN-induced starvation is associated with profound alterations of the gut microbiome, which is of critical interest given its important interactions with the host metabolism in terms of weight regulation, hormonal, immunologic and inflammatory processes, along with a direct influence on the brain and behavior ("gut-brain axis"). In this study, we investigated the composition of the gut microbiome in AN patients compared to healthy controls, and whether microbial profiles in the starved state might predict the course of disease, *e.g.* the time to achieve target weight. We compared fecal microbial profiles of twenty patients at admission to healthy matched controls, in addition to including longitudinal data at discharge for a subset (n=19) of these individuals. We find several taxa to vary in patients between admission and discharge, including *Faecalibacterium* and Lachnospiraceae. Further, these taxa also explain a portion of the variation in therapy duration across patients. The *Faecalibacterium* genus is of particular interest as it is a well-known probiotic member of the human gut microbiome, whose reduced abundance is *e.g.* a reliable indicator of Crohn's disease.

DIETARY SULFONATES DO NOT PROMOTE BACTERIA-INDUCED GUT INFLAMMATION

W. Burkhardt[1], T. Rausch[1], R. Klopfleisch[2], M. Blaut[1], A. Braune[1]

[1]Department of Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Germany
[2]Institute of Veterinary Pathology, Freie Universitaet Berlin, Germany

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

Based on the reported findings, we aimed to clarify whether the intake of dietary sulfonates also stimulates the growth of *B. wadsworthia* or other sulfite-reducing bacteria, thereby promoting intestinal inflammation in genetically susceptible mice. Dietary sources of sulfonates include cyanobacteria and chloroplasts in green vegetables, which contain the sulfolipids sulfoquinovosyl diacylglycerols (SQDG) in considerable amounts. In our study, specific pathogen-free (SPF) IL-10-deficient mice were fed either a diet supplemented with the cyanobacterium *Arthrospira platensis* (also called Spirulina) or a control diet for three weeks. Additional groups of SPF or gnotobiotic mice harboring a simplified human microbiota (SIHUMI) were gavaged with the SQDG metabolite sulfoquinovose, TC as positive control or water twice weekly in a three week intervention period.

None of the mice treated with the above mentioned sulfonates showed weight loss or macroscopic signs of inflammation. Mice fed the Spirulina diet initially even gained weight. The histopathological assessment revealed no signs of colitis and the colon barrier integrity was not affected by sulfonate feeding. The fecal cell numbers of *B. wadsworthia* remained low in all mouse groups.

In summary, neither the tested dietary sulfonates nor TC led to bacteria-induced intestinal inflammation in the IL-10-deficient mouse model, which was consistent in SPF and gnotobiotic mice.

DEVELOPMENT AND APPLICATION OF A HIGH RESOLUTION MASS SPECTROMETRY METHOD TO IDENTIFY AND QUANTIFY FAECAL LIPID SPECIES

V. Ertl[1], M. Höring[1], HF. Schött[2], G. Liebisch[1]

[1]Institute of Clinical Chemistry and Laboratory Medicine, University hospital of Regensburg, Germany
[2]Leibniz-Institute for Analytical Science – ISAS – e.V., Dortmund, Germany

It is now generally accepted that gastrointestinal system in particular the intestinal microbiome plays an important role in human health and disease. Faecal materials reflect the microbial activity and the analysis of remaining unabsorbed metabolites including lipid species and provide an estimate of metabolic interaction between gut microbiota and host. To identify subtle metabolic variations induced by diet alterations and to characterize the metabolic impact of variations of the gut microbiota, metabolic profiling is becoming increasingly popular. Here we applied flow injection analysis coupled to high resolution mass spectrometry (FIA-HRMS) to identify and quantify lipid species and their abundance in faeces.

Faecal homogenates were subjected to total lipid extraction according to the protocol of Bligh and Dyer (B/D). Analysis of crude lipid extracts was performed by FIA-HRMS. A high heterogeneity was observed in faecal sample from different subjects. However, first experiments showed high amounts of triglycerides and diglycerides in the majority of samples. Species profiles included highly unsaturated species which could be confirmed in MS/MS spectra. Therefore, we are currently validating a FIA-HRMS method for quantification of triglycerides and diglycerides species in human faeces including preanalytical conditions. To get more insight into polar lipid species profile, a three-phase extraction (Shibusawa et al., 2006) should be performed to remove excess neutral lipids by separating neutral and polar lipid classes.

In summary, FIA-HRMS offers a high throughput method to analyse and quantify lipid species profiles of faecal samples. The application of these methods in various samples should provide a comprehensive picture of the faecal lipidome and improve the understanding of the role of the microbiome in human health.

HIGH/LOW STEROL CONVERSION IS ASSOCIATED WITH DIFFERENT MICROBIOTA AND METABOLITE RESPONSES TO KETOGENIC DIET

AM. Fricker[1], AS. Jung[1], L. Ferrari[1], V. Konrat[1], P. Urbain[2], G. Liebisch[3], WF. Fricke[1]

[1]Dept. of Microbiome Research and Applied Bioinformatics, University of Hohenheim, Germany
[2]Dept. of Medicine I, Section of Clinical Nutrition and Dietetics, Medical Center – University of Freiburg
[3]Institute of Clinical Chemistry and Laboratory Medicine, Regensburg University Hospital, Germany

Ketogenic diet, which is defined by high fat, low carbohydrate and adequate protein intake, has been used as a weight-loss regimen and to treat refractory epilepsy, other neurological conditions and even cancer. However, the exact mechanism responsible for the positive effects of ketogenic diet, as well as associated cardiovascular and other risks, remain unclear. Here, we studied the effects of a six-week ketogenic diet in healthy adults on lipid metabolism and gastrointestinal microbiota. Salivary and fecal bacterial microbiota were taxonomically characterized by 16S rRNA gene amplicon sequencing and serum lipids, fecal sterols and bile acids were determined via liquid chromatography-high resolution mass spectrometry (LC-MS/HRMS). Ketogenic diet was associated with alterations in taxonomic compositions of both the oral and intestinal microbiota, most prominently a reduction in the relative abundance of *Bifidobacteria* in stool. Ketogenic diet also decreased fecal levels of total sterols and bile acids in our study population. Notably, before the intervention, study participants could be stratified based on fecal coprostanol-to-cholesterol ratios as high or low sterol converters. Low-converters were characterized by reduced fecal microbiota alpha and beta-diversity, reduced blood triglyceride (TG) and increased HDL levels. After ketogenic diet, differences between high and low-converters in fecal microbiota composition and sterol conversions were reduced. However, whereas no difference in blood TG, HDL and LDL/HDL ratio was seen in low-converters after ketogenic diet, high-converters deteriorated towards increased LDL/HDL ratios. Together our findings demonstrate broad effects of ketogenic diet on the intestinal microbiota and fecal and blood lipid profiles that correlate with pre-intervention microbiota parameters. They further suggest a potential diagnostic value of microbiota-based patient stratification to predict the metabolic and clinical consequences of ketogenic diet.

INTEGRATED MICROBIOTA AND METABOLITE PROFILES IDENTIFIED FUNCTIONAL SIGNATURES IN CROHN'S DISEASE WITH A LINK TO SULFATE METABOLISM

A. Metwaly[1], A. Dunkel[2], N. Waldschmitt[1], LF. Buttó[1], I. Lagkouvardos[3], AM. Corraliza[4], S. Schmidt[5], AC. Durai Raj [6], M. Schloter [6], T. Hofmann[5], J. Panes[4], A. Salas[4] and D. Haller[1,3]

[1]Chair of Nutrition and Immunology, Technical University of Munich, Germany
[2]Leibniz-institute for Food Systems Biology, Technical University of Munich, Germany
[3]ZIEL-Institute for Food and Health, Technical University of Munich, Germany
[4]Department of Experimental Pathology, Instituto de Investigaciones Biomédicas de Barcelona CSIC, IDIBAPS, CIBERehd, Spain
[5]Chair of Food Chemistry and Molecular Sensory Science, Technical University of Munich, Germany
[6]Helmholtz Zentrum München, German Research Center for Environmental Health, Research Unit Comparative Microbiome Analysis

Dysbiosis and metabolic alterations of the gut microbiome have been implicated in inflammatory bowel diseases (IBD). The aim of this study is to identify functional signatures associated with disease outcome or response to therapy in patients with IBD, and to mechanistically characterize their pathogenic potential using gnotobiotic humanized mice and an integrative multi-omics approach. We studied 35 IBD patients during 5-year treatment with autologous hematopoietic stem cell transplantation (HSCT) recruited in Spain. Fecal samples and tissue biopsies were collected both at baseline and at different time points during follow-up. To characterize changes in gut microbiome and metabolome, we performed 16S rRNA gene sequencing, global 16S predicted metagenomes, shotgun metagenomic sequencing and untargeted metabolomics. To address the functional impact of microbial dysbiosis, we established a humanized IBD mouse model by colonizing germfree (GF) Il-10$^{-/-}$ mice with selected fecal samples from CD patients with different disease outcome. Temporal fluctuations in gut microbiota composition and metabolite profiles reflected the individual patient-related variations and the differences in disease activity. Fecal microbiome of patients with active disease was enriched in microbial taxa involved in sulfur metabolism such as *Eschericia Shigella* & *Fusobacterium* and a high proportion of sulfate reducing bacteria such as *Desulfovibrio* and *Campylobacter*. Fecal metabolic profiling confirmed an increased abundance of sulfated metabolites (bile acids, polyphenols and biogenic amines). Predicted metagenomes from 16S rRNA gene profiling revealed enrichment of functional genes associated with sulfate and ion transport system metabolism in IBD patients with active disease. In contrast, increased abundance of several basic biosynthetic processes correlated with remission. Transplantation of microbiota from patients with active or inactive disease was reproducibly sufficient to recreate disease phenotype in recipient Il-10$^{-/-}$ GF mice. Humanized mice reflected the dysbiotic features of their respective human donors and inflammation was driven by a variety of individual community profiles. Using a machine-learning algorithm, we identified a panel of 10 taxa that discriminates humanized mice by inflammatory status, where a microbial signature characterized by an overabundance of B*acteroides fragillis* and *Desulfovibrio* classified humanized mice by inflammation with high accuracy. In accordance with the signature identified in humans, enrichment of sulfated metabolites was indicative for inflamed phenotype, together with an abundance of genes mapping to sulfate metabolism, Type II, IV and VI secretion systems. Integration of microbiota and metabolite profiles from human and mice improved the predictive modelling of disease outcome significantly and identified a network of functionally relevant bacteria-metabolite interactions linked to disease activity in CD. Our data prove that despite the heterogeneity of CD patients gut microbiome at the taxonomic level, shared functional signatures correlate with disease severity. Multi-omics data integration improved the clinical outcome prediction and identified a signature involving sulfur metabolism and detoxification to be relevant in disease outcome.

DIETARY PROTEIN SOURCES DETERMINE OBESITY DEVELOPMENT – A SYSTEMS BIOLOGICAL PERSPECTIVE

P. Rausch[1,*], L. Secher Myrmel[2,*], E. Fjære[2], Z. Xia[3], U. Rolle-Kampczyk[4], M. von Bergen[4], K. Kristiansen[1,3,*], L. Madsen[2,*]

[1]Laboratory of Genomics and Molecular Biomedicine, University of Copenhagen, Denmark
[2]Seafood in model systems, Institute of Marine Research in Norway (IMR), Norway
[3]Institute of Metagenomics, BGI-Shenzhen, China
[4]Department of Molecular Systems Biology, Helmholtz-Zentrum für Umweltforschung GmbH- UFZ, Germany
*equal contributions

BACKGROUND: The interplay of diet and microbial community has been shown to be a major determinant of metabolic syndrome and obesity. Bacterial communities play a central role in digestion and growth of the host and are strongly influenced by dietary patterns, including protein content and source. In particular, protein content and source have been shown to modify obesogenic effects in human- and rodent studies, but how the protein source affects the interplay between diet, bacteria, and host remains to be elucidated.

METHODS: To understand the dynamics leading to differential physiological host responses with regard to protein source, we challenged C57BL/6J mice with high fat/high protein diets using casein, cod, and chicken as protein sources. Using a multi-omics framework we combined data on the host's microbial communities, detailed phenotype data, as well as metabolic-, and transcriptional profiles to characterize and comprehend the complex and intimate interactions between host and bacteria that influence obesity development under different dietary conditions.

RESULTS: We detected robust differences in patterns of microbial, metabolic, and transcriptomic profiles between diets, in particular, between casein and chicken protein-based diets. These differences are expressed through differentially abundant bacteria and community modules among diets (*e.g. Sutterella*, *Oscillospira*), which are highly central and important key factors consistently associated to a multiple metabolic and physiological traits, as well as functional changes in the bacterial community. Important bacteria indicative of chicken protein (*Oscillospira*) are associated to increasing glycerophospholipds, while other central and indicative bacteria for casein (*Streptococcus, Sutterella*) associate to decreasing glycerophospholipds, patterns also paralleled at the transcriptional level.

CONCLUSION: Clusters of host-bacterial interaction types such as weight maintaining, driven by casein, and obesogenic, driven by chicken protein, emerge through the integration of multi-omics data, stressing the importance of a systems biological, holobiont perspective on food science.

POSTER

1 Full-Length 16S rRNA Sequencing Combined with the Illumina Barcode Structure Allows a Deeper Insight into Strains Present in Stool Samples

I. Abellan-Schneyder[1], Z. Sewald[1], I. Lagkouvardos[2], K. Neuhaus[1]

[1]ZIEL - Institute for Food & Health, Core Facility Microbiome, Technische Universität München, Germany
[2]ZIEL - Institute for Food & Health, Junior Research Group Microbial Bioinformatics, Technische Universität München, Germany

Based on their ubiquitous presence, evolutionary stability and changes in its variable sequences, genes of the small ribosomal subunit are the most commonly used phylogenetic marker. Nevertheless, 16S rRNA analyses are mostly performed by short amplicon sequencing for the ribosomal gene of the small subunit (SSU) and are thereby limited. An easy, cheap and user-friendly method for full-length SSU rRNA sequencing should, therefore, be developed. We described a simplified method for full-length SSU rRNA sequencing using the Illumina MiSeq platform. The method has been improved such that the standard Illumina 16S barcode primers can be used and only adaptor primers in addition, which are low in cost are needed. Moreover, the protocol is shortened and tested for a better reproducibility compared to past proposals. By using this approach, deeper insights into species and strains present in an unknown sample are possible. The method allows an easier data evaluation and is cost efficient because it can be easily adapted by all laboratories that already sequence amplicons with Illumina's standard protocol by just adding a limited amount of novel primer.

2 BREAKING DOWN BARRIERS: UNDERSTANDING ANAEROBIC BACTERIA IN THE SKIN

J. Afghani[1,2], M. Reiger[1], C. Traidl-Hoffmann[1,2,3]

[1]Chair and Institute of Environmental Medicine, UNIKA-T, Technical University of Munich and Helmholtz Zentrum München, Augsburg, Germany
[2]Institute of Network Biology, Helmholtz Zentrum München, Munich, Germany
[3]CK CARE, Christine-Kühne Center for Allergy Research and Education, Davos, Switzerland

Atopic eczema (AE) is an inflammatory skin disorder that is already affecting 20% of children worldwide and is increasing in prevalence (Williams H., et al. 2008; Asher MI., et al. 2006). Most of the research done on this topic has focused on the disturbance of the aerobic microbial layer on top of the stratum corneum, but it is known that there are anaerobic microbes lying beneath that skin layer and AE inflammation may be caused by a death of those microbes (Nakatsuji, Teruaki et al. 2013). Since there is no known list of anaerobic bacteria within the skin, an anaerobic skin bacteria catalogue was made by performing a literature search and cross-referencing to existing data from skin microbiome samples obtained at our chair. From this list, several anaerobic bacteria were ordered from the DMSZ for future characterization and experiments. A non-invasive method for culturing anaerobic bacteria from the skin was developed and anaerobic bacteria were also isolated from subjects. In order to collect data that simulates conditions which reflect the skin's physiology in the lower skin layers, we adapted a method to measure growth anaerobically in a 96 well format by using a PVC sealant. Resazurin was used as an oxygen indicator and the wavelength emitted from this chemical was cross-referenced to the optical density measured over time to determine oxygen presence. A few medias were tested in the growth curve with a known strictly anaerobic bacteria *Peptophilus koenoeniae*, and Schaedlers broth supplemented with hemin was found to be the best media for anaerobic growth. This method was then applied to determine the effects of oxygen deprivation on Staphylococcus strains isolated from AE and healthy subjects. *Staphylococcus epidermidis* strains isolated from nonlesional AE and could tolerate anaerobic conditions better than the lesional strain and the pattern of growth between the nonlesional AE strain the anaerobically isolated strain are similar. *Staphylococcus aureus* strains do not tolerate anaerobic conditions as well as aerobic conditions even after supplementation with Hemin and Vitamin K. Because anaerobic bacteria are known to have pH reducing metabolites, such as short chain fatty acids, and that SCFA presence does lower the pH in the gut and possibly other organs, further research will be performed on SCFA production and strain characterization for our select anaerobic skin isolates (LeBlanc J. et al, 2017; Besten G. et al, 2013). The SCFA production will be monitored over time by measuring the pH using bromocresol purple as a visual indicator. Metabolite characterizations will be determined by LC/MS in cooperation with Analytical BioGeoChemistry research unit at Helmholtz Centrum Munich. This research will provide a better understanding of the anaerobic skin barrier and will lead to further investigations as to how the anaerobic bacteria influence AE.

3 TOLL-LIKE RECEPTOR 1: A TARGET IN NON-ALCOHOLIC FATTY LIVER DISEASE?

A. Baumann[1*], A. Nier[1*], A. Hernández-Arriaga[2], A. Brandt[2], CJ. Jin[3], E. Pilar[1], A. Camarinha Silva[2], JM. Schattenberg[4], I. Bergheim[1]

[1]Department of Nutritional Sciences, Molecular Nutritional Science, University of Vienna, Vienna, Austria
[2]Institute of Animal Science, University of Hohenheim, Stuttgart, Germany
[3]Institute of Nutrition, SD Model Systems of Molecular Nutrition, Friedrich-Schiller-University Jena, Jena, Germany
[4]Department of Internal Medicine I, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany
[*]Authors contributed equally

An induction of certain toll-like receptors (TLR) in liver has repeatedly been attributed to the development of non-alcoholic fatty liver disease (NAFLD). However, knowledge on TLR expression patterns in blood cells of patients with NAFLD, their relation to intestinal microbiota and disease development is limited. Here, TLR expression patterns in PBMCs of NAFLD patients and controls, their relation to intestinal microbiota composition and the impact of TLRs found to be altered on NAFLD development was assessed. Blood and stool samples were obtained from 37 NAFLD patients and 14 age-matched healthy controls. Markers of intestinal permeability in blood and expression of TLR and dependent signaling molecules in PBMCs were determined. Fecal microbiota composition was evaluated using 16S rRNA illumina amplicon sequencing. In addition, TLR1-/- and C57BL/6 mice were pair-fed a liquid control or fat-, fructose- and cholesterol-rich (FFC) diet for six weeks and indices of liver damage and inflammation were assessed. Intestinal microbiota composition and markers of intestinal permeability such as zonulin and bacterial endotoxin were significantly different between groups with markers of intestinal permeability being markedly higher in patients with NAFLD than in controls. Expression of TLR1-8 and 10 mRNA was detected in PBMCs; however, only expression of TLR1 was found to be significantly higher in PBMCs of NAFLD patients when compared to controls. Expression of TLR1 was significantly positively correlated with the prevalence of Ruminococccus and Lachnospiracea in fecal microbiota. FFC-fed TLR1-/- mice were significantly protected from the development of NAFLD when compared to wild-type mice. In conclusion, results of the present study suggest that expression pattern of TLRs in PBMCs in NAFLD patients is only slightly altered. Still, targeting these alterations might be a beneficial approach in the treatment of NAFLD.

4 CHARACTERIZATION OF MICROBIAL CO-OPERATION DURING HOMEOSTASIS AND INFECTION USING METABOLOMICS AND GNOTOBIOTIC MOUSE MODELS

A. Bublitz[1,2], K. Hiller[1,2], T. Strowig[2]

[1]Bioinformatics & Biochemistry, Technical University Braunschweig
[2]Microbial Immune Regulation, Helmholtz Centre for infection research Braunschweig, Germany

The mammalian microbiota consists of several hundred species of bacteria that form a very complex food network and occupy an array of nutritional niches along the gastrointestinal tract. In healthy individuals, this community has the capability to protect the host against invading pathogens in a process called 'colonization resistance' (CR). Antibiotic treatment can disrupt the microbiota and affect the composition and variety of this community, potentially leading to a weakened CR and an environment in which pathogens like *Clostridioides difficile* might flourish. However due to the sheer limitless interactions between bacteria, host and environment, CR is still poorly understood and challenging to study in vivo. This is why I aim to utilize gnotobiotic mouse models and in vitro cultivation of defined microbial communities to characterize metabolic interactions between bacteria that can promote CR. An interesting gnotobiotic mouse model is the Oligo-Mouse-Microbiota (OMM-12), which allows the study of CR against *Salmonella enterica* serovar Typhimurium[1]. Furthermore, I want to investigate how the addition of commensals (*Prevotella* spp.) and pathogenic bacteria (*C. difficile*) can disrupt the metabolic flux within these communities. An important step in this research is the establishment of proper metabolomic analysis of microbial communities *in vivo* and *in vitro* with GC:MS, which is currently in the process.

[1] Brugiroux S. *et al, Nature Microbiology*, 2017

5 THE ROLE OF HOST GENOTYPE IN SHAPING THE OUTCOME OF ANTIBIOTIC TREATMENT

A. Cepic[1], R. Riedel[1], P. Rausch[2], G.A. Grassl[3], J.F. Baines[1]

[1]Evolutionary Genomics Group, Institute for Experimental Medicine, Christian Albrechts University, Kiel, and Max Planck Institute for Evolutionary Biology, Plön, Germany
[2]Cell Biology and Physiology, Department of Biology, University of Copenhagen, Denmark
[3]Institute of Medical Microbiology and Hospital Epidemiology and German Center for Infection Research (DZIF), Hannover, Germany

Glycans on host mucosal surfaces mediate host-microbe interactions and influence the composition of commensal microbiota. In a murine model of Salmonella-induced colitis the lack of intestinal expression of the glycosyltransferase gene *B4galnt2* was associated with reduced gut inflammation. Importantly, this mouse model requires antibiotic treatment to break the colonization resistance to S. Typhimurium, which is mediated by host commensal gut microbiota. The effect of *B4galnt2*-expression on the severity of inflammation in the experiment negatively correlated with the extent of change in microbiota composition before and after infection with *S.* Typhimurium. Fecal transfer experiments demonstrated that the effect is dependent on the *B4galnt2* genotype-specific microbiota rather than *B4galnt2* expression itself. Shotgun functional metagenomic sequencing of the fecal microbiota revealed that in mice deficient for *B4galnt2* expression in the gut, commensal microbiota have a significantly higher abundance of efflux pump genes implicated in bacterial resistance to heavy metals. We therefore hypothesized that the observed difference in severity of inflammation could result from an indirect effect, by which a higher abundance in efflux pump genes within the commensal microbiota is associated with increased residual colonization resistance, e.g. by conferring increased resistance to the streptomycin treatment used in the *S.* Typhimurium colitis model. Thus, in this project we are investigating whether the differences observed in the *S.* Typhimurium-induced colitis model are due to differences in the sensitivity of the commensal microbiota to antibiotics. Specifically, we are investigating the effect of several antibiotics for which the expected resistance mechanisms differ with respect to the involvement of efflux pumps (streptomycin, kanamycin, vancomycin and colistin).

6 INTRACELLULAR SURVIVAL OF *SALMONELLA TYPHIMURIUM* IS DEPENDENT ON HOST PROTEOGLYCANS

A. Galeev[1], A. Oktiviyari[1], A. Suwandi[1], F. Routier[2], H. Bakker[2], M. Hensel[3], G.A. Grassl[1]

[1]Institute of Medical Microbiology and Hospital Epidemiology, Hannover Medical School and German Center for Infection Research (DZIF), Hannover, Germany
[2]Institute of Clinical Biochemistry, Hannover Medical School, Hannover, Germany
[3]Division of Microbiology, Osnabrück University, Osnabrück, Germany.

Cell surface proteoglycans are heavily glycosylated proteins involved in cell-matrix interactions and cell adhesion. Previous studies highlighted a significance of proteoglycans for bacterial adhesion and invasion. Nevertheless, the role of proteoglycans in *Salmonella* pathogenesis is poorly understood. Proteoglycans consist of glycosaminoglycans (GAGs), namely, chondroitin sulfate, heparan sulfate, heparin and dermatan sulfate, attached to the core protein. An assembly on of GAGs is initiated by the β-D-xylosyltransferase enzyme encoded by the *XylT2* gene. In this work, we evaluated an effect of *XylT2* deletion on *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium) colonization of epithelial cells. Invasion and replication of *S*. Typhimurium in Chinese hamster ovary (CHO) cells were assessed by gentamicin protection assay. Gentamicin uptake by the wild-type CHO and xylosyltransferase-deficient mutant (Δ*XylT2*) was measured by ELISA. Expression of the transient receptor potential cation channel subfamily V (Trpv)-encoding genes, *Trpv1* and *Trpv4,* was measured by RT-qPCR. Intracellular localization of *Salmonella* in infected CHO cells was determined by applying the SPI-2 reporter/mutant strains of *S*. Typhimurium, immunostaining and chloroquine resistance assay. Adhesion to and invasion into CHO WT and CHO Δ*XylT2* cells by *S*. Typhimurium was comparable. In contrast, 24 hours after infection, proteoglycan-deficient cells were significantly less colonized by *S*. Typhimurium compared to WT control cells. However, the levels of intracellular gentamicin, as well as the levels of *Trpv1* and *Trpv4* expression were comparable between the studied CHO cell lines. Interestingly, chloroquine resistance assay and immunostaining revealed that an absence of proteoglycans resulted in reduced number of bacteria in *Salmonella*-containing vacuoles (SCV) within gentamicin-treated CHO Δ*XylT2* cells as compared to WT CHO cells. Infection of CHO cells with *S*. Typhimurium strains unable to establish either SCV or SIF (*Salmonella*-induced filaments) corroborated a role of proteoglycans in a survival of the bacteria in modified membranes. Taken together, our results indicate that intracellular survival of *Salmonella* in the gentamicin protection model is dependent on host proteoglycans.

7 "*IN VIVTRO*": A 3D CELL CULTURE MODEL TO STUDY THE CELLULAR IMPACT OF PROBIOTIC *E. COLI* NISSLE 1917 AND NISSLE VARIANTS ON THE HOST

J. Gonzalez[1], R. von Bünau[2], U. Dobrindt[3], C. Cichon[1]

[1]*Institute of Infectiology - ZMBE, University of Münster, Germany*
[2]*Pharma-Zentrale GmbH, Germany*
[3]*Institute of Hygiene, University of Münster, Germany*

Infectious diseases are still a major cause for death worldwide. With regard to the globally increasing problems of antibiotic resistance, there is an urgent need for the development of novel therapeutics and/or new preventive strategies (e.g. probiotics). To speed up the development, new *in vitro* cell culture models are necessary that bridge the gap between cell culture based research and more complex *in vivo* animal models. There has been a recent shift from the original "flat biology" approach (2D) to three-dimensional models (3D) of human intestinal epithelia which provide a more precise *"in vivtro"* possibility to mimic the *in vivo* host-microbe interactions and crosstalk.

Employing the mucus-producing human colonic cell line HT-29 MTX E12, we compared cellular responses after co-incubation with the probiotic *E. coli* strain Nissle 1917 and a first set of Nissle variants (*fliM*, *ycgR* and *opgH* gene), re-isolated from infants up to two years after the first inoculation.

Growing HT29 MTX-E12 cells on Transwell® Inserts ("2.5D") results in cell polarization and the formation of a functional junctional complex between host cells [1]. Nevertheless, gastrointestinal epithelial cells grown in standard transwell cultures still do not mimic a complex mucosal surface. A range of 3D cell culture systems exist by now [2]. We employ the NASA-developed rotating wall vessel (RWV) bioreactor, which has been used for studying the cellular and molecular responses of hosts and microbes. The RWV allows dynamic culture conditions of epithelial cells under low physiological fluid shear stress which influences cellular differentiation. Furthermore, cells growing on Cytodex-3 micro-carrier beads (collagen type-I-coated) express a 3D tissue-like matrix.

Our preliminary results derived from gene expression profiles of HT-29 MTX cells (RT^2 Profiler Arrays, Qiagen), revealed distinct differences in host cell responses comparing different cell culture models, strikingly when looking at "static" (24 well plates) vs. "rotating" (4ml rotating tubes) infection procedures. To approximate the molecular mechanisms we put a particular emphasis on the fine tuning of host responses by miRNAs. Our data on the one hand side might provide a better understanding of the fundamental mechanisms governing host-microbe (probiotic) interactions and in the end, pave the way for a faster translation "from bench to bedside" (application of probiotics) but they also create doubt concerning 2D cell culture infection models.

[1] Navabi N, et *al*. (2013) Gastrointestinal cell lines from polarized epithelia with an adherent mucus layer when cultured in semi-wet interfaces with mechanical stimulation. PloS One. 8:e68761.
[2] Barrila J, et *al*. (2018) Modeling Host-Pathogen Interactions in the Context of the Microenvironment: Three-Dimensional Cell Culture Comes of Age. Infect Immun. 86(11): e00282-18.

8 USING AN INTESTINAL ORGANOID MODEL TO STUDY THE IMPACT OF THE INTESTINAL MICROBIOTA ON IMMUNE-EPITHELIAL CELL CROSSTALK

K. Heiking[1], S. Freytag[1], HD. Chang[1]

[1]German Rheumatism Research Center (DRFZ) Berlin, a Leibniz institute, Germany

Several diseases of the intestinal system, such as Crohn's disease, ulcerative colitis, and intestinal cancer, have been linked to dysbiosis of the microbiota. Intestinal organoids present a useful in vitro model to study the effect of microbiota on intra- and intercellular signaling in the intestine, thus potentially allowing insight into the development of gut diseases. Understanding the complex interactions between intestinal microbiota and its host may allow the identification and characterization of bacterial subpopulations that induce pro- or anti-inflammatory reactions, thereby promoting or preventing intestinal disease.

The aim of this study is the investigation of the impact of intestinal microbiota on the cellular crosstalk between IEC and immune cells, characterizing inflammatory responses of host IECs to potentially pathogenic microbiota populations. Therefore, a two-dimensional organoid monolayer culture will be used for the analysis of IEC responses in the presence of microbiota samples from healthy and diseased donors. To single out pro- and anti-inflammatory microbiota components, the samples will be analyzed and subdivided with high resolution microbiota flow cytometry. Defined bacteria populations will then be applied to intestinal organoid monolayers, narrowing down the immune regulating properties of these populations and potentially identifying driving pathogens in certain intestinal diseases.

Furthermore, co-cultures of two-dimensional organoid monolayers with IELs and other immune cells will be cultivated with specific microbiota compositions to gain insights into the immune-epithelial cell crosstalk in health, during pathogenesis, and in response to specific pro- and anti-inflammatory microbiota.

9 MITOCHONDRIAL IMPAIRMENT IN CROHN'S DISEASE DRIVES INTESTINAL STEM CELL TRANSITION TOWARDS DYSFUNCTIONAL PANETH CELLS

S. Khaloian[1], E. Rath[1], E. Gleisinger[1], E. Berger[1], A. Metwaly[1], N. Waldschmitt[1], N. Hammoudi[2], M. Allez[2], D. Haller[1,3]

[1]Technische Universität München, Chair of Nutrition and Immunology, 85354 Freising-Weihenstephan, Germany.
[2]APHP, Hôpital Saint Louis, Department of Gastroenterology, INSERM UMRS 1160, Paris Diderot, Sorbonne Paris-Cité University, Paris, France
[3]Technische Universität München, ZIEL Institute for Food & Health, Germany.

Paneth cells (PC) reside at close proximity to Lgr5^{+} stem cells at the crypt base, providing the antimicrobial peptides and niche factors required for maintenance of the intestinal stem cells (ISC). PC dysfunction is contributing to the pathogenesis of Crohn's disease. To characterize the role of mitochondrial function on ISC niche including PC, we used TNF$^{\Delta ARE}$ mice, a model for Crohn´s disease (CD)-like ileitis, ileal tissue samples from CD patients and mice lacking Hsp60 in ISC (Hsp60$^{\Delta ISC}$). Consequences of respiratory chain and glycolysis inhibition on intestinal epithelial cell (IEC) homeostasis were further investigated using ex vivo small intestinal organoid culture. Inflammation in TNF$^{\Delta ARE}$ mice was correlating with reduced PC granularity and diffuse Lyz staining. Reduced antimicrobial peptides (*Ang4* and *Defa5*) expressions were further indicative of PC dysfunction. Inflamed ileal tissue sections from CD patients confirmed the distorted PC appearance. Beside PC dysfunction, reduced stemness, characterized by reduced Lgr5 expression in crypt base, was detected in TNF$^{\Delta ARE}$ mice as well as inflamed CD patients. Most importantly, the appearance of a low-granular PC and re-localization of Lgr5^{+} cells in non-inflamed tissues predicted disease recurrence of CD patients after surgical resection. Parallel to the inflammation, confirming the impact of the mitochondrial homeostasis on PC function and ISC maintenance, Hsp60$^{\Delta ISC}$ mice showed an elevated numbers of dysfunctional PC and transient reduction of *Lgr5* expression. Increased numbers of *Lgr5*$^{+}$-Lyz^{+} cells and HSP60^{-}-Lyz^{+} cells in absence of apoptosis or necrosis at the crypt base indicated differentiation of ISC into dysfunctional PC upon mitochondrial impairment. Reduced stemness and PC *in vivo*. resulted in an inability of crypts derived from TNF$^{\Delta ARE}$ mice to give rise to organoids *ex vivo*. However, DCA-mediated inhibition of glycolysis, rescued TNF$^{\Delta ARE}$ mice-derived crypt cultures to form sustainable organoids. Our results indicate that inflammation triggers mitochondrial dysfunction, driving the stem cell niche towards a dysfunctional PC phenotype, and is predictive for disease recurrence of CD patients after surgical resection. Maintenance of mitochondrial respiration may represent a novel drug target to antagonize PC dysfunction in the pathogenesis of CD.

10 THE IMMUNOMODULATORY ROLE OF CD14 IN PROINFLAMMATORY AND REGULATORY PATHWAYS: A LIPOPOLYSACCHARIDE STIMULATION MODEL IN BONE MARROW DERIVED MACROPHAGES

L. Magerkurth[1], S. Lienenklaus[1], N. Ruangkiattikul[2], R. Goethe[2], A. Bleich[1], S. Buchheister[1]

[1]Institute for Laboratory Animal Science and Central Animal Facility, Hannover Medical School, Hanover, Germany
[2]Institute for Microbiology, University of Veterinary Medicine, Foundation, Hanover, Germany

Disturbance of the gastrointestinal homeostasis plays a key role in inflammatory bowel disease (IBD) development. Although the exact pathomechanisms of IBD are still not fully understood, there is profound evidence that intestinal inflammation is mainly driven by proinflammatory macrophages. We have previously shown that the “cluster of differentiation 14” (CD14) protein has a protective function in experimental IBD (BUCHHEISTER, 2017). CD14 is an important co-receptor of toll-like receptors (TLR), essential for recognizing pathogen associated molecule patterns (PAMPs). Ligand induced activation results in formation of binding complexes and subsequent activation of proinflammatory and regulatory pathways. In the case of TLR4, CD14 binds the circulating lipopolysaccharide (LPS) which leads to nuclear factor ‘kappa-light-chain-enhancer’ of activated b-cells NfκB activation and production of proinflammatory cytokines like tumor necrosis factor α (TNFα). Furthermore, CD14 dependent TLR internalization results in release of type I interferons (IFN) like IFN-β. In addition CD14 has been shown to limit chronic inflammation through induction of tolerance after continuous stimulation (SAHAY et al., 2009). The aim of this study is to unravel the functions of CD14 in these two downstream pathways of TLR-signaling. Therefore we compared BMDMs, generated from wildtype and *Cd14*$^{-/-}$ mice, after LPS stimulation. To mimic the continuous presence of stimuli in the gut after barrier impairment we also performed repeated stimulation. To assess TNFα we made use of classical quantitative real time polymerase chain reaction (qRT PCR) and analyzed cell samples collected at different time points. For IFN-β analysis we established a reporter system co-expressing IFN-β and luciferase that allows life monitoring of gene expression. TNFα expression was strongly induced after exposure to LPS independent of CD14. Re-exposure to LPS hardly induced TNFα levels in wild-type controls, whereas *Cd14*$^{-/-}$-macrophages did not decrease TNFα protein production and even strikingly increased *Tnfα* gene expression. On the contrary, IFN-β induction strictly depends on CD14, both after the first and the second stimulation. Life imaging reveals IFN-β induction already after 1h and immediate induction upon re-stimulation. These findings underline that CD14 has anti-inflammatory capacities during the acute phase of inflammation and might therefore play a crucial role in immunomodulatory processes during IBD development.

11 DIETARY WHEAT AMYLASE TRYPSIN INHIBITORS MODIFY THE GUT MICROBIOME BY ANTIMICROBIAL ACTIVITY AND AGGRAVATES EXPERIMENTAL COLITIS

G. Pickert[1], J. Matzner[1], S. Wirtz[2], R. Heck[1], D. Thies[1], M. Ashfaq-Khan[1], D. Ehmann[3], J. Wehkamp[3], D. Schuppan[1,4]

[1]Institute of Translational Immunology, Research Center for Immunotherapy, Univ. Medical Center, Johannes Gutenberg University Mainz, Germany
[2]Department of Medicine 1, Friedrich-Alexander-University, Erlangen, Germany
[3]Department of Internal Medicine 1, University Hospital Tübingen, Germany
[4]Division of Gastroenterology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, USA

Background & Aims Chronic inflammation in the gastrointestinal tract, as seen in inflammatory bowel disease (IBD), arises from a failure to maintain tolerance to harmless bacterial and/or food antigens. It is likely that yet ill-defined triggers, especially certain nutrients or microbes, combined with a proinflammatory genetic predisposition, can switch the intestinal immune system from a tolerogenic to a proinflammatory state. We have recently identified wheat amylase trypsin inhibitors (ATI) as potent activators of innate immunity by engaging the toll like receptor 4 (TLR4)-MD2-CD14 complex in monocytes, macrophages and dendritic cells. We studied the effect of nutritional ATI on the intestinal inflammation in mouse models of intestinal inflammation, with a focus on their effects on the microbiome.

Methods C57BL/6 mice were fed a defined diet with ATI or a ATI-free diet for 4 weeks and challenged with 1.5% dextran sodium sulfate (DSS) for 10-14 days. ATI-feeding significantly worsened the outcome of DSS colitis, including loss of body weight, inflammatory cytokine/chemokine expression, epithelial/mucosal damage, and disease activity as assessed by endoscopy. Using 16S ribosomal RNA metagenomic sequencing, we analyzed common fecal bacteria and studied the relevance of ATI-conditioned microbiota on intestinal inflammation after broad-spectrum antibiotic treatment and fecal transplantation. Further, we studied potential antimicrobial activity of ATI on selected human gut bacteria by radial diffusion assays.

Results Nutritional ATI at a daily dose that matches human daily wheat consumption induced enhanced DSS-induced mucosal damage. They also changed the intestinal microbiota towards a dysbiotic pattern, by increasing, e.g. *Desulfovibrionaceae* and by decreasing, e.g. *Lactobacillaceae*. When tested in vitro, ATI exhibited a species-specific and dose-dependent antibiotic effect on common human gut commensals, favouring dysbiotic species and suppressing the growth of potentially beneficial species like *Lactobacillus fermentum.* When microbiota of ATI-fed mice were transplanted into gut-sterilized mice treated with DSS, the recipients developed a more severe colitis than recipients of microbiota from ATI-free mice.

Conclusions 1) Dietary wheat ATI enhance intestinal inflammation, while an ATI-free diet has a protective effect; 2) Pro-inflammatory effects of nutritional ATI go along with the induction of severe intestinal dysbiosis; 3) Wheat ATI have a species-specific and dose-dependent antibiotic effect on common human gut commensals; 4) Fecal microbiota of ATI-fed mice enhance DSS-colitis in recipients; 5) ATI (“gluten”) free diets may benefit patients with IBD.

12 SEGMENTED FILAMENTOUS BACTERIA ARE ENRICHED IN CROHN'S DISEASE AND CAUSE ILEO-COLONIC IINFLAMMATION IN TNFDARE MICE

J. Calasan[1], N. Waldschmitt[1], A. Metwaly[1], M. Ahmed[1], S. Khaloian[1], M. Basic[2], A. Mayorgas[5], J. Panes[5], A. Salas[5] H. Flint[3], A. Bleich[2], F. Cominelli[4], D. Haller[1,6]

[1]Technische Universität München, Chair of Nutrition and Immunology, Freising-Weihenstephan, Germany
[2]Hannover Medical School, Institute for Laboratory Animal Science, Hannover, Germany
[3]Gut Health Group, The Rowett Institute, University of Aberdeen, Aberdeen, United Kingdom
[4]Digestive Health Research Institute, Case Western Reserve University School of Medicine, Cleveland, OH, USA
[5]Department of Experimental Pathology, Instituto de Investigaciones Biomédicas de Barcelona CSIC, IDIBAPS, CIBERehd, Spain
[6]Technische Universität München, ZIEL Institute for Food & Health, Germany

Background. The causative role of dysbiotic intestinal microbiota in the pathogenesis of Crohn's disease (CD) remains largely elusive. CD-like ileitis in TNFΔARE/+ (ARE) mice is triggered by dysbiotic microbial ecosystems that induce a transmissive inflammatory phenotype associated with an increased expression of IL17. However, the mechanisms of microbe-host interactions in the development of TNF-driven CD-like inflammation remain to be elucidated.

Methods. Microbial communities in ARE and wildtype (WT) mice were profiled by 16S rRNA gene amplicon sequencing, FISH and qPCR. Inflammatory potential of certain bacterial species was evaluated by colonizing germfree (GF) mice with single bacterial strains, including segmented filamentous bacteria (SFB), the human SFB-like *Bifidobacterium adolescentis* (BA) L2-32, and Alistipes sp. isolated from dysbiotic ARE mice. Disease activity, cytokine expression, mucosal immune cell infiltrates as well as Paneth and goblet cells numbers were assessed in murine ileal, caecal and colonic tissue sections. Ileal and colonic biopsies from CD patients (N=112) and healthy individuals (N=45) were collected from inflamed and adjacent non-inflamed mucosal tissue regions. Microbial profiles and SFB abundance in human tissue samples were characterized.

Results. Newly imported ARE mice gradually developed CD-like inflammatory lesions in ileal tissue sections within 3 generations of breeding. Dysbiosis was associated with increasing abundance of SFB. Consistent with CD-like ileitis in ARE mice, SFB abundance was increased in both ileum and colon in CD patients with ileo-colonic phenotype. Monocolonization of GF ARE mice with SFB resulted in severe enterocolitis affecting ileum, cecum and colon, while colonization with BA L2-32 and Alistipes sp. completely failed to induce inflammation. Parallel to high tissue levels of TNF and IL-17, SPF-mediated inflammation was associated with neutrophil infiltration and the expansion of IFNγ expressing Th1 cells in the mucosa. Loss of Paneth and goblet cell function allowed SFB to penetrate mucus layers reaching close proximity to the epithelium in inflamed ARE mice.

Conclusion. We demonstrated that ileo-colonic CD in human and mice is associated with dysbiosis and increased abundance of SFB. Causality of SPF-mediated ileo-colonic inflammation was confirmed in monocolonized GF ARE mice, suggesting a new pathological role of SFB in the pathogenesis of inflammatory bowel diseases.

13 INTERPLAY OF *CANDIDA ALBICANS* AND *ENTEROCOCCUS FAECALIS* ENHANCES HOST CELL DAMAGE

M. Kapitan[1,2,3], MJ. Niemiec[1,3], M. Swidergall[4], S. Höring[5], T. Monsen[6], KA. Kline[7], B. Löffler[2,3,5], ID. Jacobsen[1,2,3]

[1]*Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena*
[2]*Friedrich Schiller University Jena*
[3]*Center for Sepsis Control and Care, University Clinic Jena, Jena*
[4]*Division of Infectious Diseases, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center Torrance, California, USA*
[5]*Department of Medical Microbiology, University Hospital Jena, Jena*
[6]*Department of Clinical Microbiology, University Hospital Norrland, Umeå*
[7]*Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore*

Life-threatening disseminated candidiasis is commonly caused endogenously by microbes colonizing mucosal surfaces. As a commensal and pathogen, *C. albicans* interacts not only with the host but also with members of the bacterial microbiota. Enterococci, especially *E. faecalis*, often accompany *Candida* during mixed infections. In critically-ill patients, and also mimicked in dysbiotic mice, *C. albicans* and *E. faecalis* dominate the low-diversity community resulting from antibiotic treatment.

We performed coinfection of human enterocytes to determine if interactions between *Candida* and enterococci affect host cell damage. This revealed that *E. faecalis-C. albicans* interactions are highly dependent on the bacterial strain. Of 20 *E. faecalis* strains 5 led to synergistic coinfection damage, while the others behaved neutral or protective compared to monoinfections. Early bacterial internalization into enterocytes was enhanced for a synergistic strain compared to a non-synergistic one; however at later time points these numbers were reversed, suggesting that synergistic coinfection damage is mainly caused by a yet unknown fungal-induced virulence factor that some *E. faecalis* strains lack.

These findings were successfully transferred into a mouse *in vivo* coinfection model of oral candidiasis. While a protective *E. faecalis* strain reduced *Candida* tissue invasion and apoptosis, a synergistic strain drastically worsened the clinical outcome with bigger lesions, more fungal invasion and host cell apoptosis.

In summary, we show high diversity in *E. faecalis-C. albicans* interactions, with strain-dependent synergistic host cell damage *in vitro* and *in vivo*. Additional data suggests that the induced damage is primarily caused by the bacteria, but both interaction partners contribute to host cell damage. Further experiments indicate metabolic cross-talk as the mechanism enhancing bacterial virulence.

14 BACTERIAL PERSPECTIVES ON EVOLVING AN ASSOCIATION WITH A HOST

N. Obeng[1] and H. Schulenburg[1,2]

[1]Evolutionary Ecology and Genetics, Zoological Institute, University Kiel, 24118 Kiel, Germany;
[2]Max Planck Institute for Evolutionary Biology, 24306 Plön, Germany

In the early stages of symbiosis, free-living bacteria shifted to a host-associated lifestyle. At this transition, bacteria follow a biphasic life cycle: switching between hosts and alternative habitats. To examine how this affects bacteria and host alike, we imposed such a life cycle on bacteria in an evolution experiment. We asked how bacteria responded to a biphasic life history, i.e., with the nematode host *Caenorhabditis elegans* and a free-living state, versus free-living only. Specifically, we evolved two key members of the *C. elegans* microbiome, *Pseudomonas lurida* (MYb11) and *Ochrobactrum* sp. (MYb71), as well as the food bacterium *Escherichia coli* OP50, to study which traits are required to establish and maintain the association with the host, and which are lost in its absence. Here, we focus on i) the transmission strategies bacteria favored in the biphasic versus free-living passaging regime, and ii) the fitness consequences of bacterial adaptation for both bacteria and their host. This study integrates the environmental context into host-microbe interactions and provides an eco-evolutionary perspective on the first evolutionary steps towards symbiosis.

15 INTERACTIONS OF THE BILE PIGMENT BILIRUBIN AND INTESTINAL BACTERIA MAY CONTRIBUTE TO COLORECTAL CANCER RISK

S. Ocvirk[1,2], B. Brandl[3], T. Skurk[3], M. Blaut[1], H. Hauner[3,4,5]

[1]Department of Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany
[2]Division of Gastroenterology, Hepatology and Nutrition, Department of Medicine, University of Pittsburgh, USA
[3]ZIEL - Institute for Food & Health, Technical University of Munich, Freising, Germany
[4]Chair of Nutritional Medicine, Technical University of Munich, Freising, Germany
[5]Institute of Nutritional Medicine, Klinikum rechts der Isar, Technical University of Munich, Munich Germany

In contrast to bile acids, the contribution of bile pigments, in particular bilirubin, to host microbial co-metabolism and colorectal cancer risk (CRC) remains unclear. Gilbert's syndrome (GS), a benign genetic condition that leads to reduced levels of bilirubin in bile, shows a significant inverse correlation with CRC risk. We hypothesize that this may be explained by altered levels of bilirubin affecting microbiota composition and metabolism in the colon.

Different commensal bacteria were incubated with bilirubin to analyze its antimicrobial activity. To recruit a GS cohort, we quantified plasma bilirubin levels in 353 healthy individuals from the *enable* cohorts. Genotyping for GS-associated polymorphisms was performed in individuals with plasma bilirubin levels higher than 16 µmol/L. Microbiota composition was determined using 16S rRNA gene sequencing and complemented with targeted analysis of functional microbial genes (by qPCR) and fecal short-chain fatty acids (using GC).

Incubation of commensal bacteria with bilirubin confirmed a dose-dependent antimicrobial effect on Gram-positive bacteria. In contrast, Gram-negative commensal bacteria were not affected by exposure to physiological bilirubin concentrations, suggesting a differential effect on microbial growth. From 72 healthy study participants showing increased plasma bilirubin levels, 19 were identified to be homozygous and 17 to be heterozygous for the main GS polymorphism. This was confirmed by significantly increased bilirubin levels in plasma compared to healthy controls (n=27) (homozygous = 24.7±1.6 (SEM) µmol/L, heterozygous = 12.6±0.7 µmol/L, control = 8.4±1.3 µmol/L). Compared to controls, the GS cohorts showed a significantly different abundance of some microbial genera in feces (e.g., *Clostridium XIVa*, *Sutterella*) and altered levels of functional microbial genes involved in bilirubin metabolism. While GS-associated bilirubin levels had overall minor effects on shifts in microbiota composition, changes paralleled the differential growth pattern observed *in vitro*.

Bilirubin shows dose-dependent antimicrobial effects. This may account for shifts in gut microbial co-metabolism observed in GS individuals that have an altered bilirubin metabolism. We aim to identify, if bilirubin-dependent changes in the gut microbiota may contribute to the significantly lower CRC risk associated with GS.

16 COMPLEX MICROBIOTA COMPOSITION DETERMINES COLITIS SEVERITY IN THE IL10-DEFICIENT MOUSE MODEL

S. Bolsega[1], L. Osbelt[2], A. Smoczek[1], KA. Odum[1], T. Strowig[2], A. Bleich[1], M. Basic[1]

[1]*Institute for Laboratory Animal Science, Hannover Medical School, Hannover, Germany*
[2]*Research Group Microbial Immune Regulation, Helmholtz Centre for Infection Research, Braunschweig, Germany*

The intestinal microbiota forms a highly complex ecosystem implicated in the host´s health and disease. Changes in the intestinal microbiota composition and function have been associated with the development of many human diseases such as inflammatory bowel disease (IBD), which is a multifactorial chronic disease of the gastrointestinal mucosa. However, it is still unclear whether changes in the composition are the cause or the consequence of the disease. Therefore, the aim of this study was to evaluate the contribution of the complex microbiota to colitis development and severity in an IL10-deficient (*Il10*$^{-/-}$) mouse model, a model of experimental IBD. To assess the impact of the microbiota on colitis onset, *Il10*$^{-/-}$ mice on B6 and C3H background housed in three different barriers (two strict specific pathogen free (SPF) and one experimental SPF) were analysed at 6, 9 and 12 weeks of age. *Il10*$^{-/-}$ mice of both backgrounds housed in strict SPF units developed with 12 weeks of age mild form of colitis, whereas mice housed in experimental SPF developed moderate to severe inflammation in the colon characterized by infiltration of inflammatory cells and epithelial hyperplasia. Furthermore, mice housed in experimental SPF displayed increased expression of pro-inflammatory cytokines such as *Ifn*□, *Tnf*□ and *Il1*□ and reduced gene expression of mucin 2 and sealing tight junctions. 16S rRNA gene sequencing of fecal microbiota showed that microbiota compositions is distinct in all three analysed barriers and that samples cluster together based on their origin and genetic background, but not disease severity. Furthermore, mice housed in experimental SPF barrier showed a higher diversity, an increased Bacteroidetes/Firmicutes ratio and lower relative abundances of Lachnospiraceae and Ruminococcaceae family than mice housed in strict SPF. Additionally, mice housed in strict SPF area showed lower abundance of family S24-7 and Verucomicrobiacecae. Moreover, microbiota composition stayed stable throughout the experiment and was not influenced by occurring inflammation. Altogether our results showed that different microbiota composition alters the disease severity in *Il10*$^{-/-}$ mice.

17 IRON-DRIVEN HOST-MICROBIOTA COADAPTATION: AN INFLUENCER DURING *MYCOBACTERIUM TUBERCULOSIS* INFECTION?

J. E. Ojong[1], E. Meyron-Holtz[2], M. Hauptmann[1], UE. Schaible[1*]

[1]Cellular Microbiology, Research Area Infection, Research Center Borstel - Leibniz Lung Center, Borstel, Germany
[2]Technion-Israel Institute of Technology, Haifa, Israel

Growth and virulence of *Mycobacterium tuberculosis* (*Mtb*), the etiological agent of tuberculosis, depends highly on iron availability. Iron is equally indispensable for growth, differentiation, survival and host defense functions of mammalian host cells, the intracellular bacterial pathogen as well as the microbiota. A deranged microbiota may contribute to exacerbation of pulmonary TB or pave the way for co-infections, which then may influence disease progression on their part. Consequently, *Mtb*, host and microbiota are engaged in a tug-of-war for iron. We therefore hypothesize that iron availability is an influencer for host-microbiota coadaptation, which in turn can modulate the susceptibility to *Mtb* infection.

To investigate the effects of iron availability on the outcome of *Mtb* infection and homeostasis of microbiota in gut and lung, we study aberrations in cellular iron metabolism by using mice deficient for iron regulatory proteins, IRP1 or IRP2. Both proteins post transcriptionally modulate iron transport and iron storage protein expression. *Irp1*$^{-/-}$ and *Irp2*$^{-/-}$ and wildtype (wt) mice were infected via aerosol route with virulent *Mtb* H37Rv. Colony forming unit (CFU) assays revealed increased mycobacterial burden in the lungs and spleens of *Irp2*$^{-/-}$ mice in comparison to wt ones. Similarly, *Irp2*$^{-/-}$ bone marrow-derived macrophages (BMMOs) were more permissive to *Mtb* infection *in vitro* than wt and *Irp1*$^{-/-}$ BMMOs. Prussian blue staining revealed cell specific iron overload in alveolar macrophages (aMOs) of both, non-infected and *Mtb* infected *Irp2*$^{-/-}$ mice at day 63 post infection (p.i.). Prussian blue positive iron overloaded Kupffer cells in *Irp2*$^{-/-}$ livers were observed at day 63 p.i., in perivascular infiltrates around the central vein and sinusoids, which was opposite to Kupffer cells of wt and *Irp1*$^{-/-}$ mice. These data indicate that macrophage iron overload promotes Mtb growth in *Irp2*$^{-/-}$ mice.

To study the influence of iron availability on commensal communities, we applied 16S rRNA sequencing of caecum microbiota from *Irp1*$^{-/-}$ and *Irp2*$^{-/-}$ mice and their respective wt-siblings after heterozygous breeding. The caecum microbiota in *Irp2*$^{-/-}$ mice demonstrated a decreased Bacteroidetes / Firmicutes ratio compared to their wt-siblings, which has been described before as predisposition to infection and enhanced inflammatory responses. Our next steps will involve 16S rRNA analysis from lung tissue of *Irp1*$^{-/-}$ and *Irp2*$^{-/-}$ mice compared to their respective wt littermates. Ultimately, changes in gut and lung microbiota will be studied in *Irp1*$^{-/-}$, *Irp2*$^{-/-}$ and wt mice upon Mtb infection. These data will provide the basis for ecological analysis regarding changes in microbial community and interactions between microbiota and *Mtb* to potentially reveal new microbiota-based therapeutic interventions in order to combat TB more effectively.

18 THE ROLE OF GUT MICROBIOTA IN INTESTINAL LIPID ABSORPTION

J. Plagge[1], G. Liebisch[2], M. Hoering[2], E. Slack[3], M. Basic[4], J. Ecker[1]

[1]ZIEL – Institute for Food & Health, Technical University of Munich, Germany
[2]Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Germany
[3]Food Immunology, ETH, Zürich, Switzerland
[4]Institute for Laboratory Animal Science and Central Animal Facility, Hannover Medical School, Hannover, Germany.

Uptake of dietary lipids which occur mainly as triacylglycerols is a three-step process comprising absorption of dietary fatty acids from the intestinal lumen, further trafficking from the apical to the basolateral site of enterocytes, and their secretion into the circulation. In the intestinal lumen, bile acids emulsify lipids, allowing their subsequent hydrolysis by pancreatic lipases to free fatty acids. After uptake into the enterocytes, fatty acids can be re-esterified to form triacylglycerols which are either stored in lipid droplets or secreted into the lymphatic system for distribution. While it is known that the gut microbiota alters the bile acid profile by converting primary to secondary bile acids, the consequences for intestinal lipid uptake are largely unknown.

To elucidate the influence of gut microbiota on dietary lipid uptake and to reveal underlying mechanisms, intestinal fatty acid absorption was tracked using a stable isotope labeling approach. A mixture of deuterated palmitic acid D_5-16:0 and tripalmitin (D_{31}-16:0)$_3$ was administered to germ-free, Oligo-MM^{12} and specific-pathogen-free mice via oral gavage. At different time points, the mice were sacrificed and sections of the small intestine, colon and liver, as well as blood and gut content samples were collected. Stable isotope-labeled fatty acids were tracked and quantified using gas chromatography-mass spectrometry (GC-MS). We found that the gut microbiota influences intestinal lipid absorption, metabolism, trafficking and secretion into the circulation. Next, mechanistic studies will be carried out to reveal underlying mechanisms including analysis and functional tests of relevant bile acid species.

19 GUT MICROBIOTA CONTROLS PERSISTENT *C. RODENTIUM* INFECTION VIA ENDOTHELIAL ACTIVATION AND NEUTROPHIL RECRUITMENT

R. Romero[1], A. Zarzycka[1], A. Visekruna[1], Bärbel Stecher[2], K. Roth[3], U.Steinhoff[1]

[1]*Institute of Medical Microbiology, Philipps University of Marburg, Germany*
[2] *Max von Pettenkofer-Institut, LMU Munich, Germany*
[3] *Institute for Molecular biology and Tumor-research (IMT)/ (ZTI) Marburg, Germany*

Citrobacter rodentium is an enteric murine pathogen that models human infections with enteropathogenic *E. coli* (EPEC). It is still largely unknown how some individuals become lifelong asymptomatic carries of EPEC and unknowingly spread the disease, while others can develop an acute, in some cases life-threatening, clinical manifestation. This immunological situation can be studied in germ-free mice, since these animals are unable to eliminate *C. rodentium* and become lifelong asymptomatic carriers of this bacterium. The asymptomatic carrier state of germ-free mice can be reversed by conventionalization, underlining the importance of the microbiota in this model. Colonization of germ-free mice with Oligo-mouse-Microbiota (Oligo MM) together with specific bacterial strains isolated from the microbiome of laboratory mice promotes angiogenesis and boosts a proper immune response, through neutrophil migration. Moreover, the active involvement of the immune system is crucial for the eradication of *C. rodentium*, meaning the microbiome alone is insufficient. These results show that a defined minimal consortium promotes pathogen clearance and immune activation and and are important for the preventive and therapeutic treatment of intestinal diseases.

20 DEVELOPMENT OF THE GUT BARRIER DURING THE POSTNATAL PERIOD

A. Schwentker[1], A. Riba[1], M. Hornef[1]

[1]*Institute of Medical Microbiology, University Hospital RWTH Aachen*

After birth the neonatal intestinal mucosa transits from a protected and sterile environment in utero to a site intensely exposed to microorganisms and environmental factors. How the neonate adapts to these changes and establishes intestinal immune homeostasis is the focus of our research. In this context, enhanced permeability of the epithelial gut barrier to macromolecules in the immediate postnatal phase has been postulated in a concept termed “gut closure” . Here we aim at a systematic analysis of the development of mucosal gut barrier integrity in mice during the postnatal period with particular focus on the permeability to bacteria and/or bacterial constituents. For this purpose we collected samples of the small intestine, colon, liver, spleen and kidney from mice at different postnatal age, starting at one day after birth and following up until adulthood. Eukaryotic reporter systems, quantitative PCR and the limulus assay were used for detection and quantification of bacterial DNA, flagellin or endotoxin, the obligate outer membrane constituent of all gram-negative bacteria, within the samples. Preliminary data indicate that systemic sites of neonatal mice are exposed to higher amounts of bacterial DNA compared to adult tissues, supporting the concept of postnatal “gut closure”. However, endotoxin quantification revealed no indication for impaired epithelial barrier integrity in the neonate host as endotoxin was barely detectable in any systemic organ at any time point and its concentration did not decline with age. This either indicates that gut barrier integrity differs for various kinds of macromolecules in the postnatal period or that the host actively compensates for the enhanced postnatal permeability.

[1] Clarke & Hardy: „An analysis of the mechanism of cessation of uptake of macromolecular substances by the intestine of the young rat (‘closure’)” *J. Physiol*. (1969), **204**. pp. 127 - 134

21 MHC I EXPRESSION DIFFERENCES LEADING TO A LOSS OF COLITOGENIC T CELLS

K.Selke[1], I. Bruesch[1], M. Buettner[1], D. Wedekind[1], A. Bleich[1]

[1]*Institute for Laboratory Animal Science, Hannover Medical School*

Inflammatory bowel disease is a disorder of highly complex etiology comprising genetic, environmental and microbial factors. A well-suited and widely-used animal model for T cell development is the adoptive transfer model. Recently, it has been noticed during the routine genetic monitoring of mouse strains that two separately imported C57BL/6J.129P2-*Il10*^{tm1Cgn}/JZtm substrains (B6-*Il10*$^{-/-}$1 and B6-*Il10*$^{-/-}$2) have a huge expression difference in H2-K1^{b,}, a MHC I molecule, but not in H2-D1^{b}, a second MHC I protein.

The aim of the study was to identify whether naturally occurring MHC I expression patterns might have an impact on T cell dependent colitis development.

Adoptive transfer of naive T cells isolated from B6-*Il10*$^{-/-}$2 (normal H2-K1^{b} expression) - but not from B6-*Il10*$^{-/-}$1 (strongly reduced H2-K1^{b} expression) - to C57BL/6J.129-*Rag1*^{tm1Mom}/JZtm (B6-*Rag1*$^{-/-}$) caused a wasting disease within four weeks with a manifestation of colitis. In detail, the T cells isolated from B6-*Il10*$^{-/-}$1 animals diminished around two weeks post transfer and were not detectable at all four weeks post transfer. Proliferation and stimulation properties of naive T cells in vitro of both strains did not reveal any difference.

It was then focused on the interaction between donor T cells and host-derived innate immune cells in particular natural killer (NK) cells. Naive T cells of both donors were transferred either in genetically NK-cell free recipients (NOD.Cg-*Rag1*^{tm1Mom} *Il2*^{tm1Wjl}/SzZtm (NRG)) or in NK-cell depleted B6-*Rag1*$^{-/-}$ mice. In both models, B6-*Il10*$^{-/-}$1 cells survived and caused colitis.

In summary, it was shown that a decreased expression of H2-K1^{b} with a normal level of H2-D1^{b} in T cells might enable host-derived NK cells to guard against the onset of colitis by effector T cells.

22 ROLE OF THE MICROBIOTA IN THE REGULATION OF IL-22BP PRODUCING CD4+ T CELLS

R. Silva de Oliveira[1], M. Sabihi[2], P. Pelczar[2], S. Huber[2], T. Strowig[1]

[1]*Department of microbial immune regulation, HZI, Brunswick, Germany*
[2]*Center for Internal Medicine, Eppendorf University, Hamburg, Germany*

The outcome and course of many human diseases including infections have been correlated with diverse changes in the gut microbiota. However, causal and mechanistic responses determined by the huge community of microbes in our body are yet under investigation. Inflammatory bowel disease (IBD) is one of many examples characterized by an imbalanced immune response, in which the microbiota members driving an excessive inflammatory immune response influencing CD4 T cell subsets. The cytokine IL-22, also highly expressed in IBD patients, is responsible to induce epithelial cell proliferation, microbial peptides production leading tissue repair consequently restoring the gut homeostasis. It has been described that the presence of an endogenous inhibitor (IL-22bp) can also play an important role lacking IL-22 function and if in an uncontrolled state might aid to colon tumorigenesis. Beyond dendritic cells, lymphocytes can express IL-22bp and modulate the outcome of a murine colitis model. Interesting, IL-22bp expression by CD4 T cells was positively correlated with TNF production in IBD patients. Currently, the contribution of the microbiota to the induction of IL-22bp by T cells is unknown. In order to address this question, this project plans to follow the schematic approach. 1- Evaluate IL-22bp production in CD4 T cells from mesenteric lymph nodes of germ-free, SPF and conventionalized mice combined with 16S rRNA gene sequencing to identify microbial signatures associated with varying IL-22bp production. 2- Possible microbiota candidates will be transferred to IL-22bp reporter mice in germ-free conditions to generate gnotobiotic lines. 3- Fecal transplant from IBD patients to mice in an attempt to establish a causal relationship of responders and nonresponders for anti-TNF therapy. 4- Transfer of candidate species or communities from humans to prove the causality of IL-22bp production. Through these approaches, we intend to develop new strategies based on microbiota manipulation to modulate IL-22bp production by T cells in the gut microenvironment.

23 MICROBIOME AND METABOLOME PROFILING IN AN ATF6 MODEL OF COLORECTAL TUMORIGENESIS AND INFLAMMATION

A. Sorbie[1], O. Coleman[1], A. Dunkel[2,3], S. Schmidt[2], D. Haller[1]

[1]*Chair of Nutrition and Immunology, ZIEL, Technische Universität München, Freising, Germany*
[2] *Chair of Food Chemistry and Molecular Sensory Science*, *Technische Universität München, Freising, Germany*
[3] *Institute for Food Systems Biology at the Technical University of Munich, Freising, Germany*

Colorectal cancer is a leading cause of cancer-related death and is strongly associated with microbiota alterations. Previously we showed that overexpression of nATF6 results in a microbiota-dependent and inflammation-independent tumor incidence of 100% at 12 weeks in homozygous transgenic (tg/tg) mice, while heterozygous (tg/wt) mice are tumor-free. To investigate the role of inflammation in nATF6-driven tumorigenesis a second model was generated, crossing tg/wt mice with IL-10 KO (tg/wt;-/-). Tumor incidence was lower in tg/wt;-/- mice than tg/tg mice (62.5% vs. 60% at 12 and 20 weeks respectively). Microbiota was characterised by 16S rRNA profiling of caecal content (CC) and tissue-associated (TA) microbiota and untargeted metabolomics of microbial metabolites in CC and faeces. We show that the two mouse models share a core microbiome and overlapping metabolite profile, but within each model, tumor and non-tumor environments differ. β-diversity plots revealed significant differences between homozygous wildtype (fl/fl) and tg/tg mice in CC and TA and similarly between heterozygous wildtype (fl/fl;-/-) and tg/wt;-/- mice. Susceptible tg/wt;-/- non-tumor mice (Non-responder: NR), clustered with fl/fl;-/- mice. Strikingly, the CC profile of NR and tumor-bearing tg/wt;-/- mice (Responder: R) is not significantly different, however, TA microbial profiles of R and NR are, suggesting TA microbiota discriminates between phenotypes where luminal cannot. This is further corroborated by supervised classification of combined microbial profiles from each mouse model. Random forests trained on the TA dataset outperform (AUC=0.83) those trained on CC at predicting phenotype (AUC=0.75). Metabolite profiles of tumor and non-tumor mice also differed in both models, with Long-chain fatty acids (LCFAs) enriched in tumor, while dipeptides were enriched in non-tumor. Several taxa enriched in tumor mice were predicted to encode for genes involved in LCFA metabolism. Together, these results suggest an important role for LCFAs in an ATF6 driven model of tumorigenesis and highlight the importance of considering tissue associated microbiota.

24 INTESTINAL DEVELOPMENT AND HOMEOSTASIS REQUIRE ACTIVATION AND APOPTOSIS OF DIET-REACTIVE T CELLS

U.Steinhoff[1], S. Hartmann[1], K. Rajalingam[2], YR. Sillke[3], S. Romero[1], F. Fischer[1] and A. Visekruna[1]

[1]Institute for Medical Microbiology and Hygiene, Philipps University of Marburg,
[2]Cell Biology Unit, University Medical Center, Mainz, Germany
[3]Medical Department for Gastroenterology, Infectious Diseases and Rheumatology, Campus Benjamin Franklin, Charité

While it is known that the microbiota shapes the development of the intestinal immune system, little information exists about the impact of dietary antigens on development and homeostasis of the intestine. We thus investigated the impact of dietary antigens on the phenotype and fate of intestinal T cells in germfree and normofloric mice.

We could show that physiological uptake of dietary proteins leads to a highly activated $CD44^+Helios^+CD4^+$ T cell population, predominantly in Peyer patches. These cells are distinct from regulatory T cells and develop independently of the microbiota. Alimentation with a protein-free, elemental diet led to an atrophic small intestine with low numbers of activated T cells, including Tfh cells and decreased amounts of intestinal IgA and IL-10. Food-activated $CD44^+Helios^+CD4^+$ T cells in the Peyer patches are controlled by the immune checkpoint molecule PD-1. Blocking the PD-1 pathway rescued these T cells from apoptosis and triggered pro-inflammatory cytokine production, which in IL-10–deficient mice was associated with intestinal inflammation. In support of these findings, our study of patients with Crohn's disease revealed significantly reduced frequencies of apoptotic $CD4^+$ T cells in Peyer patches as compared with healthy controls. These results suggest that apoptosis of diet-activated T cells is a hallmark of the healthy intestine.

In summary, these data show that dietary proteins are required for intestinal development and apoptosis of food-activated T cells warrants homeostasis.

25 CHARACTERIZATION OF PATHOGEN-DRIVEN SELECTION AT B4GALNT2 IN HOUSE MOUSE

M. Vallier[1,2], M. Belheouane[1,2], A. Suwandi[3], S. Künzel[1], K. Ehrhardt[3], G.A. Grassl[3], JF. Baines[1,2]

[1]Max Planck Institute for Evolutionary Biology, Plön, Germany
[2]Institute for Experimental Medicine, Kiel, Germany
[3]Institute of Medical Microbiology and Hospital Epidemiology and German Center for Infection Research (DZIF), Hannover, Germany

B4galnt2 is a blood group-related glycosyltransferase whose two murine alleles (driving gastrointestinal and vascular expression) are maintained by balancing selection in house mice and their relatives. The vascular allele induces a defect in coagulation and subsequent bleeding phenotype, suggesting that this fitness cost may be offset by other unknown benefits. Interestingly, despite its overall long-term maintenance, the vascular allele is absent in wild mouse populations from Germany and Northeast France, but recently increased in Southwest France as evidenced by a partial selective sweep. This suggests that geographic-dependent selective forces may be operating. Given other examples of blood group-related glycosyltransferase variation in humans, we hypothesize that resistance to pathogen(s) may mediate selection operating on *B4galnt2* over space and time. Indeed, experiments in lab mice show that the presence/absence of *B4galnt2* expression in the gastrointestinal tract influences the response to *Salmonella* infection. By applying metagenomic approaches in a wild mouse population displaying evidence of recent selection (i.e. a partial selective sweep), we found that *B4galnt2* genotype correlates with differences in intestinal inflammation and the presence of candidate pathogens, such as *Morganella* and *Citrobacter* species that could drive selection at *B4galnt2*. We thus suggest the limited dispersal of pathogens to be a potentially potent source of variation in selection.

26 GENOMIC CHARACTERIZATION OF BACTERIAL TRAITS THAT INFLUENCE THE PROBIOTIC CHARACTERISTICS OF *E. COLI* STRAIN NISSLE 1917

HT. Wami[1], U. Sonnenborn[2], R. von Bünau[2], U. Dobrindt[1]

[1]*Institute of Hygiene, Universitätsklinikum Münster, 48149 Münster, Germany*
[2]*Ardeypharm GmbH, 58313 Herdecke, Germany*

Escherichia coli strain Nissle 1917 (EcN) is one of the most well-studied non-pathogenic, Gram-negative, probiotic bacterial strains, that have been successfully used for the prevention and treatment of a range of gastrointestinal disorders. The strain used for the treatment of ulcerative colitis (remission phase), constipation and diarrheal diseases, colonization prophylaxis, and enhancing postnatal immune competence in infants. The strain possesses a number of properties which are vital to its survival, colonization potential, and persistence in the intestinal ecosystem, and therapeutic effectiveness. In order to fully understand the probiotic potential of EcN and the contributing bacterial traits, we performed a comparative genomic study amongst EcN and two of its close relatives *E. coli* strain 83972 and *E. coli* CFT073. All three isolates belong to sequence type 73 (ST73); CFT073 is a uropathogenic strain whereas 83972 is a non-pathogenic asymptomatic bacteriuria isolate with a long-term bladder colonization ability. With the intention of searching for (strain-specific) traits involved in the probiotic character of EcN, we performed a whole genome sequence analysis of EcN, 83972 and CFT073 and defined the differences in the core and accessory/flexible genome, genomic islands, virulence genes, and predicted genes that could be positively selected. In support with a previous transcriptomic study, our data validate that the genome level variation amongst the probiotic, asymptomatic and pathogenic strains is surprisingly low. While we found strain-specific gene clusters that promote the pathogenicity of CFT073 and the asymptomatic, long term colonization of strain 83972, those of EcN were mostly related to bacteriophages. The results obtained from the comparison of the three clonal *E. coli* strains encouraged us to expand our insight into the genomic comparison of a larger set of sequence type 73 (ST73) *E. coli* strains to assess the prevalence of “EcN-specific” genomic regions. These genomic regions will have to be characterized in the future to assess their impact on probiotic traits of EcN.

27 THE ROLE OF PRIMARY METABOLITES IN THE INTESTINAL ECOSYSTEM UNDER NORMAL AND INFLAMED CONDITIONS

A. Weiß[1], B. Stecher[1]

[1]*Max von Pettenkofer-Institute, Department of Medicine, Ludwig-Maximilians-University, Munich*

The composition of the gastrointestinal microbiome is not only determined by stochastic processes such as dispersal or ecological drift, but is strongly influenced by deterministic interactions between species and the individual host environment. In the healthy gut, the enteric microbiota faces frequent changes in the availability of nutrients, as the source of metabolites varies in space and time with dietary composition, as well as with host-dependent factors. Especially inflammatory processes can create metabolic niches in the gut that may affect the composition of available nutrients. Therefore, complex metabolic interaction networks between the bacterial species have evolved in the dynamic chemical environment of the mammalian gut, shaping the composition and function of the microbial ecosystem.
A prime example of bacterial metabolic interaction is syntrophic cross-feeding of primary gut luminal metabolites, specifically short chain fatty acids and intermediate reaction products as lactate, hydrogen and pyruvate. To gain a better understanding of the spatial, temporal and compositional complexity of ecological and metabolic microbial community interactions, we are investigating the role of primary metabolites in a defined subset of community members and in gnotobiotic mouse models, as well as mouse models of gut inflammation, using growth and metabolome profiling approaches.

Participants

PARTICIPANTS 2019

Mrs. Isabel Abellan-Schneyder
TUM
Core Facility Microbiome
Weihenstephaner Berg 3
85354 Freising
isabel.abellan-schneyder@tum.de

Mrs. Jamie Afghani
TUM and UNIKA-T
ZIEL and Environmental Medicine
Neusäßer Str. 47
86156 Augsburg
jamie.afghani@tum.de

PD Dr. Werner Albrich
Kantonsspital St. Gallen
Infektiologie und Spitalhygiene
Rorschacher Str. 95
CH-9007 St. Gallen
werner.albrich@kssg.ch

Mrs. Lena Amend
Helmholtz Center for Infection Research
MIKI
Inhoffenstr. 7
38124 Braunschweig
Lena.Amend@helmholtz-hzi.de

Dr. Stefanie Barnsteiner
Kantonsspital St. Gallen
Infektiologie/Spitalhygiene
Rorschacherstrasse 95
CH-9007 St. Gallen, Schweiz
barnsteiner.stefanie@gmail.com

Dr. Marijana Basic
Hannover Medical School
Institute for Laboratory Animal Science
Carl-Neuber-Str. 1
30625 Hannover
basic.marijana@mh-hannover.de

Dr. Anja Baumann
University of Vienna
Department of Nutritional Sciences
Althanstr. 14
1090 Vienna
anja.baumann@univie.ac.at

Mrs. Theresa Baumeister
Klinikum Rechts der Isar, TUM
2. Med, AG Quante
Dachauer Straße 74
80335 München
Theresa.Baumeister@tum.de

Dr. Meriem Belheouane
Max Planck Institute for Evolutionary Biology
Evolutionary genomics
August-Thienemann-Straße 2
24306 Plön
belheouane@evolbio.mpg.de

Prof. David Berry
University of Vienna
Department of Microbiology and Ecosystem Science
Althanstrasse 14
1090 Vienna
berry@microbial-ecology.net

Dr. Silvia Bolsega
Hannover Medical School
Institute for Laboratory Animal Science
Carl-Neuberg-Str. 1
30625 Hannover
bolsega.silvia@mh-hannover.de

Mr. Arne Bublitz
HZI Braunschweig
MIKI
Inhoffenstraße 7
38124 Braunschweig
abu19@helmholtz-hzi.de

Mrs. Lisa Budzinski
German Rheumatism Research Centre (DRFZ) Berlin
Microbiota & Inflammation
Charitéplatz 1
10117 Berlin
lisa.budzinski@drfz.de

Mrs. Wiebke Burkhardt
Deutsches Institut für Ernährungsforschung
Gastrointestinale Mikrobiologie
Arthur-Scheunert-Allee 114 - 116
14558 Nuthetal
wiebke.burkhardt@dife.de

Prof. Amelia Camarinha Silva
University of Hohenheim
Livestock Microbial Ecology
Emil-Wolff-Str. 10
70599 Stuttgart
amelia.silva@uni-hohenheim.de

Mr. Aleksa Cepic
Max Planck Institute for Evolutionary Biology
Evolutionary genomics
August-Thienemann-Straße
24306 Plön
cepic@evolbio.mpg.de

Dr. Erik Christen
Laves-Arzneimittel GmbH
F&E
Lavesstrasse
6247 Schötz, Schweiz
e.christen@laves-pharma.ch

Dr. Christoph Cichon
Ctr. Molecular Biology of Inflammation (ZMBE)
Institute of Infectiology
Von-Esmarch-Strasse 56
48149 Muenster
cichon@uni-muenster.de

Prof. Thomas Clavel
Uniklinik RWTH Aachen
Functional Microbiome Research Group
Pauwelsstraße 30
52074 Aachen
tclavel@ukaachen.de

Prof. Ulrich Dobrindt
University of Münster
INstitute of Hygiene
Mendelstrasse 7
48149 Münster
dobrindt@uni-muenster.de

Dr. Josef Ecker
Technische Universität München (TUM)
ZIEL - Institute for Food & Health
Gregor-Mendel-Str. 2
85354 Freising
josef.ecker@tum.de

Dr. Katrin Ehrhardt
Medical School Hannover
Microbiology
Carl-Neuberg-Straße 1
30625 Hannover
ehrhardt.katrin@mh-hannover.de

Mrs. Verena Ertl
University Hospital Regensburg
Institute of Clinical Chemistry
Franz-Josef-Strauß Allee 11
93053 Regensburg
verena.ertl@ukr.de

Mrs. Florence Fischer
University Marburg
Medical Microbiology
Hans-Meerwein-Straße 2
35043 Marburg
Florence.Fischer@staff.uni-marburg.de

Mrs. Hanna Fokt
Max Planck Institute for Evolutionary Biology
Evolutionary Genetics
August-Thienemann-Str. 2
24306 Plön
fokt@evolbio.mpg.de

Mrs. Alena Fricker
University of Hohenheim
Microbiome Research and Applied Bioinformatics
Garbenstraße 30
70599 Stuttgart
alena.fricker@uni-hohenheim.de

Mr. Alibek Galeev
Medizinische Hochschule Hannover
Institute of Medical Microbiology
Carl-Neuberg-Str., 1
30625 Hannover
galeev.alibek@mh-hannover.de

Mrs. Cordula Gekeler
Universitätsklinikum Tübingen
Medizinische Mikrobiologie
Elfriede-Aulhornstr. 6
D-72076 Tübingen
cordula.gekeler@uni-tuebingen.de

Mrs. Jessyca Gonzalez
University of Münster
Institute of Infectiology
von-Esmarch-Straße 56
48149 Münster
jessyca_gonzalez@hotmail.com

Prof. Guntram Grassl
Hannover Medical School
Institute of Medical Microbiology
Carl-Neuberg-Str. 1
30625 Hannover
grassl.guntram@mh-hannover.de

Dr. Hans-Dieter Grimmecke
Laves-Arzneimittel GmbH
R&D
Barbarastrasse 14
30952 Ronnenberg
hadigswiss@gmail.com

Prof. Dirk Haller
Technische Universität München
Ernährung und Immunologie
Gregor-Mendel-Str. 2
85354 Freising
dirk.haller@tum.de

Prof. Siegfried Hapfelmeier-Balmer
University of Bern
Institute for Infectious Diseases
Friedbühlstrasse 51
3001 Bern, Switzerland
siegfried.hapfelmeier@ifik.unibe.ch

Mrs. Danielle Harris
University of Kiel
Zoology
Am Botanischen Garten 1-9
24118 Kiel
dharris@zoologie.uni-kiel.de

Mr. Kevin Heiking
German Rheumatism Research Centre
AG Chang-Schwiete Lab for
Microbiota&Inflammation
Charitéplatz 1
10117 Berlin
kevin.heiking@drfz.de

Mrs. Angélica Hernández-Arriaga
Uni Hohenheim
Livestock Microbial Ecology Group
Emil-Wolff-Str. 8
70599 Stuttgart
angelica.hernandez@uni-hohenheim.de

Dr. Andreas Hiergeist
University Hospital Regensburg
Institute of Clinical Microbiology and
Hygiene
Franz-Josef-Strauss-Allee 11
93053 Regensburg
Andreas.Hiergeist@ukr.de

Mr. Nils Hoffard
Klinikum rechts der Isar
Dermatologie und Allergologie
Biedersteiner Str. 29
80802 München
nils.hoffard@tum.de

Mrs. Daniela Janosch
Ardeypharm GmbH
Division of Biological Research
Loerfeldstr. 20
58313 Herdecke
bio.forschung@pharma-zentrale.de

Mrs. Sevana Khaloian Sarnaghi
TUM
Nutrition & Immunology
Gregor-Mendel Straße 2
85354 Freising
sevana.khaloian@tum.de

Mrs. Hanna Klimek
University Hospital Münster
Institute of Hygiene
Mendelstraße 7
48149 Münster
hanna.klimek@ukmuenster.de

Dr. Birgit Klinkert
Ardeypharm GmbH
Division of Biological Research
Loerfeldstr. 20
58313 Herdecke
bio.forschung@pharma-zentrale.de

Mrs. Luisa Kreft
Helmholtz Zentrum München
Center of Allergy and Environment (ZAUM)
Ingolstädter Landstraße 1
85764 Neuherberg
luisa.kreft@helmholtz-muenchen.de

Mrs. Wibke Krüger
Hans Knöll Institiute
Microbial Immunology
Beutenbergstraße 11a
07745 Jena
wibke.krueger@leibniz-hki.de

Prof. Thomas Kufer
University of Hohenheim
Department of Immunology
Fruwirth Str. 12
70593 Germany
thomas.kufer@uni-hohenheim.de

Prof. François Leulier
Université de Lyon, Institute de Génomique Fonctionnelle de Lyon
Ecole Normale Supérieure de Lyon,
UMR5242 CNRS, UCBL1
46 Allée d\'Italie
F-69364 Lyon Cedex, France
francois.leulier@ens-lyon.fr

Mrs. Lena Magerkurth
Medizinische Hochschule Hannover
Institut für Versuchstierkunde
Carl-Neuberg-Straße 1
30625 Hannover
magerkurth.lena@mh-hannover.de

Dr. Lisa Maier
Universität Tübingen
Institut für medizinische Mikrobiologie & Hygiene
Elfriede-Aulhorn-Str. 5
72076 Tübingen
l.maier@uni-tuebingen.de

Dr. Frank Maixner
Eurac Research
Institut für Mumienforschung
Via A.Volta 13/A
I-39100 Bolzano, Italy
frank.maixner@eurac.edu

Mr. Andres Maser
Symrise AG
S&C Symbiosis Labs
Mühlenfeldstrasse 1
37603 Holzminden
andres.maser@symrise.com

Mr. Johannes Matzner
Universitätsmedizin der JGU
Institut für Translationale Immunologie Mainz
Obere Zahlbacher Straße 63
55131 Mainz
jmatzner@uni-mainz.de

Mrs. Amira Metwaly
TUM
Chair of Nutrition and Immunology
Gregor-mendelstrasse.2
85354 Freising
amira.metwaly@gmail.com

Dr. Klaus Neuhaus
Technische Universität München
ZIEL - Core Facilty Microbiome
Weihenstephaner Berg 3
85354 Freising
neuhaus@tum.de

Dr. Maria Joanna Niemiec
Leibniz Institute for Nat. Prod. Res. & Inf. Biol.
Hans Knöll Institute
Beutenbergstr. 11a
07745 Jena
joanna.niemiec@leibniz-hki.de

Dr. Julia Notter
Kantonsspital St. Gallen
Infektiologie/Spitalhygiene
Rorschacherstrasse 95
9007 CH St. Gallen
julia.notter@kssg.ch

Mrs. Nancy Obeng
Kiel University
Evolutionary Ecology and Genetics
Am Botanischen Garten 1-9
24118 Kiel
nobeng@zoologie.uni-kiel.de

Dr. Sören Ocvirk
Deutsches Institut für Ernährungsforschung
Intestinale Mikrobiologie
Arthur-Scheunert-Allee 114-116
14558 Potsdam-Rebrücke
soeren.ocvirk@dife.de

Mr.. Kodwo Appoh Odum
Hannover Medical School
Central Animal Facility
Carl-Neuberg-Straße 1
30625 Hannover
Odum.Kodwo@mh-hannover.de

Mrs. Jessica Ojong
Research Center Borstel-Leibniz Lung Center
Cellular Microbiology
Parkallee 1-40
23845 Borstel
jojong@fz-borstel.de

Dr. Tobias Ölschläger
University of Würzburg
Institut für Molekulare Infektionsbiologie
Josef-Schneider-Str. 2 D15
97080 Würzburg
t.oelschlaeger@uni-wuerzburg.de

Mr. Johannes Plagge
Technische Universität München
ZIEL - Institute for Food & Health
Gregor-Mendel-Str. 2
85354 Freising
johannes.plagge@tum.de

Prof. Amanda E. Ramer-Tait
University of Nebraska-Lincoln
Immunology and Microbiology - Department of Food Science and Technology
1901 N 21st Street; 260 Food Innovation Center
NE 68588-6205Lincoln
aramer-tait2@unl.edu

Dr. Philipp Rausch
University of Copenhagen
Department of Biology
Universitetsparken 13
2100 Copenhagen Ø
philipp.rausch@bio.ku.dk

Mrs. Sandra Reitmeier
Technische Universität München
Core Facility Microbiome
Weihenstephaner Berg, 3
385354 Freising
sandra.reitmeier@tum.de

Mrs. Rossana Romero
Philipps Universität Marburg
Institut für med. Mikrobiologie
Hans Meerwein Str.2
35043 Marburg
romerope@students.uni-marburg.de

Mrs. Annika Schwentker
Uniklinik RWTH Aachen
Institute of Medical Microbiology
Pauwelsstraße 30
52074 Aachen
aschwentker@ukaachen.de

Prof. Nicola Segata
CIBIO - University of Trento
Comput. Metagenomics Lab
Via Sommarive 9
I-38123 Povo (Trento), Italy
nicola.segata@unitn.it

Mrs. Kristin Selke
Hannover Medical School
Institute for Laboratory Animal Science
Carl-Neuberg-Straße 1
30625 Hannover
Selke.Kristin@mh-hannover.de

Mr. Romulo Silva de Oliveira
Helmholtz-Zentrum für Infektionsforschung
Microbial Immune Regulation
Inhoffenstraße 7
38124 Braunschweig
deoliveira.romulo@gmail.com

Prof. Emma Slack
ETH Zurich
Lebensmittel-Immunlogie; HCI E 365.1
Vladimir-Prelog-Weg 1-5/10
CH-8093 Zürich, SCHWEIZ
emma.slack@hest.ethz.ch

Dr. Ulrich Sonnenborn
Ardeypharm
Biological Research
Eichenweg 6
44799 Bochum
ulrich.sonnenborn@gmx.de

Prof. Bärbel Stecher
LMU Munich
Max von Pettenkofer Institute
Pettenkoferstrasse 9a
80336 München
stecher@mvp.lmu.de

Dr. Christoph Stein-Thoeringer
TUM
II. Med. Klinik
Ismaninger Str. 22
81675 Munich
christoph.thoeringer@tum.de

Mr. Akim Strohmeyer
Technische Universität München
ZIEL Institute for Food & Health
Gregor Mendel Straße 2
85345 Freising
akim.strohmeyer@tum.de

Dr. Marie Vallier
MPI for Evolutionary Biology
Evolutionary Genetics
August-Thienemann Str. 2
24306 Plön
vallier@evolbio.mpg.de

Mrs. Stefanie Wagner
Friedrich-Loeffler-Institut
Institut für molekulare Pathogenese
Naumburger Str. 96 a
07743 Jena
Stefanie.Wagner@fli.de

Mrs. Anna Weiß
Max von Pettenkofer-Institut
Medizinische Mikrobiologie und
Krankenhaushygiene
Pettenkoferstraße 9a
80336 München
anna.weiss@mvp.lmu.de

Mr. Adam Sorbie
Technische Universität München
ZIEL
Gregor-Mendel Straße 2
85354 Freising
adam.sorbie@tum.de

Prof. Ulrich Steinhoff
University of Marburg
Medical Microbiology and Hospital hygiene
Hans-Meerweinstr. 2
35043 GERMANY
ulrich.steinhoff@staff.uni-marburg.de

Mrs. Theresa Streidl
Uniklinik RWTH Aachen
Functional Microbiome Research Group
Pauwelsstraße 30
52074 Aachen
tstreidl@ukaachen.de

Prof. Till Strowig
Helmholtz-Zentrum für Infektionsforschung
GmbH
Mikrobielle Immunregulation
Inhoffenstraße 7
38124 Braunschweig
till.strowig@helmholtz-hzi.de

Dr. Rudolf von Bünau
Ardeypharm GmbH
Division of Biological Research
Loerfeldstr. 20
58313 Herdecke
bio.forschung@pharma-zentrale.de

Mrs. Haleluya Wami
Institute of Hygiene, UKM
Microbial Genome Plasticity
Mendelstr. 7
48149 Muenster
Haleluya.Wami@ukmuenster.de

Mr. David Wylensek
Uniklinik RWTH Aachen
Functional Microbiome Research Group
Pauwelsstraße 30
52074 Aachen
dwylensek@ukaachen.de

Mrs. Mangge Zou .
Helmholtz Centre for Infection Research
Experimental Immunology
Inhoffenstr. 7
38124 Braunschweig
Mangge.Zou@helmholtz-hzi.de