14th Seeon Conference

Microbiota, Probiotics and Host

Mikrobiota, Probiotics und Wirt

JUNE 30TH- JULY 2ND, 2022

CONFERENCE CENTER

MONASTERY SEEON / CHIEMSEE

For more information:

https://www.dghm.org/seeon/

June 30th, 2022

Dear Participants,

We warmly welcome you at the Seeon Monastery for the 14th Conference on "Microbiota, Probiotics and Host" 2022.
This meeting is annually organized by the German Society of Hygiene and Microbiology **(DGHM) section "Microbiota, Probiotics and Host"**. 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 and its role in health and disease. Past activities around this conference have substantially contributed to the creation of the **DFG-funded Priority Program "MICROBIOTA – a Microbial Ecosystem at the Edge between Immune Homeostasis and Inflammation"** (SPP 1656), which gathered >30 research groups between 2013-2019. Since then, microbiome research has continued to bloom in Germany: established in 2015, the **Collaborative Research Center CRC1182 "Metaorganisms"** in Kiel studies how resident microbes influence fitness of their plant and animal hosts to form a holobiont. In 2018, the **Cluster of Excellence CMFI - Controlling Microbes to Fight Infections** in Tübingen was funded to elucidate the mechanisms of interaction between beneficial and harmful bacteria to make them useful for targeted therapeutic interventions. Since 2019, **CRC1371 "Microbiome Signatures"** in Munich, which aims to determine the precise functional relevance of microbiome signatures in disease-specific contexts, and **CRC1382 "Gut-liver axis"** in Aachen, which dissects microbiome-derived mediators involved in organ-crosstalk, further expanded this fruitful research landscape around the microbiome. Researchers from these consortia all meet in Seeon to discuss latest advances in their field.
Seeon is a scientific event that particularly foster participation by young scientists. Besides the conference, the **Seeon Summer School on "Microbiome in Health and Disease"** was launched in 2018, with the aim to create a sustainable platform to train and promote young scientists across various disciplines, including gastroenterology, nutritional medicine, immunology, infection research, microbial ecology, synthetic biology, animal science, and computational biology in the area of basic and applied microbiome research.
This year again, we have a fantastic line-up of speakers and selected talks. We are looking forward to fruitful discussions and good science; let's have a great time together in Seeon!

Prof. Dr. Thomas Clavel, on behalf of the Organizing Committee
Institute of Medical Microbiology
Functional Microbiome Research Group
University Hospital of RWTH Aachen
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PROGRAM Thursday, June 30th

14^{00} – 16^{00} Registration
16^{00} – 16^{15} Welcoming: Thomas Clavel, RWTH Aachen, Germany

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16^{15} – 17^{00} **1st Keynote Lecture: Thaddeus Stappenbeck**, Lerner Research Institute, Cleveland, Ohio, USA:
Shaping immunoglobulin responses by the microbiome

Chair: Dirk Haller, Technical University Munich, Germany

17^{00} – 18^{00} Session 1

MICROBIOME & INFLAMMATION

***Taubenheim Jan**, Research Group Medical System Biology, Institute of Experimental Medicine, University of Kiel, Germany*
Metabolic microbiome-host interactions and its role in inflammatory bowel disease
***Häcker Deborah**, Technical University of Munich, Chair of Nutrition and Immunology, Freising-Weihenstephan, Germany*
Exclusive enteral nutrition drives protective microbiome modulation in pediatric crohn's disease
***Sander Anika**, Intestinal Microbiology Research Group, Department of Molecular Toxicology, German Institute of Human, Nutrition, Potsdam-Rehbruecke, Nuthetal, Germany*
Influence of intestinal Hyperbilirubinemia on the gut microbiota and colonic inflammation
***Steimle Alex**, Luxembourg Institute of Health, Esch-sur-Alzette, Luxembourg*
Targeted gut microbiota modulation alters autoimmune neuroinflammation

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18^{15} – 19^{45} Dinner

20^{00} – 21^{30} **Poster Slam** (2 minutes / 2 slides)

PROGRAM Friday, July 1st

09^{00} – 09^{45} **2nd Keynote Lecture: Bas Dutilh**, Viral Ecology and Omics Group, Friedrich Schiller University Jena, Germany / Metagenomics Group, Utrecht University, The Netherlands:

Mapping the Microverse and modelling its drivers

Chair: Bärbel Stecher-Letsch, Max von Pettenkofer Institute/ LMU Munich, Germany

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09^{45} - 10^{45} Session 2

ECOLOGY & MANIPULATION

***Krause Jannike L.**, German Rheumatism Research Center Berlin - A Leibniz Institute, DRFZ, Schwiete Laboratory for Microbiota and Inflammation, Berlin, Germany*
Sample logistics affect structural and functional profiles of faecal microbiota
***Ruple Hannah**, Dept. of Microbiome Research and Applied Bioinformatics, University of Hohenheim, Stuttgart, Germany*
Donor strain engraftment in patients with irritable bowel syndrome after fecal microbiota transplantation
***de Hoog Almási Éva**, Microbial Immune Regulation, Helmholtz Centre for Infection Research, Braunschweig, Germany*
Understanding the impact of sugar utilization in pathogenic-commensal Klebsiella competition in the gut with CRISPR-Cas9
***von Strempel Alexandra**, Max von Pettenkofer-Institut, LMU Munich, Germany*
Targeted design and manipulation of defined microbial consortia by bacteriophages

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10^{45} – 11^{15} Coffee Break / **Poster at the first glance**

11^{15} – 12^{15} Session 3

MICROBE & HOST

***Ortiz Diego**, Microbial Immune Regulation, Helmholtz Centre for Infection Research, Braunschweig, Germany*
Influence of probiotic-modulated microbiota on the immune system and infection susceptibility of preterm newborns
***Grießhammer Anne**, Interfaculty Institute of Microbiology & Infection Medicine Tübingen + Cluster of Excellence "Controlling Microbes to Fight Infections", University of Tübingen, Germany*
Exploring the impact of non-antibiotic drugs on colonization resistance to enteric pathogens
***Viehof Alina**, Functional Microbiome Research Group, RWTH University Hospital, Aachen, Germany*
Intestinal *Coriobacteriia* influence host metabolism
***Hoffard Nils**, Department of Dermatology and Allergy Biederstein, School of Medicine, Technical University of Munich, Munich, Germany*
Bacteria mediated immunomodulation of food allergy

12^{15} – 13^{45} Lunch

13^{45} – 14^{30} **3rd Keynote Lecture: Hauke Smidt**, Wageningen University & Research, The Netherlands :

Unlocking microbiomes diversity for human, animal and environmental health

Chair: Thomas Clavel, RWTH Aachen, Germany

14^{30} – 15^{30} Session 4

DIVERSITY, METABOLISM & EVOLUTION

***Krasenbrink Julia**, Division of Microbial Ecology, University of Vienna, Austria*
Role of the exclusive nutrient sulfoquinovose in gut microbiome-mammalian host symbiosis
***Omer Hélène**, Technical University of Munich, Chair of Nutrition and Immunology, Freising-Weihenstephan, Germany*
Impact of *Desulfovibrio* spp. and sulfur metabolism on the pathogenesis of chronic intestinal inflammation and colitis-associated cancer
***Andreani Nadia Andrea**, Max Planck Institute for Evolutionary Biology, Plön + Kiel University, Kiel, Germany*
Bacterial evolution during chronic inflammation in the intestine
***von Armansperg Benedikt**, Max von Pettenkofer-Institute of Hygiene and Medical Microbiology, Faculty of Medicine, LMU Munich + German Center of Infection Research (DZIF), Partner Site Munich, Germany*
Influence of human-targeted drugs on virulence factor regulation in EHEC

15^{30} – 16^{00} Coffee Break

16^{00} – 17^{00} Hot Topic 1 – **Pascale Vonaesch**, University Lausanne, Switzerland:

The intestinal microbiota in childhood undernutrition

Hot Topic 2 – **Lisa Maier**, University Tübingen, Germany:

Dissecting the interactions between commonly used drugs and the human gut microbiota

Chair: Mathias Hornef, RWTH Aachen, Germany

17^{15} – 17^{45} DGHM Fachgruppenmeeting 05 (only for members)
17^{15} – 19^{30} **POSTER SESSION**
19^{30} Dinner

PROGRAM Saturday, July 2nd

09^{00} – 09^{45} **4th Keynote Lecture: Florent Malard**, Sorbonne Université, Hôpital Saint-Antoine, Paris, France:

Modulation of the microbiota in patients with hematologic malignancies

Chair: Maria J. G. T. Vehreschild, University Hospital Frankfurt, Germany

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09^{45} – 10^{45} Session 5

MICROBIOME & CANCER

***Stein-Thoeringer Christoph**, Deutsches Krebsforschungszentrum (DKFZ) and Nationales Centrum für Tumorerkrankungen (NCT), Heidelberg, Germany*
A non-antibiotic disrupted gut microbiome predicts the clinical outcomes of CAR-T cell immunotherapy
***Zhang Boyao**, Structural and Computational Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany*
Gut microbiota carcinogen metabolism causes distant tissue tumours
***Brunner Valentina**, Institute of Translational Cancer Research and Experimental Cancer Therapy, Klinikum Rechts der Isar, School of Medicine + TranslaTUM, Center for Translational Cancer Research, Technical University of Munich, Germany*
Introduction of a modular and rapid approach to study microbial dependencies of colorectal tumor progression in germ-free mice
***Grant Erica T.**, Luxembourg Institute of Health + University of Luxembourg, Esch-sur-Alzette, Luxembourg*
Reduced dietary fiber intake in humans increases gut bacterial mucolytic activity

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10^{45} – 11^{15} Coffee Break

11^{15} – 12^{15} Hot Topic 3 – **Melanie Schirmer,** Technical University Munich, Germany:

Metabolic and functional disruptions of the microbiome in disease

Hot Topic 4 – **Marianne Grognot**, RWTH University Hospital, Aachen, Germany:

Biophysics of swimming: a quantitative dive into bacterial navigation in mucus-mimicking environments

Chair: Till Strowig, Helmholtz Centre for Infection Research, Braunschweig, Germany

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12^{15} – 12^{45} **Awards** & Farewell
12^{45} Lunch-to-go
13^{00} Shuttle to airport + train station

PROGRAM Thursday, June 30th

1ST KEYNOTE:
SHAPING IMMUNOGLOBULIN RESPONSES BY THE MICROBIOME

Thaddeus Stappenbeck

Lerner Research Institute, Cleveland, Ohio, USA

The host interface with the microbiota occurs at primarily at mucosal surfaces that are designed to facilitate communication and cooperation with this complex and dynamic entity. The language of this bi-directional communication is driven by secreted factors including enzymes and their products. Simple analytic tools have facilitated the association of microbes with a wide range of biologic processes and diseases. These associations have captured our imagination and suggest transformational approaches to disease. To capitalize on this work, we must effectively mine for causal microbes and microbial factors. Here, I will highlight recent studies that show approaches to functionally determine microbiota-host interactions occurring at tissue barriers. The goal is to fill fundamental gaps in current knowledge regarding the role of the microbiome in health and disease.

SESSION 1: MICROBIOME & INFLAMMATION

***Taubenheim Jan**, Research Group Medical System Biology, Institute of Experimental Medicine, University of Kiel, Germany*

Metabolic microbiome-host interactions and its role in inflammatory bowel disease

***Häcker Deborah**, Technical University of Munich, Chair of Nutrition and Immunology, Freising-Weihenstephan, Germany*

Exclusive enteral nutrition drives protective microbiome modulation in pediatric crohn's disease

***Sander Anika**, Intestinal Microbiology Research Group, Department of Molecular Toxicology, German Institute of Human, Nutrition, Potsdam-Rehbruecke, Nuthetal, Germany*

Influence of intestinal Hyperbilirubinemia on the gut microbiota and colonic inflammation

***Steimle Alex**, Luxembourg Institute of Health, Esch-sur-Alzette, Luxembourg*

Targeted gut microbiota modulation alters autoimmune neuroinflammation

METABOLIC MICROBIOME-HOST INTERACTIONS AND ITS ROLE IN INFLAMMATORY BOWEL DISEASE

Jan Taubenheim[1], Johannes Zimmermann[1], Samer Kadib Alban[1], Christoph Kaleta[1]

[1]Research Group Medical System Biology, Institute of Experimental Medicine, University of Kiel, Germany

Inflammatory bowel disease (IBD) is characterized by chronic inflammations of the gastric tract leading to severe gastrointestinal symptoms and consequently to significantly reduces quality of life and life span of patients. The etiology as well as the causes of IBDs are unknown to date, but result in an overly active immune response in the gut. It is believed that the change of the microbial composition in the intestinal tract contribute to onset and progression of IBD. With this study we aim to understand the metabolic changes associated to the alteration of the microbial community as well as changes in the gut tissue of the patients during the course of active inflammation. We used 16S, transcriptomic and metabolomics data from longitudinal cohort of IBD patients undergoing anti-TNFα or anti-IL6 treatment during active inflammation to reconstruct metabolic models for the microbial community and the gut tissue. These models were analyzed with different constrained based modeling methods to characterize metabolism of the microbiome and the patients. Results from these analyses were associated to changes in disease activity scores and response to treatment. Afterwards we used these metabolic functions and identified host-microbe interactions which could serve as possible therapeutic target. We identified the metabolism of aromatic amino acids, bile acids and fatty acids to be altered with disease activity, while the metabolism of purins and various amino acids is associated with a response to treatment in IBD patients. The microbial data revealed differences in the production of butyrate, bile acids, homocysteine and indol between different inflammatory states of the patients. Tyrosine, inosine and short chain fatty acid production in the microbiome where associated with response to treatment in our cohort. Further, we identified direct connections between microbiome and host in cholate metabolism which is associated with disease activtity. Additionally, exchange of short chain fatty acids and inosine between host and microbiome can be linked to therapeutic response in these patients. Overall, we give a thorough characterization of metabolic functions of the microbiome and the gut tissue of IBD patients and can propose targets for interventions to enhance treatment success based on microbiome-host interactions.

EXCLUSIVE ENTERAL NUTRITION DRIVES PROTECTIVE MICROBIOME MODULATION IN PEDIATRIC CROHN'S DISEASE

Deborah Häcker[§1], Kolja Siebert[§2], Amira Metwaly[1], Hannes Hölz[2], Helena Heimes[1], Federica De Zen[2], Nikolai Köhler[3], Josch K. Pauling[3], Monica Matchado[4], Markus List[4], Tobias Schwerd[2§] and Dirk Haller[1,5§]

[1]*Technical University of Munich, Chair of Nutrition and Immunology, Freising-Weihenstephan*
[2]*Department of Paediatrics, Dr. von Hauner Children's Hospital, University Hospital, LMU Munich, Germany*
[3]*LipiTUM, Chair of Experimental Bioinformatics, TUM School of Life Sciences, Technical University of Munich, Germany*
[4]*Chair of Experimental Bioinformatics, Technical University of Munich, Maximus-von-Imhof-Forum 3, 85354 Freising, Germany*
[5] *ZIEL-Institute for Food and Health, Technical University of Munich, Germany*
[§] *Both authors contributed equally to this work*

Introduction: Exclusive enteral nutrition (EEN) is a first-line induction therapy for pediatric Crohn's disease (CD). Although the protective mechanisms remain unclear, previous studies showed substantial changes in microbiome composition in response to EEN.
Aims & Methods: The aim of the study is to assess the protective function of EEN and its impact on gut microbiome signatures in pediatric CD. We prospectively followed 20 newly diagnosed paediatric CD patients who received EEN. Patients were treated according to international guidelines including EEN therapy for 6-8 weeks, followed by a gradual re-introduction of solid food and concomitant start of maintenance treatment. Disease activity and inflammatory biomarkers were monitored during 12-months follow-up. 16S rRNA gene sequencing and targeted/untargeted metabolomics was performed on stool samples collected weekly during EEN and monthly thereafter (n = 289). Dietary modulation and clinical activity of patient-derived stool microbiota were studied by ex vivo continuous faecal culturing and after faecal microbiota transplantation (FMT) and subsequent dietary intervention in germ free (GF) IL10- deficient (Il10-/-) mice.
Results: All patients achieved remission with EEN therapy but 7/20 had recurrent CD activity within 6 months. Microbiota profiling of patient-derived fecal microbiota before and after EEN confirmed the heterogeneity of gut microbiome composition and identified two bacterial community clusters dominated either by the two phyla Firmicutes and Bacteroidetes or the two

phyla Firmicutes and Proteobacteria. Longitudinal microbial profiling showed highly individualized responses to EEN without a coherent EEN-specific signature. Despite the individual variations, integrated microbiota and metabolite profiles resulted in better separation and the selection of bacterial taxa associated with certain metabolites (mostly long-chain fatty acids) improved the diagnostic classification of CD patients based on the diet (EEN vs. post-EEN). FMT into GF mice showed that humanized mice recapitulated the phenotypic and the dysbiotic features of their respective human donors. FMT using patient-derived baseline fecal microbiota after exposure to ex vivo continuous culturing with EEN media showed no colonic inflammation. In contrast, mice colonized with fermented baseline microbiota following the exposure to fiber rich (FR) media developed inflammation associated with substantial changes in gut microbiota composition, confirming that ex vivo fermentation can simulate EEN-induced remission by driving microbial shifts. Interestingly, EEN-like diet maintained the disease inactive behaviour of EEN remission-associated microbiota but induced inflammation in the Il10-/- mice when fermented under FR media. In contrast, EEN-like diet failed to mitigate the aggressive behaviour of relapse microbiota in both ex vivo culture and FMT experiments. Transfer of the same microbiota in the mouse model together with pre-treatment with an EEN-like purified diet reduced inflammation severity, suggesting that fiber might play a role in provoking inflammation after EEN cessation.

Conclusion: Our data show that clinical efficacy of EEN in pediatric CD is accompanied by temporal and individual gut microbial and metabolite changes but regardless of individual variations, integrated multi-omics data analysis identified diet-related microbiome signatures. Findings from continuous culture and gnotobiotic mouse models point towards a direct impact of diet on the composition and metabolic activity of patient-derived microbiota and on regulating intestinal inflammation.

INFLUENCE OF INTESTINAL HYPERBILIRUBINEMIA ON THE GUT MICROBIOTA AND COLONIC INFLAMMATION

Anika Sander[1], Jelena Jovic[2], Fabian Schumacher[3], Annika Osswald[1], Jannike Krause[4], Daniela Weber[5], Sasa Vukmirovic[2], Soeren Ocvirk[1,6]

[1]*Intestinal Microbiology Research Group, Department of Molecular Toxicology, German Institute of Human Nutrition, Potsdam-Rehbruecke, Nuthetal, Germany*
[2]*Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Novi Sad, Serbia*
[3]*Institute of Pharmacy, Free University of Berlin, Germany*
[4]*Deutsches Rheuma-Forschungszentrum, an Institute of the Leibniz Association, Berlin, Germany*
[5]*Department of Molecular Toxicology, German Institute of Human Nutrition, Potsdam-Rehbruecke, Nuthetal, Germany*
[6]*Division of Gastroenterology, Hepatology and Nutrition, Department of Medicine, University of Pittsburgh, PA, US*

Gilbert's Syndrome (GS) is a benign condition caused by a gene polymorphism of the UGT1A1 gene, that reduces the activity of the uridine diphosphate glucuronosyltransferase (UGT) 1A1 and is clinically characterized by elevated levels of bilirubin in the serum. Epidemiological studies demonstrated an inverse correlation of GS and inflammatory bowel diseases (IBD). We hypothesized that this may be mediated by altered bilirubin levels influencing the microbiota and the metabolism in the intestine, by acting as ligand of the aryl hydrocarbon receptor (AhR) in the intestinal epithelium.

Two rodent models of serum hyperbilirubinemia were used: Gunn rats that have an inherited hyperbilirubinemia due to a loss of hepatic Ugt1a1, and C57BL/6 wildtype mice with induced hyperbilirubinemia. Colonic microbiota was analyzed by 16S rRNA gene sequencing analysis, epithelial bilirubin was measured using LC-MS/MS and in the serum, 3-nitrotyrosine (3-NT), an inducible NO synthase (iNOS)-derived reactive nitrogen species, was measured by HPLC. Gene expression was measured by qPCR and colonic tissue was analyzed using immunofluorescence staining. Bilirubin levels in the colonic mucosa were higher in Gunn compared to control rats, indicating 'intestinal hyperbilirubinemia' conditions. Gunn rats demonstrated a distinct shift in gut microbial composition, resulting in higher numbers of Grampositive bacteria and lower levels of Gram-negative bacteria. Surprisingly, Akkermansia

muciniphila was exclusively present in Gunn but not in the control rat colonic microbiota. As bilirubin functions as an agonist of AhR, Ahr expression was elevated in the colonic epithelium of Gunn rats, whereas the gene expression of downstream targets Inf-γ and Nos2 was reduced. This was confirmed by lower levels of iNOS detected in the colonic mucosa. To confirm changes observed in the rat model, we performed intraperitoneal bilirubin injections in wildtype mice for 10 consecutive days to mimic hyperbilirubinemia. Being part of an ongoing analysis, mice in the bilirubin group (n=10) had lower bodyweight than the control group (n=9) and the small intestine and caecum of these mice were significantly lighter and shorter than the control group. Short-term DSS treatment of Gunn and control rats led to shorter colon lengths in the control group (n=6), indicating that Gunn rats were less affected by DSS-induced colitis. Lower levels of 3-NT in plasma of Gunn rats confirmed reduced iNOS activity. The Gunn colonic microbiota showed no distinct clustering with or without DSS-induced colitis, suggesting a weaker effect strength by DSS treatment under intestinal hyperbilirubinemia conditions. In contrast, the control rat microbiota showed distinct compositional spreading according to DSS treatment, suggesting that the colonic microbiota was more affected by DSS-induced colitis.

Bilirubin regulates the microbiota composition in the colon and may have beneficial effects for the homeostasis of the microbiota, potentially reducing the risk of colonic inflammation.

TARGETED GUT MICROBIOTA MODULATION ALTERS AUTOIMMUNE NEUROINFLAMMATION

Alex Steimle[1], Mareike Neumann[1], Erica T. Grant[1], Stéphanie Willième[1], Eiji Miyauchi[2], Shinji Fukuda[3], Hiroshi Ohno[2] and Mahesh S. Desai[1]*

[1]*Luxembourg Institute of Health, Esch-sur-Alzette, Luxembourg*
[2]*RIKEN Center for Integrative Medical Sciences, Yokohama City, Japan*
[3]*Institute for Advanced Biosciences, Keio University, Yamagata, Japan*

The gut microbiota of multiple sclerosis (MS) patients has been correlated with higher neurological symptoms in a mouse model of MS, experimental autoimmune encephalomyelitis (EAE). Certain gut microbes, such as *Akkermansia muciniphila,* have been reported to be associated with increased autoimmune neuroinflammation. However, this correlation of *A. muciniphila* with disease does not apply to all individuals, indicating that inter-microbial interactions in a given community crucially impact disease-driving properties of this particular species or the overall microbiota. A potential role of *A. muciniphila* in promoting autoimmune neuroinflammation might be rooted in its mucin-degrading capacity, contributing to a "leaky gut" by weakening the mucus barrier. However, inducing EAE in SPF-housed mice lacking *Muc2*, the primary mucin of the intestinal mucus layer, resulted in drastically reduced neurological symptoms compared to wild type littermates. Gut microbiota analyses revealed that the low susceptibility of *Muc2*-deficient mice to EAE was associated with increased abundances of *A. muciniphila*, which appeared contradictory to previous reports. Hypothesizing that this detrimental observation might be rooted in microbiota-specific inter-microbial interactions, we next employed the EAE model in a gnotobiotic setting. We performed multiple species-drop-out experiments, based on a "14-member synthetic human microbiome" reference microbiota including *A. muciniphila*, to investigate in detail how removing a subset of species from a characterized microbiota affected neuroinflammation-promoting properties of the microbiota. Our experiments revealed that *A. muciniphila* might indeed be considered a microbial risk factor for autoimmune neuroinflammation. However, this property appeared to be highly dependent on the presence or absence of other community members. Furthermore, we identified a combination of cellular, microbial and metabolic risk factors, which allowed prediction of the EAE disease course. However, quality of these predictions significantly depended on the precise microbiota composition.

Overall, our results indicate that disease-promoting properties of potential microbial risk factors are based on the presence rather than the relative abundance of a given species, are not determined by one hallmark species alone and are crucially influenced by the composition of

the remaining community members. These results underscore the potential of microbiome modulation for therapy-assisting purposes in the gut−brain axis but also highlight the complexity of such an approach. Thus, we conclude that personalized modulation approaches, tailored to a pre-existing indigenous microbiota in a given individual, are more likely to succeed than a “one-size-fits-all” approach.

Funding: Luxembourg National Research Fund (FNR) CORE grants (C15/BM/10318186 and C18/BM/12585940) to M.S.D.

PROGRAM
Friday,
July 1st

2ND KEYNOTE: MAPPING THE MICROVERSE AND MODELLING ITS DRIVERS

Bas Dutilh

Viral Ecology and Omics Group, Friedrich Schiller University Jena, Germany / Metagenomics Group, Utrecht University, The Netherlands

Over the past decades, diverse microbiomes have been sampled and sequenced by the global research community. Hundreds of thousands of metagenomic datasets have been gathered in public sequence repositories, analyzed with accurate bioinformatic pipelines in a standardized way. These datasets provide a bird's eye view of the Microverse, the multifarious microbial ecosystem that spans the Earth. They also contain information about the composition and functioning of individual microbiomes. I will discuss drivers of microbial community composition, and present some of our attempts at computationally modelling them. Many of the examples use "recycled data", highlighting the high scientific value of public data repositories.

SESSION 2: ECOLOGY & MANIPULATION

Krause Jannike L., *German Rheumatism Research Center Berlin - A Leibniz Institute, DRFZ, Schwiete Laboratory for Microbiota and Inflammation, Berlin, Germany*

Sample logistics affect structural and functional profiles of faecal microbiota

Ruple Hannah, *Dept. of Microbiome Research and Applied Bioinformatics, University of Hohenheim, Stuttgart, Germany*

Donor strain engraftment in patients with irritable bowel syndrome after fecal microbiota transplantation

de Hoog Almási Éva, *Microbial Immune Regulation, Helmholtz Centre for Infection Research, Braunschweig, Germany*

Understanding the impact of sugar utilization in pathogenic-commensal Klebsiella competition in the gut with CRISPR-Cas9

von Strempel Alexandra, *Max von Pettenkofer-Institut, LMU Munich, Germany*

Targeted design and manipulation of defined microbial consortia by bacteriophages

SAMPLE LOGISTICS AFFECT STRUCTURAL AND FUNCTIONAL PROFILES OF FAECAL MICROBIOTA

Jannike L. Krause*[1], Beatrice Engelmann*[2], Ulrike Rolle-Kampczyck[2], Martin von Bergen[2], Hyun-Dong Chang[1,3]

** Equal contribution*

[1] German Rheumatism Research Center Berlin, A Leibniz Institute— DRFZ, Schwiete Laboratory for Microbiota and Inflammation, Berlin, Germany

[2] Helmholtz-Centre for Environmental Research - UFZ, Department of Molecular Systems Biology, Leipzig, Germany

[3] Chair of Cytometry, Institute of Biotechnology, Technische Universität Berlin, Berlin, Germany

Many aspects in the crosstalk between the intestinal microbiota and its host is mediated by soluble metabolites, which can modulate cellular differentiation and function. However, the metabolome, comprising all low molecular weight compounds, is prone to quickly alter upon environmental changes. This poses a challenge to sample logistics and subsequent ex vivo analysis.

Here, we investigated the impact of time between defecation and analysis, oxygen exposure and storage condition on the composition and metabolome of faecal microbiota to determine most optimal conditions of sample logistics for analysis of microbial functionality. Faecal samples of six healthy individuals were put under anaerobic conditions directly after defecation and processed within 30 min for storage under different conditions: native at room temperature, 4 °C or -20 °C and -20 °C 1:10 diluted in 12.5% glycerol, both in the presence and absence of oxygen. Samples were analysed after 4 h, 24 h, 48 h and 168 h of storage. The microbiota were analysed (i) by microbiota profiling using microbiota flow cytometry (MFC) to assess changes in community structure and (ii) by short-chain fatty acid (SCFA) profiling using LC-MS/MS to determine changes in community functionality.

Hierarchical clustering of all samples revealed a donor-dependent clustering of MFC- and SCFA-profiles. To determine the effect of the different storage conditions, we calculated the Bray-Curtis (BC) similarities comparing the profiles of stored samples to the “fresh” sample for each donor. With increasing storage time, the BC similarity decreased, showing a significant negative correlation of BC similarity and time. Interestingly, our data reveal that the degree of storage-dependent change is donor-dependent, an important aspect that needs to be considered when comparing many individuals to each other. The different storage conditions as well as oxygen exposure did not have a significant effect on microbiota community structure

and SCFA abundances. Storage as native faecal sample at 4 °C or in 12.5% glycerol at -20 °C for up to 24 h best conserved MFC- and SCFA-profiles compared to the fresh faecal sample. Global metabolomics using LC-MS/MS and functional bioassays will be performed in the near future to complement our understanding on how logisitics of faecal samples affects functionality.

DONOR STRAIN ENGRAFTMENT IN PATIENTS WITH IRRITABLE BOWEL SYNDROME AFTER FECAL MICROBIOTA TRANSPLANTATION

Hannah Ruple[1], Stefan Fürst[2], Patrizia Kump[2], W. Florian Fricke[1]

[1]Dept. of Microbiome Research and Applied Bioinformatics, University of Hohenheim, Stuttgart, Germany
[2]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University of Graz, Austria

Fecal Microbiota Transplantation (FMT), the transfer of the microbial stool fraction from a healthy donor into a patient`s intestine as a treatment for microbiota-associated diseases, has been successfully tested for recurrent *Clostridium difficile* infection and, with less consistent effects, for inflammatory bowel diseases. Compared to these diseases, irritable bowel syndrome (IBS), a chronic functional gastrointestinal disorder of unclear etiology, in which patients suffer from abdominal pain or discomfort and show changes in their bowel habits like diarrhea and constipation, is associated with less severe dysbiosis and an unclear involvement of the gut microbiota. However, effective therapies for IBS are lacking and FMT could present a viable treatment option.

To study the microbiota assembly process in IBS patients after FMT, we performed strain-level fecal microbiota analyses on 12 patients and their donors from a clinical trial at the Medical University of Graz, Austria. Fecal samples were collected from patients before antibiotic microbiota depletion and at 14 and 90 days after FMT and used for metagenomic sequencing. Shared strains between donor and recipient samples, indicative of microbiota engraftment, and between pre- and post-FMT patient samples, indicative of microbiota persistence, were quantified with the SameStr tool that was recently introduced by our group.

Preliminary data indicate that IBS patients harbored comparable numbers of recipient and donor-derived strains after FMT, but donor-derived strains numbers decreased from 14 to 90 days after FMT. IBS patients with constipation (IBS-C) were characterized by an increased ratio of recipient to donor-derived strain fractions relative to IBS patients with the diarrhea type (IBS-D). Donor-derived newly engrafted *Bifidobacterium* strains were increased in patients that showed improvements at 90 days post-FMT based on the IBS severity scoring system (IBS-SSS).

Our strain-level microbiota analysis indicates that IBS patients are generally accessible to microbiota modulation with FMT after antibiotic pretreatment, but patient microbiota

remodeling and clinical response outcomes may be dependent on the IBS subtype and the engraftment of specific donor strains.

UNDERSTANDING THE IMPACT OF SUGAR UTILIZATION IN PATHOGENIC-COMMENSAL KLEBSIELLA COMPETITION IN THE GUT WITH CRISPR-CAS9

Éva de Hoog Almási[1], Lisa Osbelt[1], Marie Wende[1], Till Strowig[1]

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

A diverse gastrointestinal microbiota contributes to protecting our body from pathogens, a phenomenon called colonization resistance (CR). Certain medical interventions, e.g., antibiotic treatment, can disrupt CR, which can lead to the blooming of multidrug-resistant (MDR) pathogens in the gut. *Klebsiella pneumoniae* for instance is one of the six ESKAPE pathogens - a group of highly-virulent and antibiotic resistant bacteria – which is common among nosocomially transmitted bacteria. Colonization of carbapenem-resistant *K. pneumoniae* strains enhances the risk for blood stream and systemic infections. Hence, it is clear that antibiotic-free treatment options are necessary to counteract the rising threat posed by MDR pathogens including *K. pneumoniae*.

One such promising alternative could be the selective decolonization of MDR strains by probiotics that – unlike traditional antibiotic treatments – have little effect on the rest of the commensal community thus spares patients from long-term adverse effects. As previously reported, human-isolated strains of *Klebsiella oxytoca* are capable of decolonization of *K. pneumoniae in vivo* in ampicillin treated SPF mouse models, therefore they have the potential to be utilized as probiotics in the future. Such targeted interventions require thorough knowledge and mechanistic understanding of the interactions between pathogens and proposed probiotics. To unravel the mechanism of outcompetition by *K. oxytoca*, a CRISPR-Cas9 mediated gene deletion protocol was applied to detect loss of protection in mutant strains. As a result, casA was identified as a key gene contributing to the protective phenotype through effective utilization of β-glucosides as carbon sources in the gut. We demonstrated that ΔcasA strains of *K. oxytoca* fail to utilize arbutin and salicin as carbon sources *in vitro* in minimal media assay. Furthermore, deletion of casA in *K. oxytoca* diminishes CFU reduction of *K. pneumoniae* in minimal media assay as well as *ex vivo* in murine caecum content and *in vivo* in SPF mice. A newly developed knock-in complementation protocol by CRISPR-Cas9 confirmed the phenotype by restoration of casA function and thus the protective effect. Next, we investigated the contribution of casA in other strains against *K. pneumoniae* in the wider *K.*

oxtoca species complex. We observed that casA is present in *K. michiganensis* and *K. grimontii* as well. Application of the CRISPR-Cas9 gene deletion pipeline was successful in several human isolates of these species and we could show that casA is responsible for arbutin and salicin utilization as it is in *K. oxytoca*. However, as confirmed by *ex vivo* and *in vivo* experiments we confirm that the role of casA in competition against *K. pneumoniae* is specific to *K. oxytoca* and does not contribute to CFU reduction in *K. michiganensis*, although it might affect in vivo colonization of *K. grimontii*.

Overall, we successfully applied and optimized a CRISPR-Cas9 mediated genetic tool to a wide range of human isolated of the *K. oxytoca* species complex, which allows us in-depth analysis of interspecies competition in the Klebisella genus. Our efforts revealed the importance of competition for β-glucosides between *K. oxytoca* and *K. pneumoniae* while further mechanisms are still to be identified in *K. michiganensis*. Finally, we plan to investigate the effect of the dietary context of the competition *in vivo* as a powerful next step towards deciphering the impact of potential probiotic use in humans.

TARGETED DESIGN AND MANIPULATION OF DEFINED MICROBIAL CONSORTIA BY BACTERIOPHAGES

Alexandra von Strempel[1], Marta Salvado[1], Anna S. Weiss[1], Johannes Wittmann[2], Tom Clavel[3], Karin Kleigrewe[4], Bärbel Stecher-Letsch[1]

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The human gut is a complex ecosystem, consisting of eukaryotic cells, bacteria and viruses and alteration of this intestinal microbiota is associated with an increasing number of human diseases. Bacteriophages and viruses of Archea are important effectors and indicators of human health and disease by managing specific bacterial population structures and by interacting with the mucosal immune system.

To obtain functional insights into the gastrointestinal microbiome and its function in health and disease, we aim to establish a model to investigate the interaction of bacteriophages and cognate host bacteria in the mammalian gut. Therefore, we isolated specific phages from environmental samples for members of a minimal bacterial consortium, the Oligo-MM[14], which consists of 14 well-characterized bacterial strains that colonize gnotobiotic mice in a stable and reproducible manner and provides colonization resistance against *Salmonella*. These phages are used to analyze their effect on the stable community in the murine gut with respect to compositional and functional alterations as well as phage-host bacterial interaction over time.

We show, that phages lead to initial depletion of the target population and thereafter coexist with the bacteria over long periods of time. Furthermore, the addition of phages led to a significant decrease of the colonization resistance against *Salmonella*, indicating that community functions can be targeted by phages.

In summary, our work yields insights into phage-bacterial interactions in the gut and the effect of phages on fundamental microbiome functions, which will be important for evaluating the future use of phages for targeted microbiome manipulation.

SESSION 3: MICROBE & HOST

***Ortiz Diego**, Microbial Immune Regulation, Helmholtz Centre for Infection Research, Braunschweig, Germany*

Influence of probiotic-modulated microbiota on the immune system and infection susceptibility of preterm newborns

***Grießhammer Anne**, Interfaculty Institute of Microbiology & Infection Medicine Tübingen + Cluster of Excellence "Controlling Microbes to Fight Infections", University of Tübingen, Germany*

Exploring the impact of non-antibiotic drugs on colonization resistance to enteric pathogens

***Viehof Alina**, Functional Microbiome Research Group, RWTH University Hospital, Aachen, Germany*

Intestinal *Coriobacteriia* influence host metabolism

***Hoffard Nils**, Department of Dermatology and Allergy Biederstein, School of Medicine, Technical University of Munich, Munich, Germany*

Bacteria mediated immunomodulation of food allergy

INFLUENCE OF PROBIOTIC-MODULATED MICROBIOTA ON THE IMMUNE SYSTEM AND INFECTION SUSCEPTIBILITY OF PRETERM NEWBORNS

Diego Ortiz[1], Mangge Zou[2], Marie Wende[1], Martina Palatella[2], Lisa Osbelt[1], Gesine Hansen[3], Dorothee Viemann[3,4], Jochen Hühn[2], Till Strowig[1]

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Preterm birth complications are the leading cause of newborn morbidity and mortality worldwide. Severe infections consistently rank within the most important threats for preterm newborns, leading not only to death but also to long-term complications. In the last decades, modulation of the microbiota has emerged as a favourable intervention to prevent infections in susceptible individuals. Clinical studies correlate specific probiotic formulations with reduced risk of infections and death. Yet, the evidence on causality that this kind of studies provide is limited by the spontaneous occurrence of infection and the prompt use of antibiotics in preterm children.

We asked whether the probiotic-modified microbiome has an immunomodulatory effect on the host or confers any protection against infection. For this purpose, humanised models are the still the most comprehensive tool available. Here we present a humanised mouse model for neonatal infections and provide a glimpse of the bacterial driven shaping of the neonatal immune system.

While establishing our models, we compared the impact of preterm formula as dietary supplement in the microbial engraftment. We found out that formula negatively affects the health and the vertical transfer of microbiota in mice. We characterised the immune steady state of humanised mice at 3 weeks of age, in lymphoid organs and the gut using high dimensional flow cytometry and unsupervised analytical approaches. Our results show a marked increase on cellularity and significant changes in the distribution of different cell subtypes, especially in the B cell compartment in the gut. Focusing on relevant pathogens of preterm children, we established a gastrointestinal infection model with enteropathogenic *Escherichia coli* and we are currently working on the establishment of a sepsis model with

Staphylococcus aureus. Here we demonstrate that immune responses involving IL-17A and IL-22 production persist after complete clearance of *E. coli*, not only in the T cell compartment but also in innate cells. Altogether, our results are supporting evidence of the sustained differential effects of bacteria in the neonatal immune system.

The next logical step is to use our models as platform to study the effect of commercial and experimental probiotics in infection and immunity. Consequently, we have teamed-up with the PRIMAL randomised control trial, which evaluates the efficacy of a probiotic formulation to prevent gut dysbiosis in preterm newborns. We will have not only access to their probiotic formulation but also to faecal samples from children allocated in both the probiotic and control group of the study. Additionally, we will use the clinical metadata of the study to identify and select samples from children that were particularly susceptible to severe infections.

We expect that the results of this project will shed light on the influence of microbiota and probiotic interventions on the development of the immune system and course of infection. Moreover, we hope they will serve as motivation for further studies to address possible probiotic immunomodulatory mechanisms and characterise potential microbiome signatures associated to increased infection susceptibility.

EXPLORING THE IMPACT OF NON-ANTIBIOTIC DRUGS ON COLONIZATION RESISTANCE TO ENTERIC PATHOGENS

Anne Grießhammer[1,2], Taiyeb Zahir[1,2], Cordula Gekeler,[1,2], Katharina Schmitt[1,2] and Lisa Maier[1,2]

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Drugs have been shown to be a critical determinant of interindividual differences in the composition of the human gut microbiome, which is true not only for antibiotics but also for non-antibiotic drugs. To investigate whether these non-antibiotic compounds directly affect the growth of gut bacteria, we recently screened 835 FDA approved human-targeted drugs for their inhibitory effects on 38 prevalent and abundant species of the human gut microbiome. We found that 24% of them actually directly inhibit gut microbes, with phyla beyond proteobacteria being particularly sensitive to these drugs. However, the physiological consequences of these observations are currently poorly understood.
As many human-targeted drugs show antibiotic-like effects on gut microbes, we hypothesized that they open niches for enteropathogens, facilitating colonization by exogenous pathogens and/or promoting pathobiont growth in a similar manner as antibiotics. To test this hypothesis, we first expanded our initial screen to include a set of common enteropathogens, including *Salmonella* Typhimurium (S. Tm). We then selected drugs that inhibited a broad spectrum of commensals but spared enteropathogens. By determining MICs for both commensal and pathogenic species, we quantified their selectivity against commensals. Next, we developed an *in vitro* high-throughput assay to specifically quantify the growth of *S*. Tm in synthetic or stool-derived communities. For 50 selected drugs from our preliminary studies, we used this assay to test *S*. Tm invasion efficiency into these communities. Indeed, several drugs from different therapeutic classes were able to significantly increase the growth of *S*. Tm in synthetic communities as well as in communities derived from mice or human feces in a concentration-dependent manner. For drug candidates with particularly strong phenotypes, we are currently testing their ability to disrupt colonization resistance and increase the infection risk for *S*. Tm in gnotobiotic and SPF mice.

In summary, this study provides a systematic assessment and identification of non-antibiotic drugs that may break colonization resistance and thus increase the risk of gastrointestinal side effects and microbiome dysbiosis. Our study thus opens new tools and opportunities for a better understanding of microbiome-related (and thus personalized) infection risks.

INTESTINAL CORIOBACTERIIA INFLUENCE HOST METABOLISM

Alina Viehof[1], Sarah Just[1], Susan Jennings[1], Johannes Plagge[2], Emily Richter[1], Theresa Streidl[1], Thomas Hitch[1], Josef Ecker[2], Thomas Clavel[1]

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The intestinal microbiome influences host metabolism. Previous studies linked changes in gut microbiota profiles to diseases such as type-2 diabetes or obesity, but the bacteria involved and underlying mechanisms are only known in few cases. In studies with rodents and humans, alterations in the host metabolism have been associated with the presence of Coriobacteriia, which can deconjugate and dehydrogenate bile acids and express lipases. However, conflicting results exist and no underlying mechanisms have been reported yet. The aim of this project is to investigate whether and how Coriobacteriia mediate changes in host lipid metabolism. Gnotobiotic mice were colonised with a minimal consortium consisting of Adlercreutzia mucosicola, Collinsella aerofaciens, Eggerthella lenta, and Lancefieldella parvula (CORIO) and compared to germfree (GF) and specific-pathogen-free (SPF) mice. Those mice (n = 8-14 per group) were fed with three different experimental diets varying in amount of fat and bile acids. Independent of diet, CORIO mice showed higher cholesterol levels in plasma than GF and SPF control mice. Moreover, the white adipose tissue mass doubled and leptin levels increased in CORIO mice fed the bile acid-supplemented diet. Results from a second mouse experiment indicated inhibition of colonisation with the CORIO consortium due to composition of the experimental diets. When a standard chow was chosen as a base for diets, colonisation was achieved for subsequent experiments. To study the specific role of the lipase-expressing CORIO species C. aerofaciens, we analysed the strain-level diversity of isolates from human faecal samples. The phenotypic examination of the different C. aerofaciens isolates revealed diverse growth features, a wide range of saccharolytic and cholic acid transformation capacities, and a consistent cell-bound lipase activity. Via genome-based phylogenetic and taxonomic analysis, a great diversity and ambiguous species delineation according to classically used taxonomic cut-offs was discovered. Currently, the absorption of isotope-labelled lipids in the gut of mice mono-colonised with C. aerofaciens compared with GF and SPF mice is being investigated. Interestingly, the occurrence of dominant bacteria expressing lipases, including C. aerofaciens, in stool of 345 individuals of the KORA cohort correlated positively with host body parameters

such as BMI, visceral fat, and blood triglycerides. Our results suggest that CORIO influence host lipid metabolism, although underlying mechanisms still need further investigation. Assessment of the strain-level diversity of Coriobacteriia and their ability to affect lipid absorption in the gut will improve our understanding of gut bacterial functions that regulate host health.

BACTERIA MEDIATED IMMUNOMODULATION OF FOOD ALLERGY

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Food allergy is a common disease that not only hampers the quality of life of affected patients but can lead to fatal reactions in form of anaphylactic shocks. The prevalence of food allergies in developed countries constantly increases and some studies link a specific microbiome to the predisposition to develop allergic diseases. Still much is unknown about the exact microbiota and immune cell type driven mechanisms, whose interplay lead to tolerance to non-self-antigens or to sensitization and allergy. As a model for food allergies we chose the red meat allergy, a food allergy mediated by IgE antibodies directed against the carbohydrate alpha-gal (α-galactose- α -1-3-galactose), which is connected to proteins and lipids via α1,3-galactosyltransferase in all mammals besides humans and old world monkeys who lack the enzyme. To investigate the mechanisms behind tolerance induction, we established an alpha-gal allergic mouse model, in which mice are intracutaneously sensitized thereby mimicking tick bites, the primary cause of sensitization to alpha-gal in humans. Interestingly, we observed that our model failed to elicit allergic reactions in 15 % of sensitized mice, in spite of successful sensitization as seen by elevated amount of alpha-gal specific IgE and IgG1 antibodies. This is in line with observations in humans, since, while most sensitized and thus alpha-gal IgE+ individuals are 'resilient', only 10% display allergic reactions upon contact to red meat containing diet. We hypothesized that this resilient phenotype is caused by a difference in microbiota composition and therefore performed 16S rRNA-amplicon sequencing on stool samples from patients and ileum content of mice. Indeed, we could find differences in several bacterial species and families, including an elevated amount of Lactobacillaceae in resilient mice and Faecalibaculum in allergic mice, while Ruminococcus was elevated in resilient human patients. To further investigate the influence of microbiota on the course of allergy/tolerance, germfree alpha-gal deficient mice were created and inoculated with caecum content derived from alpha-gal sensitized mice that showed either resilience or anaphylaxis upon alpha-gal challenge. Interestingly, the group colonized with a resilience associated microbiome showed an elevated amount of FoxP3 positive Treg cells, which are associated with a tolerant reaction to non-self-antigens, in small intestinal lamina propria and peyer's patches compared to mice which received an allergy associated microbiome. This group had an elevated amount of IgE positive cells in the skin, including basophils, which are associated

with an allergic phenotype. In conclusion, our results suggest an influence of the microbiome, possibly even of single specific bacterial strains, on the course of food allergy and offers a starting point to investigate the mechanisms behind tolerance formation in detail.

3RD KEYNOTE: UNLOCKING MICROBIOMES DIVERSITY FOR HUMAN, ANIMAL AND ENVIRONMENTAL HEALTH

Hauke Smidt

Wageningen University & Research, Wageningen, The Netherlands

Microbiomes are key to solving many of the societal challenges we face, ranging from individual-based health issues related to microbiomes inhabiting our body, sustainable agriculture and biobased production, to global greenhouse gas emissions. With the large-scale facility UNLOCK, Wageningen University and Delft University of Technology have joined forces to implement and integrate experimental and data platforms, ranging from single-cell based approaches to complex community studies, enabling breakthrough research and knowledge sharing on natural and synthetic microbial communities. UNLOCK is open to excellence-driven users from universities, knowledge institutes and industries, placing them in the unique position to conduct research at unmet speed and resolution.

Specific examples will be given from studies of defined synthetic consortia representative of human intestinal microbiomes. Knowledge of the functional roles and inter-species interactions are crucial to gain mechanistic understanding of the human intestinal microbiome in health and disease. Such understanding, however, is hampered by the high complexity of the gut microbiome. To this end, synthetic minimal microbiomes provide a pragmatic approach to investigate their ecology including metabolic interactions. We designed, assembled and experimentally tested synthetic microbiomes consisting of 10-16 species that together cover selected key metabolic functionalities and ecological interactions driven by host-derived and dietary glycans. Replicate minimal microbiomes exhibited strikingly similar and reproducible ecological and metabolic patterns. By integrated analyses of metabolite production, community dynamics and strain-resolved meta-transcriptomes, we identified dynamic inter-species metabolic interactions, trophic roles and metabolic niches of human gut microbes leading to production of key metabolites such as short chain fatty acids.

SESSION 4: DIVERSITY, METABOLISM & EVOLUTION

***Krasenbrink Julia**, Division of Microbial Ecology, University of Vienna, Austria*

Role of the exclusive nutrient sulfoquinovose in gut microbiome-mammalian host symbiosis

***Omer Hélène**, Technical University of Munich, Chair of Nutrition and Immunology, Freising-Weihenstephan, Germany*

Impact of *Desulfovibrio* spp. and sulfur metabolism on the pathogenesis of chronic intestinal inflammation and colitis-associated cancer

***Andreani Nadia Andrea**, Max Planck Institute for Evolutionary Biology, Plön + Kiel University, Kiel, Germany*

Bacterial evolution during chronic inflammation in the intestine

***von Armansperg Benedikt**, Max von Pettenkofer-Institute of Hygiene and Medical Microbiology, Faculty of Medicine, LMU Munich + German Center of Infection Research (DZIF), Partner Site Munich, Germany*

Influence of human-targeted drugs on virulence factor regulation in EHEC

ROLE OF THE EXCLUSIVE NUTRIENT SULFOQUINOVOSE IN GUT MICROBIOME-MAMMALIAN HOST SYMBIOSIS

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Green-diet derived sulfoquinovose is a selective substrate for specific human gut bacteria, including the abundant commensals *Eubacterium rectale* and *Faecalibacterium prausnitzii* which have beneficial functions. Yet, complete sulfoquinovose degradation via interspecies metabolite transfer can produce hydrogen sulfide with ambivalent health impacts. This study aims to evaluate the prebiotic potential of sulfoquinovose by understanding its impact on gut microbiota diversity and function and the mammalian host. *In vitro* microcosms with human feces were incubated with increasing sulfoquinovose doses. Microbiota and metabolite dynamics were analysed using 16S rRNA gene amplicon sequencing and capillary electrophoresis, respectively. Sulfoquinovose was degraded to acetate and 2,3-dihydroxy-1-propanesulfonate (DHPS), which was further degraded to acetate and hydrogen sulfide. Irrespective of the sulfoquinovose concentration, *E. rectale* and *Bilophila wadsworthia* were the main primary and secondary degraders, respectively. This suggests no dose-dependent affinity to sulfoquinovose or DHPS of the diverse degraders. Furthermore, sulfoquinovose was not metabolized by human intestinal cells in cell culture experiments. In a first animal experiment, a single dose of sulfoquinovose was administered in two concentrations (1 and 10 mg) to C57BL/6 mice. Fecal sulfoquinovose was detectable for six hours and production of DHPS indicated a microbial conversion of sulfoquinovose in the mouse gut. *In vitro* microcosms with murine feces amended with sulfoquinovose did not produce hydrogen sulfide, which suggests alternative or lack of DHPS metabolization. The identity and metabolic activities of murine primary and secondary sulfoquinovose degraders will be further analysed by BONCAT-FACS, 16S rRNA gene amplicon sequencing, and genome-centric metagenomics and metatranscriptomics. More long-term administration of sulfoquinovose to mice and application of isotope-labelled sulfoquinovose in germ-free mice will provide further physiological evidence of its degradation and impact on the host. Our work aims to improve understanding of how individual dietary or prebiotic compounds shape the microbiota and thereby potentially influence host health.

IMPACT OF *DESULFOVIBRIO* SPP. AND SULFUR METABOLISM ON THE PATHOGENESIS OF CHRONIC INTESTINAL INFLAMMATION AND COLITIS-ASSOCIATED CANCER

Hélène Omer[1], Tingting Zheng, Daniela Wetzel, Shen Jin, Melanie Schirmer, Dirk Haller

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Sulfate-reducing bacteria (SRB) are ubiquitous bacteria, which can use inorganic sulfate as an electron donor during anaerobic respiration, producing hydrogen sulfide (H_2S) as an end product. Members of the *Desulfovibrionaceae* family, and especially *Desulfovibrio* spp., are the main SRB in the human digestive tract. They are usually considered commensals, but have been associated with various opportunistic infections as well as chronic inflammatory and tumorigenic diseases, including Crohn's disease (CD), ulcerative colitis (UC) and colorectal cancer (CRC). Increased sulfur metabolism has also been associated with active disease and relapse in inflammatory bowel disease (IBD). While endogenous H_2S is an important signal transducer and a substrate for oxidative phosphorylation in human cells, elevated exposure to H_2S may induce cellular dysfunction, aberrant tissue responses, including oxidative stress, genotoxicity and loss of epithelial barrier integrity. We recently demonstrated that SRB enrichment and increased sulfur metabolism is linked to active disease and relapse in CD patients. Fecal transfer experiments in germ-free IL-10 deficient mice (*Il10*$^{-/-}$) replicated the disease phenotype from the donor patient, with a significant increase in the accumulation of sulfated compounds and *Desulfovibrio* spp. in connection with inflammation. Though environmental isolates are available and have been thoroughly studied for their corrosion and bioremediation potential, few human isolates have been really characterized. We hypothesize that the lack of human *Desulfovibrio* spp. isolates and well-characterized reference genomes have hindered our ability to fully characterize the role of these bacteria in diseases.

To fill this gap, we started compiling a strain collection of human *Desulfovibrio* spp. We acquired strains isolated from humans and available in public bacterial strain banks or through collaborators. We sequenced those isolates, producing 14 high-quality genome assemblies. Using comparative genomic analysis, we showed that these human isolates cluster distinctly from environmental strains. In addition, we started to isolate SRB from IBD patient feces samples by using a general liquid SRB enrichment in the first steps and then specifically purifying strains of interest. So far, we isolated 3 *Desulfovibrio* spp. strains and 2 unclassified

Desulfovibrionaceae isolates. These different strains have diverse metabolic and H_2S production rates. This stepwise purification protocol further identified naturally occurring minimal consortia enriched in SRB, providing the basis for a first colonization experiments in germ-free mouse models of chronic inflammation (*Il10*$^{-/-}$) and colitis-associated cancer (nAtf6^{IEC};*Il10*$^{-/-}$). The first colonised animals confirmed good SRB engraftment in our model. SRB were furthermore detected in the colonic mucosal tissues, cecal content and feces of those mouse models in SPF conditions, and correlated with disease. In conclusion, we provide a reliable method to isolate SRB, especially *Desulfovibrio* spp., from complex human stool samples and develop the tools to better understand the functional capacities of these strains in inflammation and colitis-associated cancer.

BACTERIAL EVOLUTION DURING CHRONIC INFLAMMATION IN THE INTESTINE

Nadia Andrea Andreani[a,b], Rahul Unni[a,b], Marie Vallier[a,b], Silke Heinzmann[c], Daniel Unterweger[a,b], John F. Baines[a,b]

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The basic evolutionary principle of adaptation by natural selection applies to the natural microbial populations in our microbiomes. Disease-mediated changes in the intestinal environment would impose different selection pressures on the microbiome to what we would expect in healthy individuals, resulting in selection for disease-specific microbial traits. Inflammatory bowel disease (IBD) comprises disorders characterized by chronic inflammation of the digestive tract and an altered gut microbiome. With the aim to test the hypothesis that disease-mediated changes in the intestinal environment impose different selection pressures on the microbiome, we performed an evolution experiment with *Escherichia coli* NC101 in a mouse model of IBD to study the adaptation of the gut microbiome to chronic inflammation within a host's lifetime. Bacteria were allowed to adapt to two alternative mouse intestinal environments (healthy wild-type vs. inflamed *Il10*$^{-}$ $^{-}$) for a period of three months in two independent experiments. Fecal samples were collected during the experiment and investigated using multi-omics approaches. Evolved populations were studied by shotgun sequencing, and individual candidate parallel mutations were investigated with a combination of gene expression and phenotypic analysis. The metabolic capabilities of the evolved populations were investigated with Biolog GEN III MicroPlates and the difference in metabolites in the fecal samples were investigated by 1H-NMR metabolomics. Our results suggest that adaptation of bacterial populations to the inflamed intestinal environment could lead to changes in their metabolic repertoire, which in turn may provide new opportunities for therapeutic interventions.

INFLUENCE OF HUMAN-TARGETED DRUGS ON VIRULENCE FACTOR REGULATION IN EHEC

Benedikt von Armansperg[1,2], Martin Koeppel[1,2], Jana Glaser[1], Markus List[3], Athanasios Typas[4] and Bärbel Stecher[1,2]

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Enterohemorrhagic *Escherichia coli* (EHEC) is a food-borne human intestinal pathogen that causes haemorrhagic colitis. 5-10% of infected patients develop haemolytic uremic syndrome (HUS), which can be lethal. HUS is caused by bacterial production of Shiga toxins (Stx1, Stx2) in the gut and its systemic spread. A generally accepted causal therapy of haemorrhagic colitis in order to prevent the development of HUS is missing. Since recent outbreaks concur with the appearance of more virulent strains, there is a great need to explore novel treatment strategies. Stx2 is an AB_5 toxin encoded in the late genes of lambdoid prophages. The toxin is induced by the bacterial SOS response and expressed during the phage lytic cycle. Besides DNA-damaging antibacterials, little is known on environmental risk factors promoting Stx2 production in the human gut. Here we aimed to assess the effect of non-antibiotic drugs on EHEC growth and induction of Shiga toxin production, as they are known to substantially affect bacterial growth and physiology.

Screening a 1,200-compound drug library, we use a reporter strain expressing Gaussia luciferase (*gluc*) instead of *stx2*. This reporter (CW^{gluc}) is unique as it emulates both, Stx2 production in the bacteria and the release into the environment by phage lysis.

Out of our initial screen, we identified 161 substances, which influenced *stx*2 expression. The substances mainly belonged to the Anatomical Therapeutic Chemical Classification system (ATC) group's dermatological (D), anti-infective for systemic use (J) and sensory organs (S). To verify the effect of these compounds, we conducted a concentration dependent re-screen of 36 Shiga toxin-inducing chemicals. With this approach, we confirmed 56 % of our rescreened drugs as Shiga toxin inducing agents. Our data suggests that certain drug classes might have a strong effect on *stx2* expression during an EHEC infection, leading to the development of HUS

HOT TOPIC 1:
THE INTESTINAL MICROBIOTA IN CHILDHOOD UNDERNUTRITION

Pascale Vonaesch

University Lausanne, Switzerland

Environmental enteric dysfunction (EED) is an inflammatory disease postulated to contribute to stunted child growth and to be associated with intestinal dysbiosis and nutrient malabsorption. Yet, the microbial contribution to EED remains little studied. In the work I will present, we aimed to assess for changes in the proximal and distal intestinal microbiota in the context of stunting and EED and to test for a causal role of these bacterial isolates in the underlying pathophysiology.

Children aged 2-5 years were included in the context of the Afribiota project in Bangui, Central African Republic and Antananarivo, Madagascar from December 2016—May 2018. We analyzed gastric, duodenal and fecal samples from 627 children using a metabarcoding approach targeting the V4 hypervariable region of the 16S and 18S rRNA gene (bacteria, microeukaryotes) and the full ITS2 region (fungi) and tested for associations with clinical factors such as anaemia, intestinal inflammation, chronic undernutrition and age.

We find that the microbiome differs along the gastrointestinal tract and is strongly influenced by country of origin. Further, small intestinal bacterial overgrowth (SIBO) is extremely common (>80%) in stunted children. SIBO is frequently characterized by an overgrowth of oral bacteria, leading to increased permeability and inflammation and to replacement of classical small intestinal strains. Most importantly, these oral isolates directly decrease lipid absorption in both cultured enterocytes and mice, providing a mechanism by which they may exacerbate EED and stunting. Further, we find a specific, independent fecal signature associated with the EED markers fecal calprotectin and alpha-1-antitrypsin, which is conserved in between both study countries and may provide a useful biomarker of disease.

We observe high inter-individual diversity of the microeukaryome and overall poor correlation with clinical variables, yet slightly higher levels of Giberella intricans, a mycotoxin producing fungus, and lower levels of the common gut protist Blastocystis in stunted children.

Our results show a clear association of the prokaryotic microbiota in childhood undernutrition and mirror findings that Blastocystis is less common in individuals with inflammatory disease, thus highlighting the importance of studying populations across the world and along different

compartments of the gastrointestinal tract to uncover common features of the microbiome in health.

HOT TOPIC 2:
DISSECTING THE INTERACTIONS BETWEEN COMMONLY USED DRUGS AND THE HUMAN GUT MICROBIOTA

Lisa Maier

Interfaculty Institute of Microbiology & Infection Medicine Tübingen, Germany
Cluster of Excellence "Controlling microbes to fight infections"

In the past two decades, a fundamental role of the gut microbiome in host physiology and pathology has been established. Shifts in the microbiome signature have been associated with increased risk of a wide range of different diseases, ranging from infections to diverse non-communicable diseases. Recently, medication has arisen as one of the most potent modulators of gut microbiome composition. For the vast majority of these drugs that modulate microbiome composition, the corresponding drug targets in these microbes (if any) remain unknown. Furthermore, it is unclear whether their impact on microbial growth is part of their mode-of-action and whether they are responsible for side effects seen in humans. A detailed understanding of these interactions will lead to improvements in the efficacy of current therapies and the development of new drugs for targeted gut microbiome manipulations.
In my talk, I will present our recent findings on drug-microbiome-host interactions. In particular, I will discuss examples of how we combine state-of-the-art high-throughput screening platforms for anaerobic bacteria, bacterial genetics, and gnotobiotic animal models to understand how drugs can affect microbial communities, how such interactions impact the host, and how drugs can be used to restore a healthy microbiome balance.

PROGRAM Saturday, July 2nd

4TH KEYNOTE:
MODULATION OF THE MICROBIOTA IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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Over the last decade, a growing number of studies established a link between bacterial gut microbiota and patients' outcomes after allogeneic hematopoietic cell transplantation (allo-HCT). In particular, low of bacterial diversity at engraftment of allo-HCT is associated with a lower overall survival. Furthermore, butyrate producing bacteria have been associated with a decrease graft-versus-host disease (GVHD) related mortality. Therefore, gut microbiota manipulation seems a promising strategy to improve patient's outcome after alloHCT. Several approaches can be considered. First, antibiotic sparing strategies can be implemented to decreased their deleterious effects on the microbiota. Then use of prebiotic, probiotic and postbiotic are available. In particular fecal microbiota transplantation seems the most promising strategy at the moment for treatment, but also prevention of GVHD after alloHCT. Data available will be presented and discussed during the conference. Finally, we will provide perspective for use of FMT in others setting in patients with hematologic malignancies.

SESSION 5: MICROBIOME & CANCER

***Stein-Thoeringer Christoph**, Deutsches Krebsforschungszentrum (DKFZ) and Nationales Centrum für Tumorerkrankungen (NCT), Heidelberg, Germany*

A non-antibiotic disrupted gut microbiome predicts the clinical outcomes of CAR-T cell immunotherapy

***Zhang Boyao**, Structural and Computational Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany*

Gut microbiota carcinogen metabolism causes distant tissue tumours

***Brunner Valentina**, Institute of Translational Cancer Research and Experimental Cancer Therapy, Klinikum Rechts der Isar, School of Medicine + TranslaTUM, Center for Translational Cancer Research, Technical University of Munich, Germany*

Introduction of a modular and rapid approach to study microbial dependencies of colorectal tumor progression in germ-free mice

***Grant Erica T.**, Luxembourg Institute of Health + University of Luxembourg, Esch-sur-Alzette, Luxembourg*

Reduced dietary fiber intake in humans increases gut bacterial mucolytic activity

A NON-ANTIBIOTIC DISRUPTED GUT MICROBIOME PREDICTS THE CLINICAL OUTCOMES OF CAR-T CELL IMMUNOTHERAPY

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The gut microbiome and factors such as antibiotics or diet affecting its composition are increasingly recognized to affect the efficacy and toxicity of immunotherapies. Treatment with CD19-directed chimeric antigen receptor T cells (CARTs) represents the latest breakthrough in cancer therapy that aims to boost antitumor immunity against relapsed or progressive lymphoid malignancies. In contrast to other cancer immunotherapies, data on the impact of the microbiome and its perturbances in CART therapy are missing.

Here, we report on an international, multicentric clinical trial with centers in Germany and the US studying the effects of antibiotic treatment and gut microbiome features associated with clinical outcomes. In synopsis, we observed that broad-spectrum antibiotic administration shortly before or around CAR-T cell infusion significantly reduces overall survival and increase the incidence of tumor progression in lymphoma patients. Despite an association with microbiome dysbiosis, antibiotic treatment is identified as a surrogate for disease severity and an increased inflammatory state; it significantly interferes with the ability of microbiome features to predict clinical outcomes applying AI algorithms. However, excluding these events from our machine-learning analyses uncovers a specific gut microbiome signature at baseline that is able to segregate long-term response to CAR-T immunotherapy.

In conclusion, antibiotics are again found as strong confounder in clinical microbiome research. Despite this, we can reliably and reproducibly utilize microbiome features to predict clinical outcomes in immunotherapy in international patient cohorts.

GUT MICROBIOTA CARCINOGEN METABOLISM CAUSES DISTANT TISSUE TUMOURS

Blanka Roje[1*], Boyao Zhang[2*], Eleonora Mastrorilli[2], Ana Kovačić[3], Lana Sušak[1], Ivica Ljubenkov[4], Elena Ćosić[1], Katarina Vilović[5], Antonio Meštrović[5], Emilija Lozo Vukovac[5], Viljemka Bučević-Popović[4], Željko Puljiz[5], Ivana Karaman[5], Janoš Terzić[1] and Michael Zimmermann[2]

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Exposure to environmental pollutants and human microbiome composition are important predisposition factors for tumour development. Similar to drug molecules, pollutants are typically metabolised in the body, which can change their carcinogenic potential and impact tissue distribution through altered toxicokinetics. Although recent studies demonstrated that human-associated microbes can chemically convert a wide range of xenobiotics and influence the profile and tissue exposure of resulting metabolites, the effect of microbial biotransformation on chemical-induced tumour development remains unclear. Here we show that the depletion of the gut microbiota dramatically reduces the development and severity nitrosamine-induced urinary bladder cancer in mice, which affects the toxicokinetics of nitrosamines and which we causally linked to the metabolic activity of specific gut bacterial isolates. Further, we used microbial gut communities and individualised culture collections from different human donors to demonstrate that microbial nitrosamine metabolism strongly varies between human individuals and bacterial species. Eventually, we show that these interpersonal differences in microbiota carcinogen metabolism also exist outside the gut, such as the oral cavity and lungs. Altogether, these results suggest microbiome carcinogen metabolism as a contributing factor for chemical-induced carcinogenesis, which could open avenues to target the microbiome for improved predisposition risk assessment and prevention of cancer.

INTRODUCTION OF A MODULAR AND RAPID APPROACH TO STUDY MICROBIAL DEPENDENCIES OF COLORECTAL TUMOR PROGRESSION IN GERM-FREE MICE

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Introduction: Changes in microbiome signatures have been associated with the initiation, progression and treatment outcome of colorectal cancer (CRC). Studying the role of the microbiota longitudinally in tumor progression, however, remains a challenge due to a variety of parameters affecting the microbial composition. To elucidate the contribution of the microbiome in CRC research, germ-free mouse models have become a key tool. However, the germ-free rederivation of transgenic mouse models is time-consuming and costly and therefore not a suitable option in most cases. Alternatively, organoids can be isolated from intestinal tumors of genetically engineered mouse models (GEMMs) or human CRC biopsies and implanted into the colonic submucosa of mice. Importantly, organoids represent very versatile tools that allow the application of CRISPR-Cas9-based genome editing techniques to generate new CRC models. In addition, the orthotopic tumor model allows to study the CRC tumorigenesis in its native anatomical location and physiological microenvironment. Intriguingly, the tumor location, progression and histology in this transplantation model highly resemble those of human CRC.
Aims: We set out to develop a modular and rapid approach to provide a straightforward solution for CRC models to overcome the limitations of rederiving and breeding germ-free mouse models. Methodological challenges such as germ-free status of the animals during and after the intervention had to be overcome through a strict sterilization scheme and optimized two-person teamwork arrangements. To identify a potential microbial dependency of tumor

progression, we orthotopically transplanted tumor organoids of different pathological progression states (low- and high-grade adenomas, and carcinomas) derived from *Braf*^{V637E} and *Kras*^{G12D} transgenic mice into syngeneic immunocompetent and immunodeficient specific pathogen free (SPF) housed mice as well as into immunocompetent germ-free housed mice. To study epithelial and tumor-infiltrating immune cell compositions and activity status, in addition to the histopathological scores, a multicolor flow cytometry analysis of tumors, healthy adjacent intestine and spleen was performed.

Results: Tumor organoids injected orthotopically into the submucosa of the colon of germ-free immunocompetent mice functionally engrafted and resulted in tumor formation in the colon. Intensive hygienic monitoring after this intervention proved that we managed to maintain the germ-free status of these animals. Systematic comparisons of germ-free and SPF housed immunocompetent and immunodeficient animals implanted with the same organoid line allow us to better understand the microbial dependencies of tumor progression. While survival time after implantation with adenocarcinoma lines showed only minor differences between mice in SPF and germ-free conditions, we observed significant prolonged survival and a delay in tumor progression after implantation of low- or high-grade adenomas in germ-free compared to SPF mice. Interestingly, in germ-free mice tumor infiltration of CD8+ effector memory T-cells was increased while the proportion of lymphocytes within inter-epithelial leucocytes is decreased. Latest data focusing on cohorts implanted with organoids derived from low grade adenoma is still pending.

Conclusion and Outlook: We successfully established a colonoscopy-based implantation protocol of tumor organoids into germ-free mice and started to compare tumor growth in SPF and germfree mice. Implantation of carcinoma organoids resulted in similar survival rates in the presence and absence of the intestinal microbiota. However, germ-free mice survived longer compared to SPF mice when implanted with adenoma organoids, demonstrating an impaired tumor growth and progression in the absence of microbial triggers. Taken together, these results indicate that the intestinal microbiome may modulate tumorigenesis of colorectal cancer. Future studies will focus on exploring systematically differences of diverse oncogenic drivers of CRC, where we will assess additionally transcriptomic differences in the presence or absence of the intestinal microbiota.

REDUCED DIETARY FIBER INTAKE IN HUMANS INCREASES GUT BACTERIAL MUCOLYTIC ACTIVITY

Erica T. Grant[1,2], Hélène De Franco[1,2], Amy Parrish[1,2], Dominique Revets[1], Carine de Beaufort[3], Manon Gantenbein[1], Hanen Samouda[1], Amir Sharify[1], Michel Vaillant[1], Oliver Hunewald[1], Markus Ollert[1], Torsten Bohn[1], and Mahesh S. Desai[1]

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The global decline in dietary fiber consumption parallels an increase in prevalence of a multitude of gut-linked diseases, including inflammatory bowel disease and multiple sclerosis. Mounting evidence underscores the importance of dietary fiber consumption in the microbiome-mediated maintenance of gut barrier function to ward against such diseases (1). In a gnotobiotic mouse model colonized with a 14-member synthetic human gut microbiota, we have previously shown that deprivation of dietary fiber leads to expansion of mucin-foraging gut bacteria with detrimental effects on the host immune function (2). In order to translate these observations into humans and identify the potential early-warning microbial biomarkers of gut mucosal barrier impairment, we established a 2x2 crossover dietary fiber intervention study (Luxembourgish Fiber Cohort or LUXFICO; ClinicalTrials.gov registration: NCT04352231). Healthy adults (n=21) were provided with freshly prepared high (40 g/day)- or low (15 g/day)-fiber meals for one week and then, following a washout period, switched to the second set of meals. During each period, we measured metabolic and inflammatory markers and used cytometry by time-of-flight (CyTOF) to assess whether short-term fiber deficiency exerts detectable changes in the host immune function. We also collected stool samples during the last three days of each diet period, on which we performed 16S rRNA gene sequencing and assesed glycan-degrading activities of bacterial enzymes. Preliminary data from this ongoing study suggest that a diet low in fiber supports the growth of mucin-foraging bacteria (Akkermansia muciniphila, Ruminococcus torques), while simultaneously suppressing hostbeneficial, short-chain fatty acid-producing microbes (Eubacterium ventriosum). Additionally, during the low-fiber diet, we report increased activities of gut bacterial mucolytic enzymes β-N-acetylglucosaminidase and α-fucosidase in stool, reflecting the elevated mucin-foraging activity predicted by the microbial shifts. Despite high inter-individual variation in baseline microbiome compositions, the shifts detected on each diet are remarkably consistent, yielding promising potential biomarker targets to prevent disease linked to impaired gut barrier function.

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HOT TOPIC 3: METABOLIC AND FUNCTIONAL DISRUPTIONS OF THE MICROBIOME IN DISEASE

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The pathogenesis of autoimmune diseases, such as inflammatory bowel disease (IBD), involves complex interactions between the host, microbes and microbial metabolites. To investigate the microbial signals underlying disease heterogeneity and the spectrum of disease severity in IBD, we used multi-omics profiles and identified taxonomic, functional and metabolic signals in disease. Ectopic colonization of the gut with oral bacteria was systematically associated with disease exacerbation and patients with refractory disease retained high levels of these bacteria. In addition, disease-specific transcriptional changes and microbiome-associated metabolic shifts were identified. This highlights potential disruptions of the host-microbial interplay that may lead to immune dysregulation and development of inflammatory disease states.

HOT TOPIC 4: BIOPHYSICS OF SWIMMING: A QUANTITATIVE DIVE INTO BACTERIAL NAVIGATION IN MUCUS-MIMICKING ENVIRONMENTS

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CONTEXT. An estimated half of bacterial species can swim using one or several flagella, and bias their movement up or down chemical gradient, allowing them to navigate their environment. In the gut, navigation has long been suggested to be an important pathogenic factor, while commensals' motility is strongly quenched in health but not is some inflammatory conditions. Yet, despite its suggested importance, bacterial navigation in mucus or even simpler mucus-mimicking environments is very poorly understood.
METHODS. At the Taute Lab (Rowland Institute at Harvard, USA), we took advantage of a non-invasive high-throughput 3D tracking method, to track bacteria in homogenous medium or in defined chemical gradients. It allows access to the individual behavior of thousands of bacteria in minutes, as well as quantification of the resulting population's performances (e.g. climbing a food gradient or spreading), in liquid and simple mucus-mimicking media.
RESULTS. We interrogate how two vibrio pathogens harboring a single polar flagellum, V. alginolyticus and V. cholerae, navigate mucus-mimicking environments. We show that: (1) individual behaviors in soft agar gels, viscous polymer solutions and purified mucins can deviate from what is observed in liquid; (2) due to these altered behaviors, bacterial populations display strongly reduced diffusivity and/or chemotactic drift speeds (average speed for climbing a chemical gradient); yet (3) V. alginolyticus can alleviate this burden by expressing an additional set of flagella (lateral flagella), while V. cholerae seems to beneficiate from an intrinsic swimming asymmetry enhancing its random spreading.
CONCLUSION. Overall, our work illustrates the importance of quantitatively interrogating navigation in relevant environment(s), and how different bacteria can have different navigation strategies, leading to performances likely relevant to their pathogenicity. I aim to explore further the biophysics of host-microbe interactions in the gut through the lens of bacterial navigation, in my brand-new lab at RWTH Aachen University.
Relevant publications : Grognot & Taute, Commun. Biol., 2021 and Grognot et al., AEM, 2021


POSTER

01 MICROBIAL SIGNALS AND HOST SENSING CONTROL METABOLIC INJURY IN THE INTESTINAL EPITHELIUM

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The intestinal epithelium is the most regenerative tissue in the human body, located in close proximity to a dense and functionally diverse microbial milieu. Mitochondrial metabolism controls the phenotype and function of intestinal epithelial cells (IEC), and mitochondrial perturbation is associated with inflammatory bowel diseases (IBD). We hypothesize that disruption of microbial-metabolic circuits at the intestinal interface contributes to the initiation and progression of tissue injury and we aim to identify key players of the intestinal microbiota in this disrupted homeostasis.
Conditional deletion of the mitochondrial heat shock protein 60 in the intestinal epithelium (Hsp60Δ/ΔIEC) resulted in the loss of Lgr5+ stem cells and irregular crypt architecture causing selflimiting tissue injury (referred to as metabolic injury). Along with a complex shift in IEC metabolism, immune cell infiltrates and local inflammation were observed in the mucosa. Metabolic reprogramming of the epithelium induced a rapid drop in the species richness and changes in the bacterial community profile of specific pathogen-free (SPF) Hsp60Δ/ΔIEC mice. Shallow shotgun sequencing identified increased abundance of Bacteroides spp. and closely related Phocaeicola spp. in response to mitochondrial perturbation. Clinical relevance of metabolic injury, including mitochondrial stress signaling of the epithelium and increased abundance of Bacteroides spp., was confirmed in a combined analysis of three IBD cohorts (N=560 patients). Tissue aberration was attenuated in germ-free (GF) Hsp60Δ/ΔIEC mice, suggesting that colonic bacteria are directly involved in regulating tissue injury and regeneration. Colonization of GF Hsp60Δ/ΔIEC mice with a minimal mouse bacterial consortium (Oligo-MM12) recapitulated mitochondrial stress and intermediate tissue aberration. Importantly, we confirmed shifts in the bacterial community structure with increased

abundance of Bacteroides caecimuris and Akkermansia muciniphila to be associated with metabolic injury in colonic tissue. Mono- and dual-colonization experiments are ongoing in GF Hsp60Δ/ΔIEC mice to disentangle the role of these two bacterial strains on the disruption of epithelial homeostasis. In order to elucidate bacteria- or host-derived signals in controlling mitochondrial homeostasis, we also generated Hsp60Δ/ΔIEC x AhR-/- mice, demonstrating a dramatic acceleration of tissue pathology and disruption of regenerative processes in the absence of AhR signaling. The contribution of the microbiota in this severe phenotype is being currently investigated in antibiotic-treated mice.

In conclusion, these experimental and human data support the novel concept that microbial-metabolic circuits control intestinal tissue homeostasis. Metabolic reprogramming of the intestinal epithelium induced dysbiotic adaptation of the microbiome. Our data highlights that the xenobiotic host receptor AhR is essential in maintaining cellular plasticity upon metabolic injury of the intestinal epithelium. To conclude, mitochondria are emerging as the intriguing interceptors of milieu signals in the intestine and metabolic injury is a novel concept to be further investigated in the pathogenesis of IBD.

Key words (max 3): intestinal homeostasis; metabolic injury; host-microbiota interactions

02 CHARACTERIZING TRADE-OFFS OF THE HUMAN FUT2 GENE FOR THE IMPROVEMENT OF GUT HEALTH

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The risk of developing inflammatory bowel disease has been linked to the absence of the FUT2 gene. FUT2 is responsible for fucosylation of the mucosal lining of the gut, where fucose serves as a source of nutrition as well as provides attachment sites for various gut commensals. Differences in microbiome composition and susceptibility to intestinal pathogens with respect to host FUT2 genotype were shown in numerous previous studies. These findings suggest that differences in the host environment, as determined by FUT2 genotype, may exert different selective pressures on the part of microbes. Given the accumulating evidence that microbes evolve within individual hosts over their lifetime, it is thus likely that transmission between hosts also contributes to the evolutionary trajectories of gut microbial lineages. However, very limited experimental data exists to date, and the extent to which contrasting evolutionary trajectories between hosts could affect host health is not understood. In this study, we are using the Fut2-/- mouse model to understand the role of between-host adaptation in generating microbial strain-level diversity. This is first being studied by 16S rRNA gene analyses combined with high resolution, strain-resolved metagenomic analysis of Fut2 mouse colonies over multiple generations. Further, given the increasing interest in fecal microbiota transplantation (FMT) for therapeutic purposes, we aim to determine whether host genotype plays a potential role in the choice of optimal donor-recipient combinations. This will be determined using a DSS colitis model, followed by therapeutic FMT from donor mice of the same- or different Fut2 genotype. Finally, to infer potential signatures of local adaption with regard to FUT2 genotype in human populations, we are employing pan-genome analyses of candidate bacterial taxa from human fecal samples. The results of these studies will help provide insight into the role of host-genotype-dependent differences in the microbiome in inflammation medicine, in addition to possible novel therapeutic strategies.

03 THE IN VITRO METABOLIC NETWORK OF THE OMM12 MODEL COMMUNITY: A FUNCTIONAL EXPLORATION

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The Oligo-Mouse-Microbiota (OMM^{12}) community is a synthetic bacterial community modeling the murine gut microbiome. It includes twelve genome-sequenced, culturable strains representing five dominant eubacterial phyla and stably colonizes the mouse gastrointestinal tract. The OMM^{12} is currently widely used in multiple preclinical mouse models to investigate colonization resistance to pathogens and immune-mediated and metabolic diseases. However, the functional basis of the OMM^{12} interaction network is only beginning to be elucidated.

Recently, two studies (Weiss et al. (2021); Pérez Escriva et al. (2022)) provided the first systematic analyses of the OMM^{12} community interaction network, revealing an interconnected cross-feeding network with >100 pairwise interactions, which are shaped by exploitative and interference competition in nutrient-rich culture media. Additionally, genome-based metabolic models of the individual OMM^{12} strains have been generated, which are readily usable for *in silico* simulations and follow-up mechanistic studies.

Here, we go beyond pair-wise interactions and analyze the metabolic interplay in the full OMM^{12} community. We use *in vitro* culture of the OMM^{12} community paired with single strain and community proteome analysis to illuminate not only the metabolic *potential* of the single strains but their realized metabolic niche in the OMM^{12} in vitro. Finally, we compare these characteristics to those measured *in vivo* by community proteomics of mice colonized with OMM^{12}.

This first functional insight into the community metabolism of the OMM^{12} community provides a basis for the deeper exploration into community coexistence of bacterial strains that share a metabolic space. Furthermore, comparison to the *in vivo* metabolic network serves as a quality marker for the in vitro model, allowing for improvement of *in vitro* conditions and a possible future refinement of animal tests.

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04 THE ROLE OF PHAGE RESISTANCE IN PHAGE-BACTERIA CO-EXISTENCE IN THE GUT

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Phages and their bacterial hosts are typically engaged in an arms race, which selects bacteria for resistance and phages for an increased ability to infect its hosts. Curiously, a dynamic yet stable coexistence between the two is observed in natural communities. The ecological and evolutionary mechanisms behind this apparent stability are underexplored, as well as the impact of the arms race in bacterial communities.
In the mammalian gut, a number of plausible mechanisms are hypothesized to contribute for these dynamics. One such mechanism regards the presence of haplotypes carrying phage resistance mutations, in which phage driven selection and the fitness cost of carrying such mutations lead to a dynamic equilibrium between phage and both resistant and sensitive bacterial populations. We intend to study this mechanism and evaluate its potential contributions to gut phage-bacterium stability and on their impact on Oligo-Mouse-Microbiota (OMM12), a widely used synthetic bacterial community model. Using the mouse native strain Escherichia coli Mt1B1 as a host and recently isolated, targeted E. coli phages P3, P10 and P17, we have performed experimental evolution in vitro and in vivo to generate and select haplotypes resistant to these phages. Through whole genome sequencing of phage resistant isolates, we detected putative phage resistance mutations. We have generated single gene deletion mutants, and used phage infection assays to pinpoint the role of these genes in phage resistance. We were also able to generate wild-type E. coli Mt1B1 fluorescent backgrounds, which we will use as a reference in direct competitions. Our future prospect includes in vitro and in vivo competitions in the presence of OMM12 , between phage resistant and phage sensitive strains, in the presence and absence of the corresponding phage, to pinpoint fitness costs and test the equilibrium hypothesis. We will also monitor the abundance of each of the OMM12 member and investigate phenotypic changes at the community level. This project will contribute to enlighten the role of spontaneous phage resistance mutations in phage-host stability and to evaluate its impact in a community setting.

05 MUCOSAL MICROBIOTA ADAPTS TO ATF6-INDUCED ALTERATIONS IN HOST LIPID METABOLISM WITH PROGNOSTIC VALUE IN COLORECTAL CANCER

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Background: Colorectal cancer (CRC) is a leading cause of cancer deaths worldwide. The endoplasmic reticulum unfolded protein response (UPRER) signal transducer activating transcription factor 6 (ATF6) is a clinically relevant pre-cancerous marker in CRC and colitis-associated CRC. We clearly established the interrelated role of the microbiota and ATF6 signaling as a novel tumor-promoting mechanism in our transgenic mouse model of spontaneous microbiota-dependent ATF6-driven CRC (nATF6IEC).

Methods: To elucidate the transcriptional program initiated by acute and chronic ATF6 signaling, mRNA sequencing analyses of murine colonic intestinal epithelial cells were performed 4 days or 5 weeks after induction of ATF6, respectively. In parallel, germfree (GF) mice served to dissect the microbiota contribution to the transcriptional response. CRC patient datasets (TCGA) were used to estimate ATF6 activity and validate ATF6-driven signatures. To investigate the impact of ATF6 signaling on metabolites, untargeted metabolomics of feces was performed. Additionally, targeted lipidomics was completed in murine tissue. Mucosal microbiota was spatially characterized by 16S rRNA profiling at mm resolution along the colonic longitudinal axis. Based on 16S rRNA data, Phylogenetic Investigation of Communities Reconstruction of Unobserved States (PICRUSt2) was used to infer microbiota lipid-specific functional content.

Results: We identified an ATF6-UPR core of 368 differentially expressed genes fully activated by acute ATF6 signaling. Functional analysis using KEGG pathways showed that chronic ATF6 signaling predominantly alters UPR-related and metabolic pathways, with 22% of metabolic pathway genes classified as lipid metabolism. GF mice confirmed that the microbiota enhances ATF6-induced metabolic changes. Kaplan-Meier analyses significantly associate our microbiota-dependent ATF6-driven and lipid-specific ATF6-driven gene signatures with decreased disease-free survival in CRC patients since primary therapy. Moreover, ATF6 activity correlates with the presence of CRC-associated bacteria in TCGA samples. Tumor-susceptible mice show alterations in lipid metabolites, particularly long-chain fatty acids (FA) and an elongation of saturated FA. PICRUSt2 revealed bacterial lipid detoxification mechanisms, with an increased total abundance of oleate hydratase-positive species in the tumor niche.

Conclusion: We show that chronic ATF6-signaling alters host lipid metabolism and the lipid milieu in tumor-developing nATF6IEC mice. ATF6-driven microbiota changes are concomitant with bacterial lipid detoxification mechanisms in the tumor niche. We postulate that chronic ATF6 signaling represents a clinically relevant pathologic response that alters the intestinal lipid milieu and thus selects for a tumor-promoting microbiota.

06 AKKERMANSIA MUCINIPHILA REGULATES FOOD ALLERGY SENSITIZATION IN A DIET-SPECIFIC MANNER

Amy Parrish[1,2#], Marie Boudaud[1#], Erica T. Grant[1,2], Stéphanie Willieme[1], Mareike Neumann[1,2], Mathis Wolter[1,2], Sophie Z. Craig[1,2], Alessandro De Sciscio[1], Oliver Hunewald[1], Carsten Bindslev-Jensen[3], Markus Ollert[1,3], Mahesh S. Desai[1,3*]

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Alterations in the gut microbiome, including diet-driven changes, are linked to the rising prevalence of food allergy. However, little is known about how specific gut bacteria might impact oral tolerance. Our previous work showed that depriving mice of dietary fiber leads to the microbiota-driven erosion of the gut mucus layer. Here we hypothesized that the mucus barrier disruption mediated by the microbiota incites the breakdown of oral tolerance, leading to exacerbated allergic sensitization.

Specific-pathogen-free (SPF) mice and gnotobiotic mice hosting a defined and fully characterized synthetic human gut microbiota were fed a diet deprived in dietary fibers. Broad immunophenotyping was performed using time-of-flight mass cytometry, alongside ELISAbased assays and IgE-coating of the gut bacteria, evaluated by flow cytometry.

Here we show that depriving specific-pathogen-free mice of dietary fiber leads to an increase of the mucolytic bacterium Akkermansia muciniphila, which is associated with microbiotamediated colonic mucus barrier dysfunction, a surge in IgE-coated commensals and an increase in the colonic type 2 immune cells. These changes manifest into exacerbated sensitization of food allergens, ovalbumin and peanut. In our gnotobiotic mouse model, the presence of Akkermansia muciniphila within the synthetic microbiota, combined with fiber deprivation, resulted in stronger anti-commensal IgE coating and type 2 immune responses, which worsened symptoms of food allergy.

These results support a diet-dependent role for Akkermansia muciniphila, a commensal mucin-degrading gut bacterium, in the sensitization to food allergens. Our study supports a mechanistic link between diet and a mucolytic gut microbe in regulating food allergy.

07 PREDICTING INDIVIDUALIZED FOOD RESPONSE FROM BASELINE METABOLIC MARKERS

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Dietary fiber is one of the main dietary components used as an energy source by gut bacteria, since it is largely unaltered by host enzymes in the upper digestive tract. Several of the health benefits associated with the consumption of dietary fiber can be attributed to the metabolic activity of the gut microbiome, yet limited information is available on the mechanisms by which specific types of fiber interact with the gut microbiome. In that regard, dietary intervention is a realistic approach to understand how nutritional components modulate the gut microbiota. Moreover, due to high inter-individual microbiome differences, there is growing interest in using individual characteristics such as genotype, phenotype, gut microbiota composition and metabolic potential to develop tailored nutritional advice. In this context, investigating the effect of distinct dietary fibers on specific bacterial strains and integrating the information into personalized metabolic modeling tools provides promising avenues for identifying the gut microbiome–host interplay in this interconnected biological system. We aim to develop personalized dietary approaches, making use of data generated by the ongoing LUXFICO study, a crossover dietary intervention trial in which healthy participants are randomly assigned to an intermittent 1-week low- or high-fiber dietary intervention, separated by a 1-week washout. Bacterial abundances and functional capacity are being investigated by 16S rRNA gene and metagenomic sequencing. Participants' dietary pattern is collected through a short-form Food Frequency Questionnaire (FFQ) at the beginning of the study and detailed nutritional intake is determined through a 30-day food record comprising all foods and beverages ingested during the study. We determine participants' daily macro- and micronutrient intake based on information from the food records and the composition of the intervention diets, using published Food Composition Databases (such as Ciqual). The strength of this crossover study lies in the intervention diets, in which the meals are predefined and standardized, allowing for detailed characterizations of nutritional intake during the low- and high-fiber intervention periods. According to this data, a large-scale constraint-based reconstruction and analysis (COBRA) modelling pipeline will be leveraged to predict

individualized food responses from participants' baseline gut microbiome and nutritional inputs. The results of these models will be compared to stool and serum metabolite concentrations using broad-scale metabolomics to assess the accuracy of the model.

The dietary intervention trial will contribute to a deeper understanding of the effects of dietary fiber on human health, in particular by modulating the composition and the activity of the gut microbiome. By further exploiting the data generated in this crossover study, this project, titled Personalized Food Response Models (PERFORM), will generate novel strategies using metabolic modeling methodologies based on biological parameters for designing dietary interventions, supporting the deployment of science-based personalized nutrition.

08 INTEGRATED ANALYSIS OF MICROBIOME MULTI-OMICS WITH DISEASE AND THERAPY METADATA REVEALS DETERMINANTS OF MICROBIOME RECOVERY AFTER ALLOGENEIC STEM-CELL TRANSPLANTATION

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Background: A potentially curative treatment for patients suffering from hematological malignancies consists of cytotoxic therapy regimens followed by allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, the broader applicability of this treatment is limited by several adverse affects which immensely increase morbidity and transplant-related mortality. Still the most common life-threatening complication is the gastrointestinal graft-versus-host-disease (GvHD). Recent research has highlighted a strong correlation between the intestinal microbiota composition and the clinical outcomes after transplantation: Higher microbial diversity is linked to better prognosis after allo-HSCT. Most studies have focused on microbiota compositions during periengraftment period so far. Despite of growing evidence that the gut microbiome underlies dynamic processes regarding diversity and specific microbial composition along different time periods relative to allo-HSCT, there are no clinical studies concerned with post-transplantation microbiome recovery and its impact on overall outcomes. Thus, it seems necessary to investigate the extent of microbiome recovery after transplantation, its modulating factors and its role as a potential prognostic marker for development of GvHD and survival.

Methods: We performed translational analysis based on the ongoing prospective, observational clinical study “Metabolites and Microbiome in Acute Leukemia (M&M-AL)”. Patients analyzed in this project all suffered from hematological malignancies and underwent allo-HSCT. We obtained stool samples longitudinally starting from the day of recruitment. From the day of transplantation samples were obtained weekly until dischargement from hospital. We established additional time points 56 and 100 days after allo-HSCT as follow up care allowing us to analyze the extent of microbiome recovery. We compared metabolite profiles (analyzed by mass spectrometry) and microbiome compositions (analyzed by 16S rRNA sequencing) of stool samples obtained at day 56 and 100 with those profiles of samples

obtained shortly before transplantation (day -7). Bio samples were referenced with extensive clinical metadata (occurrence of intestinal GvHD, survival data) and potential confounding factors (diet, antibiotic and immunosuppressive therapy).

Preliminary results and outlook: As there is still high GvHD-related mortality in allo-HSCT patients and due to the growing interest in microbiome-modulating therapies, it seems important to advance the integration of microbiome-based clinical-decision-making into the daily clinical routine. Thus, we aim to identify metabolite-producing bacterial consortia in the post-transplantation microbiome, that correlate with clinical outcomes. Our first results (n=20 patients) indicate that microbiome recovery occurred in several patients on day 56 after transplantation. Sub group analyses (recovery vs. no recovery) are being made at the moment.

09 CONTACT-INDEPENDENT BACTERIA-BACTERIA INTERACTIONS BETWEEN GUT-ASSOCIATED BACTEROIDES ISOLATED FROM THE HOUSE MOUSE

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Understanding the mechanisms by which bacteria colonize and inhabit the host is key to our understanding of symbiosis in mammals. It is known that some members of microbiota form stable interactions with the host. The stability of microbial communities, in its turn, depends on the interactions between different members, as recently shown by Weiss at al. (2021) with synthetic community. However, how individual microbes in the host interact with each other is comparatively little understood. Here, we screened for interactions between Bacteroides strains isolated from the house mouse gut to systematically characterize bacteria-bacteria interactions within a genus of exclusively host-associated bacteria.

Our isolate collection contains bacteria of three known Bacteroides and one recently characterized Bacteroides muris species. To characterize bacteria-bacteria interactions, twenty-four strains were screened for contact-independent interactions in a pairwise manner, by inoculating the acceptor strain in the spent medium of the donor strain. We identified numerous positive (growth promotion), negative (growth inhibition) and some neutral (no changes in growth) interactions between members of the same and different Bacteroides species. Positive interactions prevailed representing 59% of all possible interactions. Negative and neutral interactions represented the fractions of 28% and 13%, respectively. Both, the ability to engage in positive and negative interactions, and the response to the spent media of other isolates were highly strain-specific. One Bacteroides acidifaciens isolate inhibited the growth of 18 and promoted the growth of two other isolates in the collection, whereas another B. acidifaciens isolate showed a much narrower target spectrum, inhibiting only a few isolates while promoting the growth of 20 other strains. From the perspective of the acceptor bacterium, a Bacteroides caecimuris isolate was inhibited by multiple other isolates, whereas another B. caecimuris strain was affected only by one isolate, displaying strong positive interactions with the other strains in the collection. Notably, some isolates that negatively affected the growth of the others were themselves inhibited by other antagonists. This finding suggested multiple

mechanisms of inhibition, for example by toxic molecules that might mediate growth inhibition. On the other hand, some isolates which growth was positively affected by spent media of the others, strongly promoted growth of the other strains in the collection, indicating nutritional interdependencies between certain members of the isolates collection. Moreover, coexisting strains isolated from the same five individuals display mostly neutral or slightly positive interactions.

In summary, we identified multiple positive, negative and neutral interactions among Bacteroides strains isolated from the house mouse gut. Our results highlight the specificity of these interactions, which are a powerful tool to gain a competitive advantage in microbial communities in the host.

10 High Abundance of Gut Bacteroides in Early Childhood Contributes to Microbiota Stability and Resilience after Dysbiotic Events but is Associated with Increased Risk for Ear, Nose and Throat Infections

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We investigated whether specific bacterial taxa in the early childhood microbiota

1.) predispose to the development of childhood infections, and
2.) influence the microbiota stability and resilience after dysbiotic events.

Therefore, we explored 16S rRNA-sequencing data of stool samples from children in the German LöwenKIDS birth cohort study. The cohort includes 782 children, which were followed up since birth using symptom-diaries, stool- and nasal swap-sampling. The subcohort contained 285 children of which 162 provided regular stool samples approx. every 3 months in the first two years of their lives. 49 children received a total of 66 antibiotic treatment episodes, mainly comprising Aminopenicillins (36%) and Cephalosporins (48%). Causes of antibiotic treatment were mainly ear nose throat (ENT) (73%) and urogenital infections (7.6%). 113 children did not receive antibiotics in the same timeframe. Dysbiotic events were defined as episodes of antibiotic treatment, which followed either real or suspected infections, or were prescribed prophylactically. As described before, alpha-diversity steadily increased with age in all children, and the community composition changed from a mainly Bifidobacteria-dominated to a more complex one where Bifidobacteria were still abundant but now accompanied by enhanced abundances of Firmicutes and Bacteroidetes. The average beta-diversities between individuals decreased with age.

We compared community compositions between the dysbiotic event vs. control group in four intervals before and after dysbiotic events, which are long-term pre (45 - 225 days pretreatment), immediate pre (0 – 30 days pretreatment), immediate post (0-30 days post treatment), and long-term post (>90 days post treatment and >540 days of age). To account for community differences by age, we compared cumulative probabilities of the empirical distribution within age-matched samples of the control group. This approach revealed that ENT infections were preceded by increased abundances of the genus Bacteroides in both, the long-term pretreatment, and the immediate pretreatment phase. Collinsella was decreased in the immediate post treatment phase. Blautia was increased in the long-term post treatment phase. Interestingly, low abundance of Bacteroides correlated with decreased Shannon diversity following disease plus antibiotic treatment, while high abundance of Bacteroides was associated with microbiota stability and resilience. To verify the results with an independent analysis strategy, we applied zero inflated negative binomial mixed models with individuals as random variable. This approach confirmed that increased Bacteroides abundance was associated with ENT infections. We currently analyze whether this model also confirms the role of Bacteroides for microbiota stability and resilience upon dysbiotic events.

Our data suggests an important role of members of the genus Bacteroides in the susceptibility to ENT infections and in microbiota stability and resilience after dysbiotic events. To understand the mechanistic concepts underlying our observations, metagenomics analyses have to be applied to identify differentially abundant bacterial species, to analyse gene set enrichments, and to model metabolic flows.

11 METHODS FOR QUANTIFICATION OF THE MYCOBIOME

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Introduction: Mycobiome studies are commonly based on metabarcoding of the ITS1 or ITS2 region. The fungal composition is then reported as the relative abundances of fungi in the sample. However, this representation makes it difficult to compare the abundances of fungi from different samples due to the compositional nature of the data. Thus, a relative increase of fungus X in sample A compared to sample B could be due to an increase of fungus X in sample A or a decrease of the other fungi in sample A.

Objectives: To address this problem, we are developing methods to compare fungal loads between different samples. For this purpose, we compared three quantification methods: Cultivation of stool samples, quantitative real-time PCR and spike-ins.

Methods: Stools samples were cultivated at 21°C and 37°C. DNA was isolated from stool samples and used for quantitative real-time PCR with universal ITS2 primers and specific ACT primers for Candida albicans and Saccharomyces cerevisiae. For the spike method, Pichia pastoris was spiked into stool samples before DNA isolation.

Results: We established a method for the cultivation of fungi from stool samples to determine the number of colony-forming units for fungi. For quantitative real-time PCR we used common ITS2 primers to quantify the number of ITS copies of DNA isolated from stool samples. We also designed primers suitable for detection and quantification of the most common fungi in stool samples: Candida albicans and Saccharomyces cerevisiae. For the spike method, we developed a protocol to spike stool samples with Pichia pastoris and optimized the spike level.

Conclusion: We established methods for the quantification of fungi in stool samples. The three methods are being tested on 13 stool samples to evaluate the different methods and to determine how comparable their results are.

12 ESTABLISHING THE EVOLUTIONARY FORCES THAT DRIVE THE SUCCESS OF INTEGRON RESISTANCE CASSETTES IN CLINICAL CONDITIONS

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Antibiotic resistance is among the greatest health problems of this century. The spread of antibiotic resistance genes is mainly driven by horizontal gene transfer via mobile and mobilizable genetic elements. Integrons are one of the main genetic elements causing multidrug resistance in Gram negative bacteria. These genetic platforms are able to capture, stockpile and rearrange new genes embedded in small mobile elements called gene cassettes (GC), forming cassette arrays. Integrons carry more than 130 cassettes against most antibiotic families and are hence major drivers of multidrug resistance[1,2] . These genetic platforms are successful in the clinical setting for the adaptive value of their cassettes. Yet marked differences in the abundance of individual cassettes suggest that each one has distinct evolutionary dynamics in terms of resistance levels, fitness cost, mobilization and polar effects. Our in vitro cytometry competition experiments show that closely related cassettes impose very different fitness costs to the host, with some cassettes even providing strong gains without antibiotic pressure. This suggest a potential secondary metabolic role of this cassettes. We conclude that distinct fitness effects are likely important drivers of cassette success.

In this work we aim to test if our results can be extrapolated to the clinics using more biologically relevant models. We will compete cassettes within controlled microbiota (OMM[12]) both in batch cultures and gnotobiotic mice[3] . Moreover, we want to assess the capacity of each cassette to establish in a certain environment by culturing all cassette-containing strains within a complex human microbiota derived from fecal samples. Through parallel sequencing, we will unveil the fitness effects of all cassettes in complex environments and in vivo, and its relevance in their clinical success.

Our data will help design evidence-based strategies to limit the spread of integrons and limit their impact on human health.

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13 MICROBIAL-DEPENDENT INTESTINAL TUMOURIGENESIS IN ATF6-DRIVEN COLITIS-ASSOCIATED-CANCER

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Introduction Colorectal cancer (CRC) is the third most common malignancy worldwide. Chronic intestinal inflammation, such as Inflammatory bowel diseases (IBD), represents a risk factor for the development of colitis-associated cancer (CAC). Dysbiosis of the intestinal microbiota is associated with CRC and CAC. Our transgenic mouse model overexpressing the active form of activating transcription factor 6 (nATF6) in intestinal epithelial cells (IEC), represents a microbiota-dependent model of spontaneous CRC (nATF6IEC). By crossing these mice with Interleukin-10 deficient mice, we created an inflammatory background mimicking CAC (nATF6IECxIL10-/-). The microbiota-dependency of both mouse models provides unique opportunities to study host-microbe interactions in ATF6-driven tumourigenesis.
Aims and Methods We firstly characterised our novel nATF6IECxIL10-/- CAC mouse model under specific pathogen-free (SPF) and germ-free (GF) conditions, investigating tumour formation (incidence and frequency) and inflammation (immune cell infiltration) by histological scoring, gene expression analysis and immunostaining, and mucus-producing goblet cells (PAS/AB staining). Immune cell infiltration was investigated by FACS and CHIP cytometry. Microbial profiling was performed by 16S rRNA amplicon sequencing to identify tumour-associated microbial signatures. To compare the tumourigenic phenotypes in different colonisation contexts, we colonised mice with complex human microbiota (humanisation microbiota association (HMA)) from IBD (132 stool samples) and CRC (106 stool samples) cohorts, as well as a colitis-associated simplified human microbiota (modified SIHUMI).

Results We previously showed that biallelic (tg/tg) but not monoallelic (tg/wt) nATF6IEC mice spontaneously develop tumours. IL10-deficiency increased tumour susceptibility, with tg/tg;-/- mice never exceeding a lifespan of 10 weeks, and tg/wt;-/- mice developing colon tumours with an incidence of 70%. Mucosal microbiota was significantly altered in tg/wt;-/- mice compared to controls. GF tg/wt;-/- mice remain tumour free. FACS analysis in SPF mice showed abnormalities in innate immune cells, with neutrophil and monocyte infiltrates in tumour-susceptible mice, and a decrease in NK cells in the tumour niche. CHIP cytometry was applied to specifically dissect immune cell infiltration in tumour (T) and non-tumour (NT) tissue in the tumour niche, and showed high neutrophil and cytotoxic T lymphocyte infiltration in T tissue. HMA with IBD and CRC patient stool successfully transferred the disease phenotype and reduced the lifespan of tumour-susceptible mice. HMA induced both colonic tumours (75% IBD; 86% CRC) and duodenal tumours (75% IBD; 39% CRC) in tg/wt;-/- mice. Interestingly, the colonisation with modified SIHUMI also showed a reduced lifespan and tumour incidences of 64% in the colon and 71% in the duodenum. Phenotypic characterisation of tumour-susceptible SPF and colonised mice showed elongated crypts, an increase in proliferating cells, and reduced mucin-filled goblet cells.

Conclusion Similar to our CRC mouse model, our novel CAC mouse model shows microbiota-dependent tumour development in the proximal to mid colon. A key immune cell characteristic of tumours is the alteration of innate immune cells. HMA successfully transferred the human disease phenotype, inducing inflammation and tumour formation in tumour-susceptible genotypes. Interestingly, tumour formation was also triggered following colonisation with a minimal consortium, rendering our mouse models useful tools to study host-microbe interactions in the context of inflammation and tumourigenesis.

14 EFFECT OF FOOD ALLERGY ON GUT MICROBIOMES

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There has been a striking increase of food allergies among the people of different age groups in recent years. Effects of food allergies on the gut microbiomes are known for several years already. However, any roles of different microbes in allergy are still unknown. Using published data (Goldberg, et al., Genome Medicine, 2020), we analysed associations of microbes with allergies based on 16S rRNA amplicon sequencing. The dataset contains 724 persons associated with food allergies like milk (n = 321), peanuts (n = 55), tree nuts (n = 135), sesame (n = 133), and eggs (n = 35). Furthermore, 45 people are not allergic to any of these five types of foods and serve as controls. Bacterial 16S rRNA amplicons were sequenced from faecal samples by targeting the highly conserved V4 region. From these amplicons, we first derived zOTUs. Each zOTU was aligned to the SILVA database for assignment of the probable taxa. Concerning alpha diversity, we observed no significant difference between allergic and non-allergic participants. For determining which zOTUs significantly changed in food allergy compared to controls, we performed analysis using a discrete False Discovery Rate (dsFDR) at the significance level of 0.1. To validate the zOTUs found discriminating allergic and non-allergic, we performed a supervised machine learning. Using these two methods, we observed bacteria from the families of Muribaculaceae, Lactobacillaceae, Lachnospiraceae, Bacteroides, and Sutterellaceae to be significantly affected (both positive and negative) in food allergy. Multiple studies have reported a link between the abundances of these microbes and food allergy. These findings re-emphasize that the composition of the intestinal bacteria changes in food allergies. However, further research is needed to turn correlation into causation, which we expect in the ongoing ABROGATE study.

15 BENCHMARK OF METAGENOME PREDICTION TOOLS: ON THE LIMITATIONS OF 16S-BASED FUNCTIONAL PROFILING

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While being accessible and cost effective, 16S rRNA gene sequencing cannot directly provide insights on the functional profiles. With a number of tools proposed for functional prediction from 16S rRNA gene profiles, it is important to understand how they perform compare to shotgun metagenome sequencing (MGS) and if they are able to capture subtle functional differences related to human health. We tested the reliability of the prediction tools such as PICRUSt2, Tax4Fun2, PanFP and Metgem using matched 16S rRNA and MGS human datasets as well as simulation datasets for different functional categories. Our contribution is three-fold: (i) Focusing on type 2 diabetes, inflammatory bowel disease, colorectal cancer and obesity, we establish if health-related differential abundance measures of functional categories are concordant between 16S-predicted and MGS-derived across multiple cohorts from different geographic location; (ii) we investigate if technical differences related to the assay or sample preparation lead to biases based on artificial 16S profiles extracted from simulated MGS data; (iii) we studied if the performance of existing tools could be improved by replacing the builtin copy number normalisation with a normalisation based on the rrnDB database.

Predictions of these tools deviated significantly from actual metagenome gene profiles across all samples that were analysed. Our results showed that metagenome prediction tools generally do not have the necessary sensitivity to delineate health-related functional changes in the microbiome and should thus be used with care. Furthermore, we delineate important differences in the individual tools tested and offer recommendations for tool selection.

Keywords: Microbial functional profiles prediction, 16S rRNA gene sequencing, metagenome prediction tools, 16S rRNA gene copy number normalization

16 TNF-DEPENDENT INTESTINAL INFLAMMATION IN XIAP-DEFICIENCY INDUCES MICROBIAL DYSBIOSIS LEADING TO A LOSS OF BUTYRATE-PRODUCING *CLOSTRIDIA* SPECIES

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Loss of the X-linked inhibitor of apoptosis protein (XIAP) can lead to a very early-onset Crohn's like intestinal inflammation in male patients. A risky hematopoietic stem cell transplantation is the only available treatment as of now.
We employed an *Xiap*$^{-/-}$ mouse model to investigate the underlying mechanism of this inflammatory phenotype. *Xiap*$^{-/-}$ mice from a conventional mouse facility display markers of tissue inflammation in their terminal ileum, characterized by villus edema and T cell infiltration, and a loss of mucus-producing Goblet cells resulting in impaired epithelial barrier integrity. Analysis of mouse intestine deficient in Tumour necrosis factor receptor (TNFR)1 and TNFR2 showed that both receptors contribute to inflammation downstream of excessive *Tnf* expression in absence of XIAP.

Interestingly, germfree or SOPF *Xiap*$^{-/-}$ mice do not present with an intestinal phenotype, and co-housing wildtype and *Xiap*$^{-/-}$ mice rescued inflammation. Introduction of a conventional microbiome into germfree WT and *Xiap*$^{-/-}$ mice via fecal matter transplantation induced ileal inflammation in *Xiap*$^{-/-}$ mice only. Therefore, we analyzed the cecal content of conventionally housed and SOPF-housed *Xiap*$^{-/-}$ mice in comparison to WT animals via 16S rRNA sequencing. *Xiap*$^{-/-}$ mice from conventional housing show a marked loss of bacteria of the *Firmicutes* phylum, in particular members of the class *Clostridia*. NMR-based analysis of fecal content showed a reduction of both butyrate and its metabolite β-hydroxybutyrate, known to be produced by *Clostridia* species. Additionally, the presence of *Helicobacter rodentium* was confirmed in inflamed *Xiap*$^{-/-}$ mice, potentially acting as a trigger in establishing intestinal inflammation.

Hence, Crohn's like ileitis in *Xiap*$^{-/-}$ mice from conventional housing is induced by aberrant TNF-signalling driving intestinal tissue inflammation. Ultimately, this leads to the development of microbial dysbiosis featuring a distinct loss of butyrate-producing *Clostridia*.

17 PROTECTING BACTEROIDES/PHOCAEICOLA SPP. FROM MACROLIDE ANTIBIOTICS BY ANTAGONIZING DRUG INTERACTIONS

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Medication, especially antibiotics, has been identified as a major factor impacting the human gut microbiota composition. The collateral damage of antibiotics and the simultaneous increase of antibiotic resistance are major health care concerns. Hence, ways to prevent the effects of antibiotics on human gut microbes could improve current antibiotic therapy. Culture-based methods to investigate the effect of antibiotics on gut microbes can be instrumental and recently revealed that, text book bacteriostatic antibiotics, such as macrolides, exert bactericidal effects on commensals. Knowing that drug-combinations can have species-specific outcomes, we investigated to what extent antagonistic drug interactions ("antidotes") can be used to selectively protect the highly abundant genera Bacteroides and Phocaeicola from macrolides, without compromising the macrolide's effect on relevant pathogens. From ~1,200 drugs tested we identified three antidotes (Dicoumarol, Benzbromarone & Tolfenamic Acid) that could selectively protect Bacteroides/Phocaeicola spp. from the macrolide Erythromycin in both pure culture and in microbial communities.
Still, the mechanism and the protective extent of these antagonistic interactions remains to be elucidated. For this, we investigated strain-to-strain variation of these antagonistic drug interactions for the highly abundant commensal Phocaeicola vulgatus and also expanded the repertoire of antidote-antibiotic combinations for which antagonism occurs. Further, analysis of structurally similar antidotes as well as drug accumulation and/or efflux assays will contribute to the elucidation of the underlying mechanism and prerequisites for the selective antagonism. Overall, our results reveal novel strategies to circumvent the collateral damage of antibiotics on the gut microbiota to reduce the side effects currently associated with antibiotic treatment.

18 RIBOSOME PROFILING OF MINIMAL CONSORTIA OBSERVES DIFFERENT LAYERS OF GENE REGULATION

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Ribosome profiling is a sequencing technique used to analyse mRNAs under translation (i.e., revealing the 'translatome'). Ribosome-covered mRNAs are RNase protected (i.e., producing footprints) and these fragments are sequenced. Thus, differentially expressed proteins are indirectly detected via translation. Analysis of complex gut communities is difficult since the obtained sequencing reads are short and non-ambiguous mapping to genomes is challenging. We reduced complexity by using a simplified human gut microbiome (SIHUMI) consortium with seven strains. Mapping with BBMap and adjusted parameters was found performing best in minimizing ambiguous mapping of footprints to the bacterial genomes, allowing determining translational profiles for each strain separately. At first, meta-ribosome profiling of SIHUMI was conducted in in-vitro cultures at pH 7 and pH 5.5. Secondly, meta-ribosome profiles from faeces of SIHUMI-colonized wild-type mice were compared to profiles from interleukin-10-deficient mice. For this, the protocol was adapted in order to use lower amounts of bacteria. Standard RNA-seq was performed as comparison in all experiments. We analysed specific gene groups involved in bacteria-bacteria interactions in further detail (e.g., quorum sensing, toxin-antitoxin systems, toxins including bacteriocins, motility, and protein export) and additionally hypothetical genes. Meta-ribosome profiling enhances our knowledge about gene regulation and novel genetic elements expressed in culture and in a mouse model.

19 DEOXYCHOLIC ACID PROMOTES COLONIC TUMORIGENESIS IN GNOTOBIOTIC MICE COLONIZED WITH A BILE ACID-CONVERTING SIMPLIFIED MICROBIOTA

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Objective: The secondary bile acid deoxycholic acid (DCA) is produced by human gut bacteria and was shown to be positively correlated with colorectal cancer (CRC). Mechanisms underlying the tumor-promoting function of DCA are still not clear and the effect of in vivoproduced DCA on CRC development was not demonstrated to date. Here we investigate the tumorigenic effects of DCA in the colon using gnotobiotic mice colonized with a simplified microbial consortium. We further analyze the effects of the presence/absence of DCA on markers associated with CRC, bile acid metabolism and intestinal regulatory T-cells (Treg).
Methods: Germ-free wildtype mice were colonized with a simplified bile acid-converting microbiota with (BACOMI) or without (BACOMI-S) Clostridium scindens, which has a 7-adehydroxylating activity, resulting in DCA production. A subgroup of BACOMI colonized mice was treated with AOM/DSS to induce colonic tumorigenesis. Bacterial abundance in colon content was measured using qPCR and bile acids were quantified by LC-MS/MS. In the colon, expression of genes related to tumorigenesis and bile acid metabolism were analyzed using qPCR and proliferation of epithelial cells assessed by Ki67 staining. Treg were isolated from colon and iliac lymph nodes and analyzed using flow cytometry.
Results: Germ-free wildtype mice were successfully colonized with the microbial consortium and DCA was only present in mice colonized with BACOMI but not in BACOMI-S mice lacking C. scindens. In mice treated with AOM/DSS, colonization with BACOMI led to higher numbers

of colonic tumors compared to mice colonized with BACOMI-S. Even without chemicallyinduced tumorigenesis, CRC-associated genes (e.g. Ccnd1, Ptgs2 and Myc), as well as genes involved in bile acid metabolism (e.g. Slc10a2, Nr1h4 and Slc51b) were upregulated in the colonic mucosa of BACOMI compared to BACOMI-S colonized mice. BACOMI mice had higher numbers of Ki67 positive cells in the colon and showed reduced colon lengths compared to BACOMI-S mice. Foxp3+, Foxp3+ CD103+ and Foxp3+ CD304+ Treg were more abundant in the iliac lymph nodes of BACOMI compared to BACOMI-S mice, but no differences in these Tcell populations were detected in the colon.

Conclusion: Colonization of germ-free mice with different BACOMI consortia is a suitable model to investigate the effects of DCA on colonic tumorigenesis and intestinal immune cells in vivo. The presence of DCA in this gnotobiotic model system leads to increased levels of different mucosal markers associated with CRC and higher numbers of colonic tumors, demonstrating the tumor-promoting function of DCA.

20 IMNGS2: A WEB PLATFORM THAT PUTS THE GLOBAL AMPLICON WEALTH AT THE RESEARCHER'S FINGERTIPS FOR INTEGRATIVE MICROBIOME STUDIES

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The SRA (Sequence Read Archive), as a primary repository for primary Next Generation Sequencing data, currently host over 1,400,000 16S rRNA gene amplicon-based microbial profiles from various host habitats and environments. This wealth of information is underutilized, mainly due to difficulties in integration of data from different studies. The first step for a seamless integration would require a quick and versatile compatible dataset recognition and a flexible modification of the list of prospective samples for analysis. Furthermore, the analytical pipeline itself should be able to handle thousands of samples and deliver the results in reasonable time. Responding to this challenge, here we present IMNGS2, the evolution of Integrated Microbial Next Generation Sequencing (IMNGS) web-based platform that focuses on offering to its users a unified platform for integrative analysis. IMNGS2 systematically and uniformly preprocess all available Illumina based amplicon datasets (>1 Million) down to denoised sequences. Users can easily retrieve lists of samples fitting to selected criteria to analyze or combine with own data. Our platform incorporates state of the art analytical tools and custom-made solutions for enhanced insights into community structure. Combined with the former functionalities for sequence or taxa querying this massive integrated resource IMNGS2 offers a unique open platform for microbiome research that is accessible at www.imngs2.org.

21 ROLE OF COMMENSAL FUNGI IN A MOUSE MODEL OF ANTIBIOTIC-INDUCED DYSBIOSIS

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Resilience of microbiota describes the capacity to overcome dysbiotic disruption of a given consortium by antibiotic therapy and regain its previous species diversity. We study resilience in mice rendered dysbiotic by treatment using an antibiotic cocktail of Vancomycin, Metronidazole, Neomycin, and Ampicillin (VMNA) followed by intranasal applications of four bacterial strains originally isolated from lungs of wild mice (Muribacter, Corynebacterium, Ligilactobacillus, Staphylococcus (MCLS; Yun et al., 2014) or a mock solution. Our preliminary data revealed low Shannon diversity indices in antibiotictreated, but significantly increased diversities in recolonized mice. In an adapted model, we applied the antifungal agent Amphotericin B in addition to VMNA to prevent antibiotic-induced fungal overgrowth. This led to increased caecum weights indicative of gut dysbiosis. In contrast to former experiments, MCLS-recolonized animals maintained high caecum weights and did not recover from gut dysbiosis. Based on these results, we hypothesize that fungi play a greater role in the stability of the microbiome than previously appreciated. To determine whether fungi foster resilience of the microbiota after induction of antibiotic induced dysbiosis, we will directly compare the different treatments and determine the restoration of the murine microbiome based on 16S and ITS sequencing.

As dysbiotic microbiomes are often associated with gastrointestinal and respiratory tract infections, we have established a minimal infectious dose Klebsiella pneumoniae (Kp) infection model in mice to analyze immune mechanisms contributing to microbiota resilience. In brief, mice were infected oropharyngeally with 10 CFU. Kp infections have previously been shown to cause a stronger disease course in mice when pre-treated with VMNA compared to non-dysbiotic mice. Using this model, we will challenge dysbiotic mice with Kp and initiate recovery with MCLS before or after infection to determine, whether and when recolonization with MCLS can ameliorate immunological control over a Kp infection, and whether commensal fungi contribute to it.

A better understanding of cross-kingdom and the mutual interactions between microbiota and the immune system could improve probiotic approaches to treat both, gastrointestinal- and respiratory tract infections.

22 UNDERSTANDING THE FUNCTIONAL ROLES OF WITHIN-HOST EVOLUTION OF THE GUT MICROBIOTA

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The mammalian gut is a highly dynamic microbial ecosystem that impacts host fitness. The metabolic networks established between intestinal bacteria affect several microbiome functions including colonization resistance (CR) against enteric pathogens, which is in part mediated by competing for nutrients that are essential for the invading pathogens such as Salmonella enterica serovar Typhimurium (S.Tm). One of the factors that strongly influence these metabolic networks and bacterial interactions is within-host evolution. While community composition and function have been extensively studied, it remains unclear how these are affected by within host evolution. In this study, we use experimental evolution as a tool to get insights into the adaptation of a defined microbial consortium to the gut environment and its functional relevance for CR against S. Tm. Our preliminary results show that during colonization of the mouse gut key strains involved in CR adaptively evolve and accumulate extensive, non-synonymous mutations in functional gene classes. We also show that this community evolution translates into an increased colonization resistance to S. Tm enteric infection. Further understanding the mechanisms driving evolution will allow engineering microbial communities towards a healthy state of the host.

23 EFFECTS ON HUMAN FECAL MICROBIOME AFTER IN VITRO FEREMENTATION OF FUNGAL POLYSACCHARIDES FROM CLOSELY RELATED GANODERMA SPP.

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β-glucans are macromolecules with glycosidic bonds recalcitrant to human enzymes. Therefore, these polysaccharides are not absorbed by enterocytes and thus considered as dietary fiber. β-glucans differing in molecular weight and chemical branching are present in cereal, baker´s yeast, mushrooms, and seaweed. These chemical differences confer different health functions such as immunomodulatory, cardiovascular effects and gut health. We hypothesize that their functionality towards gut microbiome shaping depends on their chemical features. Here, we compare the chemistry and the effects on the human fecal microbiome after *in vitro* fermentation of polysaccharides extracted from *Ganoderma sessile*, a fast growing strain, and a commercial standardized β-glucan extract from the medicinal mushroom *G. lucidum*.

Polysaccharides of *G. sessile* (GSP) were extracted from supernatants by precipitation with ethanol and further characterized by size exclusion chromatography and nuclear magnetic resonance (NMR). Molecular weight distribution and monosaccharide composition were also assessed. Polysaccharides from *G. lucidum* (GLP) were extracted from a dietary supplement available in the market and characterized by the same methods. Stool samples were collected from ten healthy volunteers. The *in vitro* fermentation assays on stool samples were performed in anaerobic conditions at a final concentration of polysaccharides of 10 mg/mL (1%). Before and after 8 hours of fermentation with the said polysaccharides, DNA was extracted for further analysis of the microbial composition. Variable regions of the 16S rRNA genes in each sample (i.e., V3-V4 regions) were amplified and sequenced. Ecological parameters and correlations with meta variables (e.g., SCFA, β-glucan chemistry) of the samples are being analyzed using

the existing pipelines of IMNGS and Rhea, and SCFAs assessed by GC-MS after derivatization with BSTFA to form trimethylsilyl (TMS) derivates.

GLP was exclusively composed of glucose, while GSP contained additional monosaccharides such as galactose, mannose, arabinose, rhamnose and ribose. NMR revealed the presence of both alpha and beta glycosidic bonds in both polysaccharides, being the later more abundant in GLP. Significant differences were found between SCFA profiles after *in vitro* fermentation of GSP and GLP ($p<0.05$).

While the analyses on microbial composition and SCFAs are ongoing, these early results reveal that the chemistry of polysaccharides differ as well as the metabolic responses of the fecal microbiota. Differences in microbial metabolic responses between GSP and the reference polysaccharide GLP, suggest that a higher chemical heterogenicity of the fiber would be associated to an increase in functionality.

24 THE NUTRITIONAL ENVIRONMENT INFLUENCES SPECIES INTERACTIONS AND COMMUNITY ASSEMBLY IN A SYNTHETIC GUT BACTERIAL COMMUNITY

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The nutritional environment and bacterial species membership are key determinants of bacterial community function, both mutually dependent on each other. To elucidate cause and consequence of bacteria interacting with each other and their environment, studying bacterial mono- and pairwise co-cultures provides a good basis for characterizing individual interactions in low-dimensional communities. However, in multispecies consortia, pairwise interactions can influence each other, increasing complexity of dependencies and leading to non-linear higher-order interactions. Here, we used “drop-out” communities to develop a deeper understanding of the role of individual species, as well as the nutritional environment on the assembly and ecology of the Oligo-Mouse Microbiota (OMM^{12}), a synthetic bacterial community.
Different single-species dropout communities were established in two different culture media in an *in vitro* batch culture approach, as well as in germ-free mice. To resolve the effect of individual species on the overall community assembly and composition, community structure was analyzed using quantitative real-time PCR. As a parameter for environmental modification, pH measurements and metabolomics analysis of the spent culture media, as well as in the different compartments of the murine gut, provided insights to the capability of the individual strains to influence community metabolic profile.
Thereby, it was shown that the nutritional environment is a key determinant of community assembly of the OMM^{12} community and, directly depending on their potential to modify the corresponding metabolic environment, individual species exert a particularly strong influence on the abundance of other species. Specifically, *Bacteroides caecimuris* I48 and *Enterococcus*

faecalis KB1 were identified as key species in driving community ecology *in vivo* and *in vitro* by resolving their capacity to manipulate their environment in a mechanistic fashion.

In conclusion, these findings help to create a more comprehensive understanding of the OMM^{12} community ecology, the driving forces for community assembly and the influence of the nutritional and metabolic environment *in vivo* and *in vitro*.

25 PROMOTING GUT DECOLONIZATION OF MULTI-DRUG RESISTANT BACTERIA VIA THE MICROBIOME

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The fight against multi-drug resistant (MDR) Enterobacteriaceae has been declared as a high priority by the WHO. Colonization of the human gut with MDR Enterobacteriaceae, including MDR E. coli, is associated with an increased risk of infection and also dissemination within the community. Several experimental interventions have been explored to promote decolonization of the gut of MDR Enterobacteriaceae, for example treatment with antibiotics or fecal microbiota transplantation (FMT). However, both potentially exhibit adverse effects on the gut microbiota (for example diarrhea and loss of colonization resistance). In contrast, probiotics developed to selectively decolonize the microbiota of carriers from MDR strains are a promising alternative, specifically, if they achieve their aim without affecting health-promoting commensals. Previous studies showed that closely related commensal Enterobacteriaceae can compete against each other in the murine gut resulting in the displacement of the losing species from the ecosystem. We hypothesize that the human gut is a great resource for probiotics, which show the potential to selectively decolonize MDR Enterobacteriaceae.
To identify commensal strains with protective properties we established an ex vivo assay, spiking candidate probiotics and a MDR E. coli strain into cecum content of mice or humans. This assay can be used as a universal screening-tool, which enables a screening of commensal isolates. As a novel resource for the identification of potentially probiotic bacteria, a strain collection with nearly 400 strains was generated from 250 donors from three cohorts comprising individuals from different age groups and nationalities. We were able to show growth reduction of MDR E. coli after co-cultivation with specific commensal strains (42/ 232 tested strains). To further verify the protective effect, our goal was to implement competition experiments in mice. To demonstrate the probiotic effect of the respective bacterial strain, we show that SPF mice treated with ampicillin, were able to promote a 100 % clearance of MDR E. coli, after administration of a probiotic strain. Since MDR E. coli strains show a high genomic diversity, it should be also verified that the probiotic bacteria can protect against a variety of different strains.

For promising candidates we intend to identify their metabolic niche and potential cooperation partners as well as to gain mechanistic insights using loss-of-function genetic screens. On top of that, it should be verified that the probiotic strains fulfill all required characteristics. The strains need to show a protective effect, should have a GRAS status (generally-regarded-as-safe) and not express any virulence factors or antibiotic resistance genes.

26 A PORCINE MODEL OF INFLAMMATORY BOWEL DISEASE

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Inflammatory bowel disease (IBD) is a global burden with increasing incidence and rising prevalence, projected to reach up to 1 % of the local population in many regions by 2030. Rodent models of IBD are often not ideal for translational research as they differ in anatomy, physiology and diet form their human counterparts. Therefore, our goal was to develop a physiologically relevant porcine model that would reflect the phenotype of human IBD as closely as possible and which would support the transfer of therapeutic interventions into the clinic. Based on the mouse model for a Crohn's disease-like microbiome-driven ileitis pathophenotype, the TNFα$^{\Delta ARE/+}$ mouse, we generated pigs with a 93 bp deletion of the adenosine-uracil rich element (ARE) and a constitutive decay element (CDE) within the 3' UTR of the porcine TNFα gene. A comparative analysis of physiological, molecular, histological and microbial characteristics was performed on WT, TNFα$^{\Delta ARE/+}$ and TNFα$^{\Delta ARE/\Delta ARE}$ animals. Increased TNFα half-life and elevated TNFα protein levels were observed in TNFα$^{\Delta ARE}$ pigs compared to control animals, in a gene dose-dependent manner. Enhanced activity of genes encoding proinflammatory cytokines and immunohistochemical markers of intestinal inflammation were detected throughout the gut, but particularly in the proximal colon and distal ileum. Comparative analysis of 16s rRNA sequencing data revealed specific compositional and functional alterations of bacterial communities in inflamed pigs. Our results demonstrate that the TNFα$^{\Delta ARE}$ pig resembles a Crohn's disease-like pathophenotype, making it a physiologically relevant model for translational studies in human IBD research.

27 APPLICATION OF MICROBIOTA FOR PROBIOTICS PROMOTING GUT DECOLONISATION OF MULTI-RESISTANT GRAM-NEGATIVE BACTERIA

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The World Health Organisation has reported increasing numbers of antibiotics-resistant bacteria with healthcare significance. Especially, the spreading of Carbapenem-resistant Enterobacteria and multidrug-resistant nosocomial bacteria of the ESKAPE panel has remained an ongoing challenge. To evade the continuous cycle of developing new antibiotics and consecutive bacterial resistances, alternative treatment options have to be considered.
Similar to fecal microbiota transplantion (FMT) for *Clostrioides difficile* infections, using protective properties of the human intestinal microbiota for preventing intestinal colonization and infection could be a promising approach. Moreover, the identification and utilisation of single species or of a defined multi-species cocktail of bacteria would avoid FMT attributed risks of transferring an unknown combination of bacteria.
A previous study from the lab identified that commensal *Klebsiella oxytoca* strains have protective characteristics decreasing gut colonization of multi-resistant *Klebsiella pneumoniae* strains. To broaden the possible application of this strain as a probiotic, decolonisation efficiency and protective mechanism were tested against a variety of multi-resistant pathogenic bacteria, such as *Enterobacter cloacae, Proteus mirabilis* and *Acinetobacter baumannii*. For bacterial co-cultures of commensal *K. oxytoca* and pathogenic bacteria different competition assays were evaluated in vitro aiming to minimize in vivo experiments in mice. Preliminary data suggests that the probiotic *K. oxytoca* strain has broadly protective properties. Ongoing experiments are aimed to identify potential mechanisms, f. e. niche or carbohydrate competition or potentially selective bacterial toxicity.
In summary, this study aims to gain mechanistic insights into microbial competition between commensal Klebsiella strains with MDR bacteria with the aim to achieve broad protection against colonisation with diverse multi- resistant nosocomial bacteria.

28 WITHIN HOST EVOLUTION OF A SYNTHETIC BACTERIAL COMMUNITY DRIVES INTER- AND INTRA-SPECIES DIVERSIFICATION

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Genetic evolution is the major driving force during adaptation to changing environmental conditions in bacterial ecosystems. In the mammalian gastrointestinal tract, limited availability of nutrients such as carbohydrates and electron acceptors ignites a harsh competition between members of the microbiota, inducing rapid within-host evolution. While adaptive evolution of single bacterial species is well characterized, the impact of genetic adaptation in bacterial communities on the fine-tuning of the bacterial metabolic network remains poorly understood. The Oligo Mouse Microbiota 12 (OMM12) is a defined consortium, consisting of 12 bacterial species with diverse functionalities regarding carbohydrate degradation, secondary metabolite production and short-chain fatty acid biosynthesis. It represents the five most abundant phyla of the mammalian gut and stably colonizes germ-free mice over time, making it a well-suited model system to study evolution of bacterial communities in vivo. In order to explore evolution of a bacterial community on a single-species genomic level, we established a diverse set of tools for the re-isolation of bacteria from OMM12 mice, including magnetic-activated cell sorting-based techniques, selective cultural methods and high-throughput screens. In total 130 bacterial isolates from seven different bacterial species were obtained from three different mice and subjected to genome resequencing and phenotypic characterization. Next-generation sequencing revealed the accumulation of non-synonymous single nucleotide polymorphisms (SNPs) in metabolic pathways in all isolates, as well as partial genome duplications and deletions in several species. The targeted genetic loci included genes involved in carbohydrate transport, nucleotide metabolism, glycolysis, amino acid degradation and the stringent response. Among the seven isolated species, we found a varying degree of intra-species

diversity, which partially correlated with drastic differences in mutation rates. Phenotypic characterization of bacterial isolates using Biolog Phenotype MicroArrays identified inter- and intra-species differences in the carbon source utilization potential. Collectively, this study implements a workflow for the characterization of evolutionary trajectories within bacterial communities in vivo and identifies key functions and pathways under selection of a mouse-adapted community. Further research is needed to elucidate how the observed metabolic changes impact microbiome-pathogen interactions and host physiology.

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