3rd Summer School Seeon

13th Seeon Conference

Microbiota, Probiotics and Host

JULY 1ST- 3RD, 2021

HYBRID CONFERENCE

For more information: www.DGHM.org/Seeon

July 1st, 2021

Dear Participants,

We warmly welcome you at the Seeon Monastery for the 13th Seeon Conference "Microbiota, Probiotics and Host", an event that is annually organized by the section ***Microbiota, Probiotics and Host*** of the ***German Society for Hygiene and Microbiology (DGHM)***.

Since its kick-off in 2008, the Seeon Conference has become an important ***hub for microbiome research in Germany***, with topics that range from fundamental principles of microbial ecosystems to effects on the host in the clinics. Since 2018, the conference is accompanied by a ***Summer School*** on "Microbiome in Health and Disease" to train and promote young scientists in the fields of gastroenterology, nutritional medicine, immunology, infection research, microbial ecology, and computational science.

Multiple research consortia in Germany have contributed to and continue to animate activities at and around the Conference. The DFG-funded Priority Programme "MICROBIOTA – a Microbial Ecosystem at the Edge between Immune Homeostasis and Inflammation" (***SPP 1656***) was instrumental for laying the foundation of this event. Further development is boosted by several other microbiome-centred research consortia and initiatives in Gemany, including (but not restricted to): ***NFDI4Microbiota***, Collaborative Research Center ***(CRC) 1182*** 'Metaorganisms' in Kiel; ***Cluster of Excellence CMFI*** (Controlling Microbes to Fight Infections) in Tübingen; ***CRC 1382*** 'Gut-liver axis' in Aachen; ***CRC 1371*** 'Microbiome Signatures' at the TU Munich in Freising. These consortia taken together gather >200 researchers and even more young scientists that all actively contribute in bringing this very dynamic field of research further.

We are delighted to have you as a participant of this great event and happy that you will be given the opportunity to interact with experienced and younger scientists in the field. Due to the current pandemic situation, the 2021 Seeon Conference will follow a ***hybrid design*** with a core group of scientists on-site to animate discussions, an outstanding line-up of speakers who will give keynote lectures online coupled to stimulating discussion with a broad audience of participants.

We are looking forward to hear about good science and have a great time in (virtual) Seeon!

Prof. Dr. Thomas Clavel, on behalf of the Organizing Committee
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Functional Microbiome Research Group
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FINANCIAL SUPPORT

Many thanks to our supporters!
Our meeting would not be possible without them:

PRE-RECORDED LECTURES 3RD SUMMER SCHOOL

ACCESS-LINK WILL BE PROVIDED VIA EMAIL (ACCESSIBLE ONLINE FROM 24.06.-09.07.21)

1. **Thomas Clavel** (University Hospital of RWTH Aachen, Germany)
Basics in gut microbiome research

2. **John Baines** (Kiel University / MPI for Evolutionary Biology, Plön, Germany)
Evolutionary perspectives on the human gut microbiome

3. **Nicola Segata** (CIBIO - University of Trento, Povo, Italy)
In-depth and large-scale metagenomic profiling of the human microbiome

4. **Bärbel Stecher** (Max von Pettenkofer Institute, LMU Munich, Germany)
Microbial interactions & synthetic communities

5. **Mathias Hornef** (University Hospital of RWTH Aachen, Germany)
Ontogeny of the enteric microbe-host interaction -
postnatal establishment of the enteric microbiota

6. **Dirk Haller** (Technical University Munich (TUM), Germany)
From observational population-based studies to causation

7. **Till Strowig** (Helmholtz Centre for Infection Research, Braunschweig, Germany)
Imprinting of immune responses by gut microbes: on the verge of inflammation

SUMMER SCHOOL PROGRAM

Thursday, July 1st – Live via Zoom

14^{00} – 14^{05}	Welcoming to the Summer School: **Thomas Clavel** (University Hospital of RWTH Aachen, Germany)
14^{05} – 15^{30}	Meet the lecturers
15^{30} – 15^{45}	Break
15^{45} – 17^{45}	Online Live-Lectures and Discussion **Cynthia Sears** (John Hopkins School of Medicine, Baltimore, USA): ***Colon Cancer and the Microbiome: Evolving Data*** **John Penders** (Maastricht University, The Netherlands): ***Does our Microbiome Travel well?*** **Ed Kuijper** (Leiden University Medical Center, The Netherlands): ***How to restore the disturbed microbiome of patients with Clostridioides difficile infections?***
17^{45} – 17^{50}	Closing Words: **Thomas Clavel** (University Hospital of RWTH Aachen, Germany)

CONFERENCE PROGRAM
Friday, July 2nd – Live via Zoom

10^{00} – 10^{10} Welcoming to the Conference: **Thomas Clavel**
(University Hospital of RWTH Aachen, Germany)

SESSION 1

*Chair: **Dirk Haller** (Technical University Munich (TUM), Germany)*

10^{10} – 10^{55} Keynote Lecture
Shinichi Sunagawa (ETH Zurich, Switzerland):
Multi-layered strategies of host and environmental microbiome analysis

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10^{55} – 11^{25} 2 Selected Abstracts

Fátima Pereira *(University of Vienna, Austria)*
SRS-FISH: High-Throughput Platform Linking Microbiome Function to Identity at the Single Cell Level
Thomas Hitch *(University Hospital of RWTH Aachen, Germany)*
Automating the description and naming bacteria in the 21st century

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11^{25} – 11^{40} Break

SESSION 2

*Chair: **Maria Vehreschild** (University Hospital Frankfurt, Germany)*

11^{40} – 12^{25} Keynote Lecture
Dario Valenzano (MPI for Biology of Ageing, Cologne, Germany):
Host-microbiome interactions during aging

12^{25} – 12^{55} 2 Selected Abstracts

Louise Thingholm *(University Kiel, Germany)*
Altered gut microbiome and microbial metabolism in primary sclerosing cholangitis
Anna Weiss *(LMU Munich, Germany)*
Exploring the interaction network of a synthetic gut microbial community

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12^{55} – 14^{00} Lunch Break

SESSION 3

Chair: ***Bärbel Stecher*** *(Max von Pettenkofer Institute, LMU Munich, Germany)*

14^{00} – 14^{45} Keynote Lecture
Nassos Typas (EMBL, Heidelberg, Germany):
The interface of drugs and the human gut microbiome

14^{45} – 15^{15} 2 Selected Abstracts

Marie Wende *(Helmholtz Centre for Infection Research, Braunschweig, Germany)*
Promoting gut decolonization of multi-drug resistant bacteria via the microbiome
Alyson Hockenberry *(ETH Zürich, Switzerland)*
Microbiota-derived metabolites inhibit Salmonella virulent subpopulation development by acting on single-cell behaviors

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15^{15} – 15^{30} Break

SESSION 4

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

15^{30} – 16^{15} Pre-Recorded Keynote Lecture
Eran Elinav (Weizmann Institute of Science, Rehovot, Israel):
Host microbiome interactions in health and disease

16^{15} – 16^{45} 2 Selected Abstracts

Henriette Arnesen *(Norwegian University of Life Sciences (NMBU), Oslo, Norway)*
Naturalizing the microbiome by housing mice in a farm environment confers protection against colorectal carcinogenesis
Miriam Ecker *(Technical University Munich (TUM), Germany)*
High fat diet induces ATF6-dependent colorectal tumorigenesis in murine models

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16^{45} – 17^{00} Break

POSTER SESSIONS

17^{00} – ca. 18^{45}	Discussions in Breakout Rooms:
Author	Short Titel

SESSION 1 - 17^{00} – 17^{20} – Breakouts:

1 Afrizal Afrizal	Anaerobic Single-Cell Dispensing
2 Lena Amend	Microbiome in arthritis
3 Mohamed Tarek Badr	Metagenomics for diagnostics
4 Sophia Fabiana Göldel	Microbiome & hematopoietic stem cell transplantation
5 Martin Köberle	Skin microrbiome sequencing

SESSION 2 – 17^{20} – 17^{40}– Breakouts:

6 Arne Bublitz	Combinatorial microbiome against C.diff
7 Amira Metwaly	Diet/SFB in experimental Crohn's disease
8 Klaus Neuhaus	Meta-ribosome profiling
9 Diego Ortiz	Probiotics in preterm newborns
10 Annika Schwentker	Gut barrier in postnatal period

SESSION 3 – 17^{40} – 18^{00}– Breakouts:

11 Sandra Bierwirth	ATF6 and lipid metabolism in colonic tumorigenesis
12 Celina Prosch	Probiotic ACLS in mouse models
13 Aayushi Shah	Microbiota/Tcells in IBD
14 Esther Wortmann	Secondary Bile Acids in Colorectal Cancer

SESSION 4 – 18^{00} – 18^{20}– Breakouts:

15 Sudip Das	Human Lung Microbiota
16 Nina Heppner	GOS and Bifidobacteriaintervention in infants
17 Claudio Neidhöfer	Carbapenemase-encoding bacteria
18 Felix Sommer	Hexokinase 2 in colitis
19 Iris Stolzer	Intestinal epithelial necrosis

SESSION 5 – 18^{20} – 18^{40}– Breakouts:

20 Jamie Afghani	Sampling for skin metabolomics
21 Olivia Coleman	Mucispirillum in ATF6-dependent colorectal tumorigenesis
22 Tina Eismann	Microbiome in hematological malignancies
23 Julia Notter	Microbiome & drugs in HIV patients
24 Stefanie Wagner	Quantitative microbial sequencing

CONFERENCE PROGRAM
Saturday, July 3rd – Live via Zoom

SESSION 5

Chair: ***Mathias Hornef*** *(University Hospital of RWTH Aachen, Germany)*

10^{00} – 10^{45} Keynote Lecture
Debby Bogaert (Edinburgh Medical School, UK):
Early life microbiome assembly: a window of opportunity

10^{45} – 11^{15} 2 Selected Abstracts

Cristina Kalbermatter *(University of Bern, Switzerland)*
The role of maternal microbiota in durably shaping intestinal immunity and gene expression in the offspring through epigenetic mechanisms
Tim Rollenske *(University of Bern, Switzerland)*
Parallelism of the intestinal secretory IgA response modulates functional microbial fitness

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11^{15} – 11^{30} Break

HOT TOPICS

Chair: ***Thomas Clavel*** *(University Hospital of RWTH Aachen, Germany)*

11^{30} – 12^{00} **Michael Zimmermann** (EMBL, Heidelberg, Germany):
Identifying microbiome contributions to drug metabolism and toxicity

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12^{00} – 12^{30} **Mercedes Gomez de Agüero** (University Würzburg, Germany):
Maternal microbiota and neonatal barrier function

12^{30} – 12^{35} Closing Words: **Thomas Clavel**
(University Hospital of RWTH Aachen, Germany)

SUMMER SCHOOL

Pre-Recorded Lectures - Abstracts

BASICS IN GUT MICROBIOME RESEARCH

Thomas Clavel
University Hospital of RWTH Aachen, Germany

Microbiome research is a blooming scientific field. Body microbiomes play a very important role in many aspects of host physiology and thereby influence health maintenance and the development of infection and noncommunicable diseases. Microbiome research strategies have been dominated by high-throughput sequencing methods in the last two decades, but cultivation is now catching up with speed. In all, but especially blooming fields of research, it is essential to maintain a foundation of basic knowledge and methodologies to be able to interpret state-of-the art research output. In this short presentation, to set the stage for the following talks in this Summer School, I will touch upon a few background notions on gut microbial ecosystems, including the diversity of microbes, intestinal biogeography, technical approaches and cultured fractions of the gut microbiome.

EVOLUTIONARY PERSPECTIVES ON THE HUMAN GUT MICROBIOME

John Baines
Kiel University / MPI for Evolutionary Biology, Germany

The renewed interest in human gut microbiome research spawned by modern developments in metagenomics resulted in many fascinating new results, but confusion and seeming contradictions are still common in this nascent field. As for other subdisciplines of biology, evolutionary biology serves as a unifying principle in studying host-microbe interactions. However, the range of perspectives offered by evolution is often not considered or fully appreciated in human gut microbiome research. In my lecture I will provide a broad overview of evolutionary perspectives on the human gut microbiome, which range from the origin of holobionts to microbial evolution within a host's lifetime.

IN-DEPTH AND LARGE SCALE METAGENOMIC PROFILING OF THE HUMAN MICROBIOME

Nicola Segata
CIBIO - University of Trento, Italy

Metagenomic analysis of the human microbiome has uncovered multiple links between our microbial complement and our health. However, several challenges have to be overcome to exploit the human microbiome in personalized medicine strategies, including its inter- and intra-personal variability, its extensive genetic diversity, and its still hidden components. In my talk I will introduce such challenges and discuss how large-scale high-resolution integrative metagenomics can address them and have an impact on biomedical applications.

MICROBIAL INTERACTIONS & SYNTHETIC COMMUNITIES

Bärbel Stecher
Max von Pettenkofer Institute, LMU Munich, Germany

The human gut is populated by highly diverse microbial communities that break down dietary compounds, help train our immune system, and influence human health in different ways. Most research on microbiomes to date has been limited to correlative analyses, for example, the identification of “Microbiome signatures” correlating with health or disease. However, community functionality cannot be predicted from these signatures, i.e. the sum of single organisms present in the community. The reason is that inter-species interactions, in particular higher-order interactions (behavior or functional impact of species X is different within a community versus alone) can have a huge impact on community functionality, membership and stability. Currently, the nature of these interactions is largely unresolved and limited to a few examples. Inter-species interactions can encompass metabolite exchange, physical association, signaling and secretion of antimicrobial compounds. On top of that, the environmental conditions play a critical role in influencing microbial interactions. Advancing past correlations to a point where we understand the nature and underlying mechanisms of microbial interactions is critical to predict and control microbiome function. In my lecture, I will introduce different types of pair-wise interaction between microbes and point out, how this behavior can change in complex ecosystems or depending on the environment. I will illustrate potentially medically relevant microbial interactions by means of examples from the recent literature. Lastly, I will highlight the value of reductionist experimental models and synthetic communities to identify and study microbes and molecular mechanisms involved in complex interactions.

ONTOGENY OF THE ENTERIC MICROBE-HOST INTERACTION - POSTNATAL ESTABLISHMENT OF THE ENTERIC MICROBIOTA

Mathias Hornef
Institute of Medical Microbiology, RWTH University Hospital, Aachen, Germany

The establishment of the enteric microbiota starts immediately after birth. During the immediate postnatal period the bacterial density increases rapidly followed by compositional alterations and a more delayed rise of the bacterial diversity ultimately leading to a stable and complex microbial ecosystem. The general process of the establishment of the enteric microbiota after birth has been described in a number of studies both in humans and animals models and a variety of factors have been identified that significantly influence the early microbiota composition. Also, the critical importance of the early microbiota for mucosal and systemic immune maturation and long-term disease susceptibility has been demonstrated. However, it remains incompletely understood how the early microbiota is shaped and which molecular and cellular mechanisms are involved to establish immune homeostasis and promote long-term health. Here we will review the studies that describe the establishment of the early enteric microbiota and discuss the controversial topic of prenatal colonization and the existence a placental microbiome. We will also talk about the exogenous and endogenous factors that influence the early microbiota and the effect of early microbial exposure on the host's immune maturation and homeostasis. Finally, we will deal with the role of the enteric microbiota in the pathogenesis of necrotizing enterocolitis, a mucosal inflammatory disease of preterm human neonates, and illustrate the consequences of an interventional administration of probiotics to neonates in order to promote the establishment of a beneficial microbial and host-microbial heomeostasis.

FROM OBSERVATIONAL POPULATION-BASED STUDIES TO CAUSATION

Dirk Haller
Nutrition and Immunology, Technical University of Munich, Germany

The intestinal microbiome is suggested to play an essential role in the regulation of human health and disease. Large-scale population studies and patient cohorts established first correlations between microbiome alterations and multiple diseases, including inflammatory and metabolic disorders. Metagenomic resolution and bioinformatic analysis considerably improved over the past years, allowing even strain level analysis, but the search for microbial risk patterns in human cohorts is often confounded by environmental factors (e.g. geographic location, diet, medication) and the individual host status. Comparing individual microbiota compositions and phylogenetic relationships across cohorts and disease entities confirmed diverse ecosystems and the absence of global disease-specific clusters. Nevertheless, we recently demonstrated that the availability of metadata information allowed diagnostic risk profiling in type-2 diabetic patients at the population but not at the individual patient level (Reitmeier et al. 2020, Cell Host Microbe). Understanding the functional relevance of microbiome alterations in patients or individuals at risk would allow a more comprehensive insight to the causal role of microbiome changes in disease development. Analysis of Crohn's disease patients confirmed the dynamic individual changes in microbiome profiles across the course of chronically remitting intestinal inflammation. Human fecal transplantation into germ-free mouse models became a popular and highly disputed area of translation microbiome research (Walter et al. 2019, Cell). Efficiency of microbial engraftment and the choice of models are key in the interpretation of data. We here demonstrate that fecal transplants establish in IBD mouse models and recapitulate human disease activity in a model-dependent fashion. Implementing multi-omics approaches allow the identification of disease-relevant mechanisms and bacterial targets (pathobionts), illustrated by the enrichment of Desulfovibrio ssp. in response to substrate availability (Metwaly et al. 2020, Nature Commun.). In summary, a broader insight into microbiome signatures for inflammatory and metabolic disorders will be provided, including a critical reflection of standing challenges in effective microbiome biomarker discovery and validation.

IMPRINTING OF IMMUNE RESPONSES BY GUT MICROBES: ON THE VERGE OF INFLAMMATION

Till Strowig
Helmholtz Centre for Infection Research, Braunschweig, Germany

The mucosal immune system contains the largest number of immune cells in the body and has long been recognized to protect the host against invading pathogens. In the recent years it has become clear that the mucosal immune system is also critical to maintain a symbiotic relationship between the microbiota and host. In turn, in numerous human disease conditions pathological changes in immunity have been associated with imbalances in the composition of the gut microbiota, a state often referred to as dysbiosis. However, whether the changes in the microbiota contribute directly to the development of the disease-causing alterations in immunity or rather reflect an altered physiology of the host remains debated in many instances. In the lecture, I will introduce key players of the mucosal immune system and illustrate how they interact with the microbiota during health and disease. Several examples of disease-associated microbiota alterations and corresponding changes in local and systemic immunity will be utilized to highlight the caveats of designing and interpreting experiments aiming to dissect the imprinting of immune responses by gut microbes.

Recommended reading:

Belkaid, Y. & Hand, T. Role of the Microbiota in Immunity and Inflammation. Cell (2014). https://doi.org/10.1016/j.cell.2014.03.011

Zheng, D., Liwinski, T. & Elinav, E. Interaction between microbiota and immunity in health and disease. Cell Res 30, 492–506 (2020). https://doi.org/10.1038/s41422-020-0332-7

SUMMER SCHOOL PROGRAM

Thursday, July 1st

COLON CANCER AND THE MICROBIOME: EVOLVING DATA

Cynthia L. Sears
Johns Hopkins University School of Medicine, Baltimore, MD, USA

The colonic microbiome is hypothesized to contribute to the induction and progression of colon cancer. Both select bacterial species and bacterial community organization and composition have been implicated as procarcinogenic in the colon. Human translational studies and use of murine models have advanced our understanding of the epidemiology of procarcinogenic bacteria and potential mechanisms by which these bacteria and microbiota communities contribute to sporadic CRC with implications for the emerging early onset CRC. This talk will present an update on the intersection of individual microbes, biofilms, host genetics and mechanisms of colon carcinogenesis. Together our studies support a model by which specific bacteria with their virulence genes as well as microbiota organization act with host immune responses and genetics to contribute to colon cancer pathogenesis.

DOES OUR MICROBIOME TRAVEL WELL?

John Penders
Maastricht University, The Netherlands

Antimicrobial resistance (AMR) is one of the biggest global public health treats, jeopardizing the effective prevention and treatment of an ever-increasing range of. Infections. International travel can substantially contribute to this problem when AMR bacteria and their AMR genes hitchhike in human guts. The extent to which international travel contributes to AMR remains, however largely unknown, as the presence of AMR bacteria in the human microbiota following travel remain undetected unless they cause manifest infections.

Within several cohorts of international travelers as well as local populations in AMR hotspots, we studied the dynamics of the human gut resistome by the application of functional, sequence-based as well as targeted metagenomic approaches. Together these analyses provide information on the detection of novel AMR genes and the abundance and diversity of AMR gene acquisition during travel. Moreover, by comparing the gut resistome of travellers and local populations in AMR hotspots we will discuss to what extend travelers can serve as sentinels to gain insight into prevailing AMR genes across the globe.

HOW TO RESTORE THE DISTURBED MICROBIOME OF PATIENTS WITH *CLOSTRIDIOIDES DIFFICILE* INFECTIONS?

Ed J. Kuijper, Liz Terveer, Joffrey van Prehn and Romy Zwittink
Leiden University Medical Center, The Netherlands

In healthy individuals, there is a symbiotic relationship between the host and the gut microbiota. The microbiota fulfils many functions which are of benefit for the host. Similarly, host factors are required to maintain a balanced microbiota. Colonization resistance is the mechanism whereby the microbiota protects against colonization of exogenous and potentially pathogenic microorganisms, such as Clostridioides difficile. C. difficile is a Gram-positive, spore forming, obligate anaerobic bacterium. Afterr germination of the spores in the small intestine under the influence of bile salts, vegetative bacteria enter the colon where they can remain inactive (asymptomatic colonization) or cause an infection (CDI, C. difficile infection) varying from self-limiting and mild diarrhoea to life-threatening pseudomembranous colitis. Infections relapse in approxiimately 20% of the patients. The two most important virulence factors of C. difficile are the exotoxins that are produced, toxin A and B. Both toxins are cytotoxic for enteric epithelial cells and increase vascular permeability by opening tight junctions between cells. In addition, the toxins induce an inflammatory response mediated by tumour necrosis factor-alpha and pro-inflammatory cytokines which contribute to the characteristic formation of pseudo-membranes. Recurrent CDI is associated with an impaired immune response to C. difficile toxins and more importantly, with a persistent and severely perturbed colonic microbiota. The propensity of C. difficile spores to colonize the intestinal tract and subsequently outgrow and produce toxins, is highly influenced by the host microbiota and metabolome. Upon disruption of the microbiota due to exogenous factors such as antimicrobials or other medication as proton pump inhibitors or chemotherapy, colonization resistance decreases and C. difficile can proliferate, produce toxins and cause disease. The precise microbes associated with inhibition or progression from C. difficile colonisation to (re)infection have not been recognised, but a few suggestions have been made. For example, some bacteria, like for instance Clostridium scindens, convert the C. difficile growth enhancing primary bile acids to the inhibiting secondary bile acids. Several studies have reported the recovery of Bacteroidetes and members of the Firmicutes phylum; the families Lachnospiraceae (formerly known as Clostridium cluster XIVa) and Ruminococcaceae (formerly known as Clostridium cluster IV), including Faecalibacterium prausnitzii, Eubacterium rectale, Roseburia intestinalis and other known butyrate-producing bacteria along with successful clinical recovery from CDI. These observations will guide the further development of live biotherapeutic products (LBP) to prevent and treat rCDI and will replace the currently recommended “Fecal Microbiota Transplantation” treatment with better defined products.

CONFERENCE PROGRAM

Friday, July 2nd

Session1
10^{10}-11^{25}

MULTI-LAYERED STRATEGIES OF HOST AND ENVIRONMENTAL MICROBIOME ANALYSIS

Shinichi Sunagawa
ETH Zurich, Switzerland

The use of DNA and RNA sequencing data from environmental and host-associated samples has enabled researchers to study of microbial community diversity and function in unprecedented ways. This increase in potential possibilities, however, has also posed new methodological challenges such as profiling taxonomic community compositions, reconstructing microbial gene and genome sequences, and accurately predicting genomic coordinates of inducible prophages, all starting from short sequence fragments. Overcoming these challenges shows great promise not only in identifying metagenome-wide associations with disease states or environmental factors, but also uncovering the biosynthetic potential harbored within an ecosystem and previously unsuspected microbial groups.

INVITED ABSTRACT NO. 1:
SRS-FISH: HIGH-THROUGHPUT PLATFORM LINKING MICROBIOME FUNCTION TO IDENTITY AT THE SINGLE CELL LEVEL

Fátima C. Pereira[a,1], *Xiaowei Ge*[b,1], *Matthias Mitteregger*[a], *David Berry*[a], *Meng Zhang*[b], *Michael Wagner*[a,c], *and Ji-Xin Cheng*[b,d]

[a]*Centre for Microbiology and Environmental Systems Science, Department of Microbiology and Ecosystem Science, University of Vienna, 1090 Vienna, Austria*
[b]*Department of Electrical & Computer Engineering, Boston University, Boston, Massachusetts, USA*
[c]*Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark*
[d]*Department of Biomedical Engineering, Photonics Center, Boston University, Boston, Massachusetts, USA*

One of the biggest challenges in microbiome research in environmental and medical samples is to better understand functional properties of microbial community members at a single cell level. Single cell isotope probing has become a key tool for this purpose, but the currently applied detection methods for measuring isotope incorporation into single cells do not allow high-throughput analyses. To overcome this limitation, we developed an imaging-based approach termed stimulated Raman scattering - two-photon fluorescence in situ hybridization (SRS-FISH) for high-throughput structure-function analyses of microbial communities with single cell resolution. SRS-FISH enables chemical imaging of metabolically active cells, whose identity is revealed using fluorescently-tagged oligonucleotide probes targeting the rRNA of different bacteria. SRS-FISH has an imaging speed of 10 to 100 milliseconds per cell, which is two to three orders of magnitude faster than spontaneous Raman-FISH. Using this technique, we delineated metabolic responses of thirty thousand individual cells to various mucosal sugars in the human gut microbiome via incorporation of deuterium from heavy water as an activity marker. Application of SRS-FISH to investigate the utilization of host-derived nutrients by two major human gut microbiome taxa revealed that response to mucosal sugars tends to be dominated by Bacteroidales, with an unexpected finding that Clostridia can outperform Bacteroidales at foraging fucose. We further show that pattern of mucosal sugar foraging differs between the murine and human microbiome. By allowing the scanning of

[1]These authors contributed equally.

multiple samples in a fast and sensitive manner, SRS-FISH is well-suited to reveal fine-scale temporal, individual, and spatial patterns in microbiome samples, which can otherwise be missed by existing methods due to their low-throughput.

INVITED ABSTRACT NO. 2:
AUTOMATING THE DESCRIPTION AND NAMING BACTERIA IN THE 21ST CENTURY

Thomas C. A. Hitch, Thomas Riedel, Aharon Oren, Jörg Overmann, Trevor D. Lawley, Thomas Clavel

The study of microbial communities is hampered by the large fraction of still unknown bacteria. However, many of these species have been isolated, yet lack a validly published name or description. Without a valid name, these species cannot be referenced in a standardised way, causing confusion and preventing the consistent study of these species. The process of validating names for novel bacteria requires that the uniqueness of those taxa is demonstrated, the properties of the taxa are described, and the name must be in either latin or latinised greek. The accepted format for this is the protologue, which can be time-consuming to create. Both the naming, and description of these novel taxa is ripe for automation, allowing for improved standardisation for this process.

As such, we have developed Protologger (www.protologger.de), a bioinformatic tool that automatically generates all the necessary readouts for writing a detailed protologue. By producing multiple taxonomic outputs, functional features and ecological analysis using the 16S rRNA gene and genome sequences from a single species, the time needed to gather the information for describing novel taxa is substantially reduced. Integration of GAN (Great Automatic Nomenclature) allows for the automatic generation of novel names based on the ecology and functional features of the studied isolate. By combining both Protologger and GAN, novel taxa can be described and named in record time while also reducing the manual workload on the user.

Session 2
11^{40}-12^{55}

HOST-MICROBIOME INTERACTIONS DURING AGING

Dario Valenzano
MPI for Biology of Ageing, Cologne, Germany

Gut bacteria occupy the interface between the organism and the external environment, contributing to homeostasis and disease. Yet, the causal role of the gut microbiota during host aging is largely unexplored. Using the naturally short-lived African turquoise killifish (Nothobranchius furzeri), a naturally short-lived vertebrate, we show that the gut microbiota plays a key role in modulating vertebrate life span. Recolonizing the gut of middle-age individuals with bacteria from young donors resulted in life span extension and delayed behavioral decline. This intervention prevented the decrease in microbial diversity associated with host aging and maintained a young-like gut bacterial community, characterized by overrepresentation of the key genera Exiguobacterium, Planococcus, Propionigenium and Psychrobacter. Metabolomic analysis of intestine, brain, liver, hearth, skeletal muscle, serum and stool indicate that important metabolic pathways are modified during killifish aging and that these changes are in part reversed by gut microbiota transfers from young donors. Our findings demonstrate that the natural microbial gut community of young individuals can causally induce long-lasting beneficial systemic effects that lead to life span extension in a vertebrate model. Our work provides a novel set of candidate bacterial taxa and metabolites that connect commensal microbial composition and function with host systemic aging.

INVITED ABSTRACT NO. 3:
ALTERED GUT MICROBIOME AND MICROBIAL METABOLISM IN PRIMARY SCLEROSING CHOLANGITIS

Louise. B. Thingholm[1], Martin Kummen[2], Alesia Walker, Malte[3]. C. Rühlemann[1], Corinna Bang[1], Wolfgang Lieb[4], Johannes R. Hov[2], Christoph Schramm[5], Andre Franke[1], German Clinical Research Unit in PSC (KFO306).

[1]Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, Kiel, Germany; [2]Norwegian PSC Research Center, Department of Transplantation Medicine, Oslo University

Hospital Rikshospitalet, Oslo, Norway; 3Department of Environmental Sciences (DES), HelmholtzZentrum München, Deutsches Forschungszentrum für Gesundheit und Umwel, Germany; 4Institute of Epidemiology, Christian-Albrechts-University of Kiel, Kiel, Germany; 5Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Primary sclerosing cholangitis (PSC) is a rare chronic progressive cholestatic liver disease. PSC is characterized by multifocal inflammation and fibrosis of the biliary tree, with strong links to pathology of the GI tract including IBD as is observed in 80% of PSC patients. The etiology of PSC remains unknown, there is no cure and treatment options remain limited. Increasing our understanding of the disease is therefore strongly needed. PSC has been linked to compositional changes in the gut microbiome, predominantly in cross-sectional studies with amplicon data, and with the increasing understand of the importance of the GI microbiome for inflammatory diseases, further research in this field is needed.

In the setting of the German Clinical Research Unit in PSC (KFO306), we have generated shotgun metagenomic sequencing data on two cohorts (one Norwegian and one German), in total comprising 136 patients with PSC (58% with IBD), 158 HCs and 93 IBD patients, and via characterizing the genetic potential of the gut microbiome in PSC, identified robustly associated species as well as marked differences in the abundance of microbial genes related to vitamin B6 synthesis and branched chain amino acid (BCAA) synthesis. We have followed along these findings with targeted metabolomics and shown clear associations between the disease state and outcome and several potentially microbiome-derived metabolites. Subsequently, we have generated untargeted metabolomics on serum and stool, as well as targeted SCFA measures, on the German subset, and this data is now being analyzed.

We would like to present the latest findings from these analyses, as they relate to differentially abundant microbial species, metabolites, and their inter-relations in PSC, IBD and HC.

INVITED ABSTRACT NO. 4:
EXPLORING THE INTERACTION NETWORK OF A SYNTHETIC GUT MICROBIAL COMMUNITY

Anna S. Weiss [1], Anna G. Burrichter [1], Abilash Chakravarthy Durai Raj [1], Alexandra von Strempel [1], Chen Meng [2], Karin Kleigrewe [2], Philipp C. Münch [1,3], Luis Rössler [4], Claudia Huber [4], Wolfgang Eisenreich [4], Stephanie Göing [5], Kirsten Jung [5], Alvaro Sanchez [6,7], Bärbel Stecher [1,8]

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A key challenge in microbiome research is to predict and understand functionality from microbial community composition. As central microbiota functions are determined by complex bacterial community networks it is important to gain insight into the principles that govern bacteria-bacteria interactions. In this line, we focused on metabolic interactions of the Oligo-Mouse-Microbiota (OMM^{12}) synthetic bacterial community, which is increasingly used as model system in gut microbiome research.

So far, little is known about the ecological structure and metabolic capabilities of this synthetic bacterial community, both of which determine community assembly, population dynamics and bacterial community functionality. Using a bottom-up approach, we uncovered the directionality of strain-strain interactions in mono- and pairwise co-culture experiments, as well as in community batch culture. Metabolomics analysis of spent culture supernatant of individual strains in combination with genome-informed pathway reconstruction provided insights into the metabolic potential of the individual community members. Thereby, we could show that the OMM^{12} interaction network is shaped by both, exploitative and interference competition *in vitro*. In particular, *Enterococcus faecalis* KB1, a low-abundant member of the mammalian gut

microbiota, was identified as important driver of community composition by affecting the abundance of several other consortium members *in vitro*.

Together, our work provides a detailed understanding of the mode of *in vitro* strain-strain interactions within the OMM^{12} consortium, which serves as a knowledge base for future mechanistic studies and the fundamental basis for targeted community manipulation to probe a wide range of microbial community functions *in vivo*.

Session 3
14^{00}-15^{15}

THE INTERFACE OF DRUGS AND THE HUMAN GUT MICROBIOME

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Drugs impact the human gut microbiota, but also their efficacy is affected by gut microbes. Here, I will be presenting recent highlights from coordinated efforts at EMBL to systematically map this drug-microbiome interface, understand its impact on the gut microbiota, address the emergence of communal behaviors against drugs, and to discover ways to mitigate the collateral damage of drugs, and especially of antibiotics, on gut microbes. A particular focus will be on how this knowledge can change our view on use of anti-infectives and present new opportunities for effective and tailored therapies.

INVITED ABSTRACT NO. 5:
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 K. pneumoniae, 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 microbiomes with protective properties we established an ex vivo assay, spiking candidate probiotics and a bioluminescent K. pneumoniae strain into cecum content of mice or humans. This assay can be used as a universal screening-tool, which enables a high-throughput screening of commensal isolates. It is of great interest to screen as many strains as possible regarding the high genetic diversity of bacterial isolates. As a novel resource for the identification of potentially probiotic bacteria, a strain collection 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 K. pneumoniae after cocultivation with specific commensal strains. To further verify the protective effect, our goal was to implement competition experiments in humanized microbiota mice. For this approach, human stool samples were classified, regarding their ability to promote outgrowth of MDR K. pneumoniae, as susceptible or protected. This in vitro phenotype could be transferred in vivo. Mice humanized with susceptible stool samples could stably integrate the MDR K. pneumoniae into

their microbiota, whether mice humanized with protected stool samples could clear MDR K. pneumoniae. To demonstrate the probiotic effect of the respective bacterial strain, we show that mice humanized with susceptible stool samples were able to promote a 100 % clearance of MDR K. pneumoniae, after administration of this probiotic strain. 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. Also, it is of great interest to extend the screening to other MDR Enterobacteriaceae, like E. coli.

INVITED ABSTRACT NO. 6:
MICROBIOTA-DERIVED METABOLITES INHIBIT SALMONELLA VIRULENT SUBPOPULATION DEVELOPMENT BY ACTING ON SINGLE-CELL BEHAVIORS

Alyson M Hockenberry[*1,2], Gabriele Micali[1,2], Gabriella Takács[1,2], Jessica Weng[1,2,3], Wolf-Dietrich Hardt[4], & Martin Ackermann[1,2]*

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Abstract: *Salmonella spp.* express *Salmonella* pathogenicity island 1 Type 3 Secretion System 1 (T3SS-1) genes to mediate the initial phase of interaction with their host. Prior studies indicate short-chain fatty acids, microbial metabolites at high concentrations in the gastrointestinal tract, limit T3SS-1gene expression. A number of reports show only a subset of *Salmonella* cells in a population express these genes, suggesting short-chain fatty acids could decrease T3SS-1 population-level expression by acting on per-cell expression or the proportion of expressing cells. Here, we combine single-cell, theoretical, and molecular approaches to address the effect of short-chain fatty acids on T3SS-1 expression. Our results show short-chain fatty acids do not repress T3SS-1 expression by individual cells. Rather, these compounds act to selectively slow the growth of T3SS-1 expressing cells, ultimately decreasing their frequency in the population. Further experiments indicate slowed growth arises from short-chain fatty acid-mediated depletion of the proton motive force. By influencing the T3SS-1 cell-type proportions, our findings imply gut microbial metabolites act on cooperation between the two cell-types and ultimately influence *Salmonella*'s capacity to establish within a host.

Significance Statement: Emergence of distinct cell-types in populations of genetically identical bacteria is common. Furthermore, it is becoming increasingly clear that cooperation between cell-types can be beneficial. This is the case during *Salmonella* infection, in which cooperation between inflammation-inducing virulent and fast-growing avirulent cell-types occurs during infection to aid in colonization of the host gut. Here, we show gut microbiota-derived

metabolites slow growth of the virulent cell-type. Our study implies microbial metabolites shape cooperative interactions between the virulent and avirulent cell types, a finding that can help explain the wide array of clinical manifestations of *Salmonella* infection.

Session 4
15^{30}-16^{45}

HOST MICROBIOME INTERACTIONS IN HEALTH AND DISEASE

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The mammalian intestine contains trillions of microbes, a community that is dominated by members of the domain Bacteria but also includes members of Archaea, Eukarya, and viruses. The vast repertoire of this microbiome functions in ways that benefit the host. The mucosal immune system co-evolves with the microbiota beginning at birth, acquiring the capacity to tolerate components of the community while maintaining the capacity to respond to invading pathogens. The gut microbiota is shaped and regulated by multiple factors including our genomic composition, the local intestinal niche and multiple environmental factors including our nutritional repertoire and bio-geographical location. Moreover, it has been recently highlighted that dysregulation of these genetic or environmental factors leads to aberrant host-microbiome interactions, ultimately predisposing to pathologies ranging from chronic inflammation, obesity, the metabolic syndrome and even cancer. We have identified various possible mechanisms participating in the reciprocal regulation between the host and the intestinal microbial ecosystem, and demonstrate that disruption of these factors, in mice and humans, lead to dysbiosis and susceptibility to common multi-factorial disease. Understanding the molecular basis of host-microbiome interactions may lead to development of new microbiome-targeting treatments.

INVITED ABSTRACT NO. 7:
NATURALIZING THE MICROBIOME BY HOUSING MICE IN A FARM ENVIRONMENT CONFERS PROTECTION AGAINST COLORECTAL CARCINOGENESIS

Henriette Arnesen[1,2], Thomas C. A. Hitch[3], Christina Steppeler[1], Mette H. B. Müller[1], Linn Emilie Knutsen[1], Inga L. Angell[2], Ida Ormaasen[2], Knut Rudi[2], Jan Erik Paulsen[1], Thomas Clavel[3], Harald Carlsen[2], Preben Boysen[1]

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Mammals have co-evolved with the billions of microbes surrounding them and harboring their bodies. Thus, it is paradoxical that disease modelling studies using research mice take place under strictly hygienic conditions, far-away from the typical lifestyle of the end goal for such studies, humans. To close the gap between the preclinical mouse model and human lifestyles, we have established a system where laboratory mice are raised under a full set of environmental conditions present in a naturalistic, farmyard habitat. We call this process feralization, and the resulting mammal display a more mature states of immune cells and a diverse gut microbiota, likely surpassing conventional laboratory mice in resembling responses of free-living mice. We used the feralization approach with two different mouse models of colorectal cancer, showing that the mice feralized in a farm environment were protected against carcinogenesis. In contrast to conventionally reared laboratory mice, the feralized mouse gut microbiota structure remained stable and resistant to mutagen- and colitis induced neoplasia. Moreover, the feralized mice exhibited signs of a more mature immunophenotype, indicated by increased expression of NK and T cell maturation markers, and a more potent IFN-γ response to stimuli. In our study, conventionally born and raised mice subsequently feralized post-weaning were protected to a similar level as life-long exposed mice, downplaying the need for neonatal exposure. Collectively, we show protective implications of a farm environment on colorectal cancer development and demonstrate the utility of a novel animal modelling approach that recapitulates realistic disease responses in a naturalized mammal.

INVITED ABSTRACT NO. 8:
HIGH FAT DIET INDUCES ATF6-DEPENDENT COLORECTAL TUMORIGENESIS IN MURINE MODELS

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Introduction: Colorectal cancer (CRC) is the third most common cause of cancer death worldwide, with highest incidence rates seen in industrialized countries. This implicates environmental factors as drivers in the pathogenesis of CRC. Westernized diets with low fiber and high fat content have been identified as main contributors[2,3]. The complex interplay between diet, host and intestinal microbiota unarguably plays a key role in gastrointestinal pathologies. The unfolded protein response effector Activating Transcription Factor 6 (ATF6) has been associated with early changes in sporadic and colitis-associated CRC (CAC)[4]. In our transgenic mouse model for spontaneous CRC (nATF6^{IEC}), homozygous overexpression of ATF6 drives microbiota-dependent tumorigenesis[5]. To investigate the role of ATF6 in inflammation-driven tumorigenesis, we generated a model for CAC by crossing nATF6^{IEC} mice with IL10-deficient mice (nATF6^{IEC}xIL10$^{-/-}$). In otherwise tumor-free heterozygous ATF6 mice, deficiency of IL10 induces strong colonic inflammation and tumors with an incidence of 75 %.
Aims & Methods: To validate our mouse models in the context of human fecal microbiota transplantation (FMT), germfree (GF) mice were colonized with stool from lean (BMI 25) and obese (BMI 39) age matched CRC patients. In SPF housed nATF6^{IEC} and nATF6^{IEC}xIL10$^{-/-}$ mice, microbial communities were characterized by 16S rRNA sequencing of mucosal content in colon. To assess the impact of lipid metabolism on tumor development we used two different approaches: first, untargeted metabolomics of cecal content at different tumor stages (pre tumor (5 week), tumor (12 week) and late-tumor (20 week)); second, dietary lipid interventions

via a westernized high-fat low-fiber diet (HFD) containing 48 energy% fat based on lard and 50 g/kg cellulose for 6 weeks after weaning. A low-fat low-fiber diet (LFD) served as a control. Results: Colonization of GF mice with stool from CRC patients successfully transferred the phenotype, inducing colonic tumors in genetically susceptible mice. Preliminary results following FMT from an obese donor led to a tumor incidence of 71 % in nATF6^{IEC}xIL10$^{-/-}$ mice. 16S rRNA sequencing showed that mucosa-associated microbiota differentiates tumor-bearing from non-tumor-bearing mice in our CAC model under SPF conditions. Furthermore, spatial analyses along the colon revealed a tumor-tissue specific microbial signature, identifying Mucispirillum as a pathobiont. Untargeted metabolomics revealed elongation of saturated and unsaturated fatty acids (FA) already at the pre-tumor stage, which was further pronounced at the late-tumor stage. Especially very long chain FA, such as C22 and C24 species, were increased compared to non-tumor mice. In nATF6^{IEC} mice, saturated FA and the ratio of C20 to C22 FA correlated with tumor number. In tumor-bearing nATF6^{IEC} mice, alterations in microbial community and FA form a close network, which was absent at the pre-tumor stage. HFD feeding increased tumor incidence in nATF6^{IEC}xIL10$^{-/-}$ mice to 100 % compared to 75 % on a standard Chow diet and LFD. For nATF6^{IEC} mice, preliminary results suggest an impact on tumor growth with trends towards increased tumor number (5.5 on HFD compared to 3.3 on LFD) and volume (38.3 mm³ on HFD compared to 29.8 mm³ on LFD). Unexpectedly, otherwise tumor-free heterozygous nATF6^{IEC} mice developed colonic tumors with an incidence of 71 % on HFD and 67 % on LFD.

Conclusion: Taken together, alterations in lipid metabolism driven by ATF6 are associated with colonic tumorigenesis. Further, dietary fats play a role in ATF6-dependent tumorigenesis, especially tumor initiation and growth. Findings revealed that low fiber content in our diets is an important contributor to tumor initiation. Our current research effort focusses on identifying the specific role of dietary fiber and fat on tumorigenesis in our CRC and CAC mouse models. Additionally, ongoing FMT with stool samples from lean and obese CRC patients aims to elucidate BMI in the context of tumorigenesis.

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Poster Session 1

17^{00}-17^{20}

1 AFRIZAL AFRIZAL
ANAEROBIC SINGLE-CELL DISPENSING FACILITATES THE CULTIVATION OF HUMAN GUT BACTERIA

Afrizal Afrizal[1], *Alina Viehof*[1], *Thomas C.A. Hitch*[1], *Thomas Clavel*[1]

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The intestinal microbiota influences host health. However a large fraction of gut bacteria are still undescribed. Cultivation is essential to capture these species but classical agar plate (CAP) approaches, which are widely used, are time-consuming. As single-cell dispensing (SCD) allows high-throughput, label-free bacterial sorting, we aimed to benchmark a new anaerobic SCD workflow for efficient cultivation of human gut bacteria. Initial studies with pure cultures and faecal slurries under aerobic or anaerobic conditions demonstrated the feasibility of this approach. Speed and efficacy were then compared to a CAP workflow using faecal communities from four donors on three rich culture media. Cultured fractions were determined via 16S rRNA gene amplicon sequencing.

The SCD approach decreased the experimental time to obtain pure cultures from 17 ± 4 to 5 ± 0 days. The growth fraction ranged from 19% to 51% of 800 sorted cells depending on the sample and culture medium. The diversity of isolates obtained by SCD was comparable to the CAP method (21 ± 2 species identified from 45 colonies). Both methods were also equivalent regarding the fraction of molecular species (16S rRNA gene amplicons) captured by sequences from the isolates (28.9 ± 5.1% for SCD, representing a cumulative relative abundance of 48.1 ± 12.0%). This cultured fraction increased to 43.5 ± 15.4% (73.5 ± 13.8% rel. abundance) when sequencing directly bulk sorted-communities after growth without downstream identification of single strains. SCD cultivation also captured species (16 ± 5 per sample) not detected by sequencing. From this work, a collection of 80 bacterial species across five phyla (*Actinobacteriota, Bacteroidota, Desulfobacterota, Firmicutes, Proteobacteria*) and 22 families was established, including 13 potential novel genera and 11 novel species that are currently being characterized taxonomically using www.protologger.de.

Anaerobic SCD cultivation accelerates the recovery of pure cultures of anaerobic microbes. Next steps include the automation of identification methods. With the increasing awareness of

the significance of inter-individual microbiota diversity, the SCD approach will facilitate the establishment of personalised culture collections.

2 LENA AMEND
THE ROLE OF THE MICROBIOME IN IMMUNE-MEDIATED TYPES OF ARTHRITIS

Lena Amend[1, 2], Aida Iljazovic[1], Eric J.C. Galvez[1, 3], Torsten Witte[2, 3], Axel Finckh[4], Till Strowig[1, 2, 3]

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The human gut microbiome plays an important role in host's health status and various alterations in these complex microbial communities have been associated with diverse diseases. Particularly, in immune-mediated types of arthritis including rheumatoid arthritis (RA), spondyloarthritis and psoriatic arthritis, the involvement of the gut microbiota in disease initiation and progress has been highly debated. Specifically, several studies investigated the role of *Prevotella copri*, a prevalent colonizer in the human intestine, in these types of diseases and especially in patients with RA[1–3]. However, these studies reported contrasting associations. Despite the ambiguous role of specific gut bacteria, i.e., *P. copri,* in RA development, recent research has provided evidence for a molecular mimicry between autoantigens and microbiota-derived peptides triggering autoimmunity[4]. The additional observation that specific microbial signatures have the potential to modulate treatment response against disease-modifying anti-rheumatic drugs identifies the microbiome as an important potential target in disease therapy of immune-mediated types of arthritis[5].
Using human fecal samples from the ongoing human cohort "RheumaVOR" comprising newly diagnosed patients with different rheumatic diseases, we investigate the microbial composition of these patients to identify potential disease-specific microbial signatures by performing 16S rRNA gene sequencing. Analyzing longitudinal samples of these patients taken after treatment initiation will allows us to identify disease-dependent microbial shifts in the gut. Additional combination with medical metadata of the patients will enable the correlation of microbial composition and treatment response. Of note, serum from different patient groups could not identify significant differences between distinct patient groups and controls when used to

measure pathogen-specific IgG levels against multiple *P. copri* strains and gut commensals adapting an ELISA method. Therefore, by performing IgA sequencing from the patients stool samples, we aim to identify bacteria targeted by secretory IgA in the intestine in an unbiased manner and thereby reveal specific host-microbe interactions in the pathogenesis of these different forms of immune-mediated arthritis.

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3 MOHAMED TAREK BADR

APPLICATION OF CLINICAL METAGENOMICS IN STANDARD MICROBIOLOGICAL DIAGNOSTICS (DISSECTING ASCITIC MICROBIOTA COMPOSITION AS A MODEL)

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Introduction: Ascites is an unnatural accumulation of fluid in the abdominal cavity. Cirrhosis is the most common cause, but other possible causes include heart failure, tuberculosis, pancreatitis, and cancer. Infection of the ascetic fluid is a serious complication that can lead to peritonitis and sepsis and is associated with high morbidity and mortality. Around 70% of hospitalized patients remain without an identifiable infectious source. The prompt identification of the related pathogen is essential for the patients' prognosis. Here we aim to establish metagenomics next- and third-generation sequencing workflows to aid the clinical diagnosis and treatment.

Methods: Different DNA isolation protocols/kits have been tested with various optimization steps to compare their efficiency. Various high variable regions of the 16s rDNA gene have been compared for their sensitivity in ascitic materials. The V1-2 region of the 16S rDNA has been used to target bacterial communities in the ascitic fluid. The DADA2 informatic pipeline and the Genome Taxonomy Database have been used to analyze and identify bacterial taxonomy from the sequences.

Results: Over the time between October 2019 and June 2021, a cohort of 30 ascitic samples has been collected from intensive care patients. Clinical characteristics of the curated cohort showed a higher prevalence of male patients of 63% with a 10% death rate. From the 30 samples, six were culturally positive. Using V1-2 targeted PCR, 23 samples were positive including all culturally positive samples. Sequencing analysis showed evidence of anaerobic bacteria in 10 samples and other pathogenic bacteria such as E. faecalis and Klebsiella.

Conclusions: New sequencing techniques can be used for rapid and efficient diagnosis of critical ill patients, as they offer higher sensitivity in comparison with culture-based methods. Further modification for sample preparation and algorithms for the identification of clinical relevant pathogens are essential for the full implementation of the technology.

4 SOPHIA FABIANA GÖLDEL

CLINICAL COURSE OF A PATIENT UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION, COMPARING MICROBIOTA PATTERNS IN BLOOD AND STOOL

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Background: In this exceptional case report, we are highlighting the clinical course of a patient undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT) who developed bacteraemia, sepsis and Graft-vs-Host-Disease (GVHD) and are correlating the clinical course with microbial patterns detected in their stool and blood samples. The patient, a 64-year-old woman with acute myeloid leukaemia underwent allo-HSCT from a mismatched unrelated donor. On -d3 the patient experienced fever and diarrhoea but no pathogen could be determined via blood or urine culture nor stool testing. We started empiric antibiotic treatment with meropenem and escalated this to ceftazidim and vancomycin after recurring fever. At d2 we detected *Enterococcus faecium* in the blood cultures, together with the clinical presentation this established the diagnosis bacterial sepsis. Esophageal candidiasis occurred on d4 and was treated with isovuconazol. At d8 *Candida albicans* could be detected in the blood cultures. The central venous catheter was removed, and the therapy expanded to anidulafungin. On d15 an acute GVHD (skin grade II; GIT grade I) was diagnosed and treatment with etanercept started, which led to a fast recovery. After d44 at the stem cell transplantation unit the patient was released into after care at a rehabilitation center.

Methods: We performed calendar and event driven collection of blood, saliva, and stool samples. These samples were taken over the course of several weeks from the day of initial admission at our bone marrow transplantation unit up to 4 weeks after transplantation in weekly intervals. Parallel to bio-sampling, we recorded clinical parameters in weekly follow-ups. We considered important blood values, like CRP or leukocytes, administered medication as well as vital parameters, like blood pressure, heart rate or fever. We performed a comprehensive analysis of microbiome profiles in all stool and blood samples using 16S rRNA sequencing methods to assign the found bacteria to OTUs (operational taxonomic units). We will look at alpha diversity, beta diversity and taxonomic binning. The task is to detect infections before

clinical occurrence so species abundance in each sample will be calculated. Blood and stool samples collected at d0 should show a predominance of *Enterococcus faecium* as the blood cultures were positive on d2. The samples collected on d7 could show a higher colonisation with *Candida albicans*. At d14 we should be able to detect a loss of diversity as this promotes occurrence of GVHD. Taxonomic binning will enable us to get an overview of the estimated taxonomic composition during these defined time points.

Conclusions: We are going to evaluate if it is possible to use stool microbiome signatures to predict a developing infection complication. Dysbiosis with overabundance of pathogenic species may be associated with systemic infection complications such as bacterial sepsis. Detection of the signatures could be useful for initiation of early and more targeted (narrow band) antibiotic treatment. This should enable a less aggressive approach to treatment of bacterial infections and even prevent such. Microbiome based clinical decision making could result in less severe side effects of an allo-HSCT and more patient specific treatments, reducing the excessive use of antibiotics, thereby reduce the risk for GVHD and other antibiotics-related side effects and thus benefit the patient's recovery process. To confirm this hypothesis a higher number of cases is needed and currently collected in our non-interventional prospective clinical study at Klinikum Rechts der Isar.

5 MARTIN KÖBERLE

PRE-DIGEST OF UNPROTECTED DNA BY BENZONASE IMPROVES THE REPRESENTATION OF LIVING SKIN BACTERIA READS IN BOTH 16S RRNA GENE SEQUENCING AND METAGENOMICS

Yacine Amar[1,2], Ilias Lagkouvardos[4,5], Rafaela L. Silva[1,2], Oluwaseun Ayodeji Ishola[3], Bärbel U. Foesel[3], Susanne Kublik[3], Anne Schöler[3,7] Sebastian Niedermeier[1], Rachela Bleuel[1,2], Alexander Zink[1,2], Klaus Neuhaus[5,6], Michael Schloter[3,6], Tilo Biedermann[1,2], Martin Köberle[1]*

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Next generation sequencing (NGS) based microbiome analyses have dramatically improved our understanding of microbial communities in health and disease. However, microbiome sequencing intrinsically cannot differentiate between DNA of living and dead microorganisms. In environments with low microbial loads, such as the skin where host defense mechanisms including antimicrobial peptides and low cutaneous pH result in high microbial turnover. Potentially the DNA of high numbers of dead bacteria cells is still present. Therefore, NGS analyses may lead to inaccurate estimations of microbiome structures and functional capacities.

To overcome this weakness, we investigated whether the approach to digest unprotected DNA by Benzonase before microbial lysis and subsequent DNA preparation (BDA), results in a more accurate assessment of the living microbiome. A skin mock community as well as skin microbiome samples were analyzed using 16S rRNA gene sequencing and metagenomics

after DNA extraction with and without prior Benzonase digest. BDA resulted in less reads from dead bacteria, both in the skin mock community and in skin swabs spiked with either heat inactivated bacteria or bacterial free DNA. This approach also efficiently depleted host DNA reads in samples with high human DNA content, with no obvious impact on the microbiome profile.

In conclusion, the BDA approach enables both a better assessment of the living microbiota and effective depletion of host DNA reads.

Poster Session 2

17^{20}-17^{40}

6 ARNE BUBLITZ

COMBATING *C. DIFFICILE* INFECTION THROUGH COMBINATORIAL MICROBIOME EDITING AND MICROBIOME-SPARING ANTIBIOTICS

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The mammalian microbiota consists of several hundred species of bacteria that form a complex network occupying an array of nutritional niches and performing biotransformation of diverse molecules along the gastrointestinal tract. In the undisturbed state, this community has the capability to protect the host against invading pathogens in a process called 'colonization resistance' (CR)1 . Broad-spectrum antibiotic treatment can disrupt the microbiota affecting its composition and functionality, potentially leading to a weakened CR. Nosocomial pathogen like Clostridioides difficile can expand into these newly created niches and cause diseases like pseudomembranous colitis, which are further complicated by high recurrence rates2 . In order to reduce the risk of starting the vicious cycle of antibiotic treatment, breakdown of CR and infection, novel approaches specifically strengthening CR by microbiome editing or sparing CR through novel antibiotics should be explored. The OMM12 gnotobiotic mouse model 3 is naturally susceptible to CDI infection as its members lack the potential to convert host-derived primary bile acids into C.difficile inhibiting secondary bile acids (secBA). In line with this observation, colonization with a single 7α-hydroxylating bacteria Extibacter muris significantly increases the amount of secBAs in the intestinal tract, therefore promoting CR against CDI. Interestingly, colonization with additional six commensal strains, without the genetic potential to produce secBAs, significantly boosts secBA production by Extibacter muris. Ongoing metabolomics analysis addresses the role of these six commensal bacteria on the multi-step

intestinal bile acid conversion and their contribution to the colonization resistance against Clostridioides difficile. In parallel, we are investigating the effect of a new antibiotic candidate against C. difficile, Chlorotonil A and its derivative Chlorotonil B, on the microbiota, the intestinal bile acid pool, and infection susceptibility to CDI as well as their therapeutic potential to treat CDI. Understanding the functional contribution of commensal bacteria to intestinal bile acid metabolism will provide new strategies in probiotic development and antibiotic treatment to combat nosocomial pathogens like C. difficile.

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7 AMIRA METWALY

DIET CONTROLS SEGMENTED FILAMENTOUS BACTERIA IN DRIVING CROHN'S DISEASE-LIKE ILEO-COLONIC INFLAMMATION IN TNF^{DELTAARE} MICE

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Crohn's disease (CD)-like inflammation in TNFΔARE (ARE) mice is triggered by dysbiotic gut microbial communities. Similar to the therapeutic effect of exclusive enteral nutrition in Crohn's disease, dietary intervention using semi-synthetic experimental diet prevents disease development in ARE mice, yet the role of diet-microbiota interactions in disease initiation and progression remains unclear. The aim of this study is to identify the causal microbial cues responsible for CD-like inflammation and to dissect the protective role of diet in ARE mice. Microbial communities in ARE and wildtype (WT) mice were profiled by 16S rRNA gene amplicon sequencing, FISH and qPCR. Germfree WT and ARE mice were colonized with single bacteria and bacterial communities, including segmented filamentous bacteria (SFB),

human SFB-like *Bifidobacterium adolescentis* (BA) L2-32, *Alistipes sp*. isolates from dysbiotic ARE mice and fecal microbiota from CD patients. Disease activity, cytokine expression, mucosal immune cell infiltrates as well as Paneth and goblet cells numbers were assessed in murine ileal, cecal and colonic tissue sections. The influence of diet on disease development was evaluated by feeding ARE mice either chow or the purified semi-synthetic experimental diet (SED). Impact of SFB on ileal-colonic inflammation was tested in additional mouse models ($XIAP^{-/-}$ and $IL10^{-/-}$). Mucosal biopsies from adult and pediatric IBD patients (N=407) were screened for the presence of human SFB using previously published SFB-specific PCR primers. Our results showed that dysbiosis was associated with increasing abundance of SFB and correlated strongly with the severity of inflammation in ARE mice. Monocolonization of germfree ARE mice with SFB resulted in severe enterocolitis affecting ileum, cecum, and colon, while WT mice remained disease-free. *BA L2-32* and *Alistipes sp*. completely failed to induce inflammation. Parallel to high tissue levels of TNF and IL-17, SPF-mediated inflammation was associated with neutrophil infiltration and the expansion of IFNγ expressing Th1 cells in the mucosa. Loss of Paneth and goblet cell function allowed SFB to penetrate mucus layers reaching proximity to the epithelium in inflamed ARE mice. Treatment of ARE mice with SED reduced SFB abundance and completely abolished CD-like inflammation in ARE mice, suggesting a crucial role of dietary components in preventing disease development. Monocolonization of GF $XIAP^{-/-}$ and $IL10^{-/-}$ mice with SFB failed to induce inflammation, emphasizing host specificity to the mucosa-adherent SFB. Presence of SFB was confirmed in 12 ileal and 11 colonic mucosal biopsies from IBD patients with active or inactive disease at different timepoints during disease progression. In conclusion, our work provides evidence for a novel pathogenic role of SFB in driving severe CD-like ileo-colonic inflammation characterized by loss of Paneth and goblet cell functions in ARE mice. Detection of SFB in mucosal biopsies of patients with IBD opens new perspectives about therapeutic strategies targeting SFB-mediated processes. Purified diet antagonized SFB abundance and prevented disease development in ARE mice, clearly demonstrating the important role of diet in modulating a novel IBD-relevant pathobiont.

8 KLAUS NEUHAUS

META-RIBOSOME PROFILING OF A SIMPLIFIED GUT-BACTERIA-CONSORTIUM ENABLES NOVEL INSIGHTS IN INTERACTIONS *IN VITRO* AND *IN VIVO*

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Ribosome profiling is a precise technique analysing mRNA under translation (i.e., revealing the 'translatome'). It allows sequencing ribosome-covered mRNA, indirectly detecting differentially expressed proteins in various treatments. Analysis of complex gut communities is difficult since the obtained sequencing reads are short and non-ambiguous mapping to genomes a challenge. We used a simplified human gut microbiome (SIHUMI) consortium, which reduces complexity, but bacteria-bacteria interaction can still be determined for the strains included. Next, the mapping software BBMap with adjusted parameter settings was found performing best in minimizing ambiguous mapping of sequenced short footprint reads to various bacterial genomes. Thus, meta-ribosome sequencing allowed determining gene expression profiles of each strain used. Here, we performed, first, meta-ribosome profiling of the SIHUMI consortium of *in-vitro* cultures at pH 7 and pH 5.5 and, second, a further improved protocol allowed *in vivo* meta-ribosome profiling using faeces of wild type mice compared to interleukin-10-deficient mice. Interestingly, more genes were found to be regulated in translation than in transcription. We analysed specific groups of genes involved in bacteria-bacteria interactions in detail (e.g., quorum sensing, toxin-antitoxins, toxins, bacteriocins, motility, and protein export). In addition, we found larger fractions of hypothetical genes in each SIHUMI genome being regulated for the mouse conditions compared to the cultures. Thus, meta-ribosome profiling enhances our knowledge about interactions between organisms (e.g., hypothetical genes, genes involved in interactions) compared to common single-strain cultures used otherwise.

9 DIEGO ORTIZ

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

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Every year an estimate of 15 million preterm children are born. Preterm birth complications are the leading cause of newborn morbidity and mortality worldwide, accounting for up to 1 million deaths per year. The most important threats for preterm newborns include sepsis as well as respiratory and gastrointestinal infections that can lead to long-term complications. Modulation of the microbiota has emerged as an beneficial intervention to prevent infections in susceptible individuals. Many randomised control trials and observational studies correlate probiotic interventions with reduced risk of necrotizing enterocolitis, sepsis and other infections and overall mortality. For instance, PRIMAL is a German randomised control trial that addresses the efficacy of a probiotic intervention to prevent gut dysbiosis in preterm newborns[1]. In the frame of the study, we want to answer the question whether the probiotic-modified microbiome has immunomodulatory effects on the host or confers any protection against infection.
For this purpose, we will work in two phases. In the first phase, we will establish a preterm faecal transfer mice model (humanised model) and compare the dietary impact in the microbiota engraftment[2]. This model will further serve to develop a *Staphylococcus aureus* model of sepsis and an EPEC model of gastrointestinal infection both in neonatal mice. During the second phase of the project, humanised mice with faeces from PRIMAL newborns that received either the probiotic intervention or a placebo will be challenged to infection. Survival will be monitored for up to 3 weeks after infection. At this time point, the bacterial burden will be quantified in blood, small intestine, spleen, lung and liver. To address possible immunomodulatory effects we will perform immunophenotyping by flow cytometry from small

intestine, spleen, blood and mesenteric lymph nodes. This analysis will comprise innate and adaptive cell types, including different Th subsets, Treg, ILCs, macrophages and neutrophils. Additionally, we will have access to preterm faecal samples from the Clinic for Pediatric Pneumology, Allergology and Neonatology of the Medical School Hannover. Using the previous established models, humanised mice with faeces from healthy individuals and individuals that later developed late-on-set sepsis will be compared. This will provide us of evidence on whether a particular preterm microbiota can affect infection susceptibility and outcomes.

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 expect they will serve as motivation for further studies to address possible probiotic immunomodulatory mechanisms and characterise potential microbiome signatures associated to increased infection susceptibility.

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10 ANNIKA SCHWENTKER
GUT MUCOSAL BARRIER INTEGRITY DURING THE POSTNATAL PERIOD

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Starting with the rupture of membranes at birth the neonatal host leaves the sterile and protected environment in utero and faces the challenge to rapidly adapt to intense microbial and environmental exposure. The neonatal intestinal mucosa is populated by high numbers of microbes within hours after birth. Simultaneously, the neonate has to adjust to the shift from parental to enteral nutrition during a period of extensive energy requirement. The need of efficient intestinal energy absorption while ensuring the containment of the rapidly rising luminal concentration of microbial stimuli during a time period where immune homeostasis is established poses a critical task for the neonatal host.

Previous reports have described enhanced permeability of the gut mucosal barrier during the early postnatal period. Using fluorescence spectroscopy, immunostaining and microbial ligand quantification, we confirmed enhanced levels of orally applied macromolecules in the systemic circulation in neonatal mice prior to weaning. We observed macromolecular uptake of the tracer molecules by enterocytes, which appeared to be restricted to the distal regions of the small intestine. Intriguingly, this impaired integrity of the gut barrier also extended to immune stimulatory substances such as endotoxin (lipopolysaccharide, LPS). Additionally, the neonatal

small intestinal microbiota showed enhanced relative abundance of endotoxin producing members of the phylum Proteobacteria.

A possible compensatory mechanism of the neonatal host was identified by transcriptional analysis of LPS-detoxifying enzymes in the intestine. The highly conserved acyl-oxy-acyl hydrolase (AOAH) is capable of removing secondary fatty acids from the lipid A moiety of LPS, thus transforming hexa-acylated LPS into less immunostimulatory tetra-acylated LPS. Interestingly, expression of AOAH was significantly increased in the small intestinal epithelium of neonatal mice before weaning. Altogether, these results deepen our understanding of the complex processes that maintain gut barrier integrity and establish immune homeostasis during the critical early postnatal period.

Poster Session 3

17^{40}-18^{00}

11 SANDRA BIERWIRTH

CHRONIC ACTIVATION OF ATF6 IN THE EPITHELIUM PROMOTES COLONIC TUMOR SUSCEPTIBILITY VIA MICROBIOTA-DEPENDENT CHANGES IN LIPID METABOLISM

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Introduction: Prolonged activation of the endoplasmic reticulum unfolded protein response (erUPR) promotes functional changes in the epithelium and is associated with several diseases such as inflammatory bowel diseases (IBD) and colorectal cancer (CRC). Mechanistically, the contribution of the transcriptional responses of the three distinct erUPR-signaling arms to such pathologies are not fully understood and require focused research efforts. The erUPR signal transducer activating transcription factor 6 (ATF6) was recently associated with early dysplastic changes in CRC and colitis-associated CRC. We showed that intestinal epithelial cell (IEC)-specific expression of ATF6 ($nATF6^{IEC}$) induces spontaneous

colon tumorigenesis in homozygous nATF6^{IEC} mice in a microbiota-dependent manner, while heterozygous nATF6^{IEC} mice are tumor-free.

Aim & Methods: To investigate the impact of short-term (acute) and long-term (chronic) activation of ATF6 signaling on tumor initiation in the epithelium, we performed mRNA sequencing analyses on isolated colonic IECs of specific-pathogen-free (SPF) nATF6^{IEC} mice at pre tumor timepoints. To dissect the impact of microbial signaling on the ATF6-dependent transcriptional program, colonic mRNA from germ-free (GF) nATF6^{IEC} mice was also sequenced.

Results: Based on the 2180 genes regulated in all tumor-susceptible mice, we generated a gene signature of the 20 highest regulated transcripts. Kaplan-Meier analysis of a published CRC patient cohort shows significant association of this ATF6-dependent gene signature with a decreased disease-free survival, indicating a prognostic value for ATF6 target genes. To functionally define downstream targets of the ATF6 signaling response, we identified a core ATF6-driven signature comprising 328 genes present in mice with at least one transgenic nATF6 allele, of which most genes are associated with ER stress responses (e.g. *Grp78, Grp94* or *Pdia4*). Interestingly, while acute ATF6 activation only initiates a program to restore cellular homeostasis, chronic activation of ATF6 leads to a pre-tumor increase of lipid synthesis related gene expression (*Fasn, Dgat2*). In line with this, lipidomics analyses show an elongation of saturated fatty acids in response to ATF6 activation. Comparison of the transcriptional profiles of GF and SPF housed nATF6^{IEC} mice showed that these ATF6-dependent changes in lipid metabolism are microbiota-dependent. Investigation of metabolites of tumor-susceptible mice via untargeted metabolomics of luminal content could validate an enrichment in fatty acids, particularly long chain fatty acids, and links fatty acid metabolism to bacterial lipid detoxification in the tumor niche.

Conclusion: Our data shows that microbiota-dependent chronic ATF6 signaling induces a metabolic shift towards lipid metabolism, which contributes to a pro-oncogenic microenvironment and increased tumor-susceptibility.

12 CELINA PROSCH
THE EXPERIMENTAL PROBIOTIC ACLS IN MOUSE MODELS OF ANTIBIOTIC-INDUCED DYSBIOSIS AND RESPIRATORY INFECTION - A PROJECT OUTLINE

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Disruption of the microbiome (dysbiosis) can lead to disease formation, in particular, gastro-intestinal but also respiratory tract infections (RTIs). However, it remains unclear which changes in the microbiome are responsible for influencing the immune response and more specifically, lead to altered immune responses in the lungs.

Our preliminary data indicates that C57BL/6 mice, treated with the antibiotic cocktail VMNA (Vancomycin, Metronidazole, Neomycin, Ampicillin) recovered more quickly from a gut dysbiosis when they received intranasal applications of three bacterial strains, which had been isolated from murine lungs, either individually or as a combination (ACLS, confidential strain composition). Published data shows that mice infected with *Klebsiella (K.) pneumoniae* develop a stronger disease course when they had been pre-treated with VMNA (Brown, Sequeira and Clarke, 2017).

Here we outline our strategy to determine whether immunological mechanisms contribute to the microbiota resilience, and whether this ameliorates immunological control over *K. pneumoniae* infection. Therefore, we will investigate whether resilience depends on administration of live bacteria, or if inactivated ones have a similar effect. Further, we will analyze the *K. pneumonia* disease course in mice that are rendered dysbiotic with VMNA and where recovery is initiated with ACLS.

A better understanding of the reciprocal interaction between microbiota and the immune system of the intestine and lungs could lead to improved probiotic therapies, which in turn offer new treatment options for RTI.

Brown, R. L., Sequeira, R. P., and Clarke, T. B. (2017). The microbiota protects against respiratory infection via GM-CSF signaling. Nature communications, 8(1), 1512. https://doi.org/10.1038/s41467-017-01803-x

13 AAYUSHI SHAH
UNDERSTANDING THE ROLE OF MICROBIOTA POPULATIONS IN LICENSING OF PATHOGENIC T CELLS IN INTESTINAL BOWEL DISEASES

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The intestinal health is reflected by the composition and the diversity of the microbes that reside in it. In state of intestinal bowel diseases (IBD), there is most often a dysbiosis that occurs, causing either an enrichment or decline of certain populations, thus there is a need to define and understand the changes in the microbiota and how it translates to the changes in immune compartment. Using a Tbet-deficient T cells in a transfer colitis model, we observed that contrary to previous findings colitis is induced in mice. Colitis induction heavily dependent on the microbiota composition. Although clinically very similar, the pathomechanisms between Tbet-deficient and Tbet-sufficient colitis differed drastically. We now want to dive deeper and identify which members of the microbiota license colitis in the absence of Tbet and how they do so molecularly. We are using flow cytometry staining with markers reflecting immune recognition of bacteria *in vivo* (eg. IgA) to dissect the different bacterial populations. Understanding the specific crosstalk between the pro- and anti-inflammatory bacteria and the Th cells will help to understand the mechanisms underlying development of chronic inflammatory bowel diseases and could lead to better stratification of IBD patients based on specific immune-microbiota interactions.

14 ESTHER WORTMANN
CAUSAL EFFECTS OF SECONDARY BILE ACIDS PRODUCED BY THE GUT MICROBIOME IN COLORECTAL CANCER

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Western diet is a major etiological factor for colorectal cancer (CRC) and the gut microbiome is implicated in disease development. Several studies have highlighted a link between secondary bile acids (SBA) and CRC, but causation has not been addressed.
We performed two feeding trials in the *APC*$^{1311/+}$ pig model of colorectal adenomas (n = 4-9 pigs per group) to investigate diet-microbiota interactions in CRC. The first trial tested the effects of feeding a diet enriched in red meat and lard (RL) for 3 months on the pathology, faecal microbiota, and metabolome. The RL pigs were characterized by significantly increased polyp size and count, which was associated with substantial shifts in faecal microbiota profiles. Faecal concentrations of the SBA deoxycholic acid (DCA) and 12-ketolithocholic acid (12-KLCA) were 6- and 2-fold increased in the RL group after feeding. To elucidate the causal role of SBA, the second feeding trial included supplementation of the diet with the bile acid scavenger cholestyramine (COL). We observed decreased epithelial proliferation in the COL vs. RL diet after 3 months of feeding (15-25% vs. 37-47% Ki67-positive cells in distal colonic crypts) accompanied by a trend towards lower faecal concentrations of DCA and 12-KLCA. Bile acids measurement in the blood, lipidomics, and 16S rRNA amplicon analysis are being performed.

These experiments provide further experimental evidence about diet-microbiome interactions and the link between secondary bile acids and CRC development. Ongoing faecal microbiota transplantation experiments in germfree *Apc*$^{1638N/+}$ mice and targeted colonization with synthetic communities, including SBA-producing bacteria, will help dissecting causality and the effects of SBA on the gut epithelium at the single-cell level.

Poster Session 4

18^{00}-18^{20}

15 SUDIP DAS
HUMAN LUNG MICROBIOTA-DRIVEN INNATE IMMUNE LANDSCAPE IN AN ALVEOLAR-LIKE MACROPHAGE MODEL

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The human lower respiratory tract was long thought to be sterile, but recent evidence show presence of a lower airway microbiota and its impact on lung health. Recently, we have characterized the microbiota dynamics of longitudinally-acquired human bronchoalveolar lavage fluids (BALF). We define the prevalent and viable lung bacteria by combining sequencing and culturing approaches, resulting in identification of four distinct microbiota profiles or pneumotypes. We establish links to bacterial and viral loads, host gene expression, lung function and clinical stability[1]. We show that the predominant "balanced" pneumotype resembles a healthy lung and consists of a diverse bacterial community. We also establish a large collection of primary lung isolates called the Lung Microbiota culture Collection (LuMiCol) containing bacteria from major phyla in human lower respiratory tract, including the most prevalent bacteria and important lung pathogens[2].

Alveolar Macrophages (AM) are surveying cells in the human alveolar space with a unique phenotype. However, there is a lack of a standardized in vitro model for studying the largely unknown AM-lung bacteria interactions. Therefore, we repurpose THP-1 monocytes harboring a reporter for NFkB activation, to differentiate into macrophages. Using mass cytometry and cell-based assays, we show that these macrophages are phenotypically similar to AMs i.e. no background NFkB activation and similar surface markers. Thereby, we establish an AM-like macrophage model in vitro and a fast, easy, scalable screening strategy to investigate the inflammatory potential of diverse bacteria from human lung. By doing so, we discovered differential recognition of lung bacteria by pattern recognition receptors, varied NFkB activation and contact-dependent cytokine production in AM-like macrophages (Das et al. Unpublished). We also observe differential priming of macrophages by bacteria belonging to the balanced pneumotype, prior to pathogen challenge. We show that these differences can be partially

explained by surface lipopolysaccharide variants. Next, we used Small Artificial Communities (SACs) of beneficial bacteria and discover a novel role of commensal Streptococci in dampening inflammation in AM-like macrophages. Overall, we discover novel interactions of human lung bacteria and the innate immune system. We also uncover the potential of LUMICOL as a resource for individual bacteria or tailor-made communities with therapeutic potential in lung disease.

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16 NINA HEPPNER

INFANT FORMULA SUPPLEMENTATION WITH GALACTOOLIGOSACCHARIDES AND BIFIDOBACTERIA EFFECTIVELY PROMOTES GROWTH OF ACTINOBACTERIA IN THE GUT OF TERM INFANTS

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Introduction: The naïve early life microbial composition of the infant's gut is continually changing and developing in the first months of life. Factors at the very beginning of life (e.g. birth mode, feeding type) may have an influence on the long-term development of the gut microbiota. The bacterial composition of infants fed on formula compared to breastfed infants has been shown to differ significantly, especially concerning the abundance of Bifidobacteria.
Aims and Methods: Infantibio-II is a double blind, placebo-controlled study designed to investigate the effects of different supplements in infant formula on the gut microbiota composition during the first year of life. A cows-milk based formula was either supplemented with two bifidobacteria strains, previously isolated from stool of fully breastfed infants, galactooligosaccharides (GOS), both Bifidobacteria and GOS or no supplements. 210 infants (106 females, 104 males) were recruited in the greater Munich area in Germany. They were randomized to one of the four formula groups but had the option to breastfeed (exclusively or in combination with formula). A third of the infants were exclusively breastfed throughout the entire study period. During the first three months of their life, 60 of the study participants obtained the majority of their caloric intake from formula ('high-feeders'). Stool samples from infants at five time points and parental samples at one time point were sequenced targeting the V3V4 region of the 16S rRNA gene (Ø 21484.05 ± 6136.8 high quality reads per sample).
Results: The number of observed species increased over time across all feeding groups. Both supplementation with the strains *B.longum infantis* and *B.breve* and galactooligosaccharides (GOS) led to a significant increase in the relative abundance of Actinobacteria, even surpassing the relative abundance in the breastfed group at early time points. The placebo formula group consistently showed the lowest levels of Actinobacteria. After 7 months, breastfed babies and babies in the placebo formula group (corr. p-value 0.011) showed a

significantly different microbial composition, whereas no significant difference between the formula group containing Bifidobacteria and GOS and breastfed babies could be observed (corr. p-value 0.17). After stratifying the cohort according to ‘high-feeders’ at early time points (2 weeks, 1 month and 3 months), a distinct clustering according to formula group as well as to birth mode (44% C-section) was observed. In ‘high-feeders’, *B.longum* showed an increased relative abundance in all non-placebo formula groups which further increased over time. The relative abundance of *B.breve* was reduced in formula groups lacking GOS supplementation. A homogeneous phylogenetic distribution of microbial composition in infants with different feeding type (breastfed, bottle or mixed) was observed. However, parental samples formed a distinct adult cluster. Conclusion Bifidobacteria and GOS supplementation effectively increased the relative abundance of Actinobacteria in infants during the first year of life. In particular, GOS supplementation sufficiently enhanced its abundance. Metabolomics and metagenomics will provide further information about the importance of particular strains and their functional association

17 CLAUDIO NEIDHÖFER
GLOBAL DISTRIBUTION PATTERNS OF CARBAPENEMASE-ENCODING BACTERIA IN A NEW LIGHT: CLUES ON A ROLE FOR ETHNICITY

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Background: The rapid global spread of opportunistically pathogenic carbapenemase-encoding bacteria (CEB) poses a growing threat to human health. Epidemiological data is of primary importance to understand the scope of the problem and to design effective control measures. The human microbiome has become a significant reservoir of resistance genes and has been correlated with factors such as age, ethnicity and geography. We investigated to what extent microbiome-associated parameters are also CEB-associated and offer new insights on factors that may be taken into account when optimizing preventive sanitary precautions and developing safe hospital environments.
Methods: We analyzed demographic and clinical data on 397 CEB detected at our University Hospital (Bonn, Germany) between September 2014 and December 2019, investigating correlations between microbiome-associated demographic parameters and the presence of certain types of CEB. Data on detected CEB was combined with patient's demographic and clinical information for each isolate. Multiple regression techniques were applied to estimate the predictive quality of observed differences.
Results: Our findings confirm an important role of age and gender in CEB colonization patterns and indicate a role for ethnicity and domicile in the type of CEB patients were colonized with. Also, carbapenemase-encoding A. baumannii was most frequently introduced to the hospital, while the risk of colonization with VIM-encoding P. aeruginosa rose with the length of hospital stay.
Conclusion: We obtain first evidence on a role for ethnicity in the type of CEB patients were colonized with, which necessitates confirmation and further attention for a better understanding of their global distribution, for improving health policies, for better tailoring diagnostic

approaches and therapies to individual patients and to prevent ethnicity from being a cause of disadvantage in routine medical practice. OXA-encoding CEB being harder to detect in routine screening, targeted preventive measures, such as culture media selective for carbapenem-resistant bacteria, would be opportune for selected patient groups, for example. Not all hospital-acquired pathogens can be equally well contained. Carbapenemase-encoding A. baumannii was most frequently introduced to the hospital but could efficiently be controlled from spreading at hospital admission, while the risk of colonization with VIM-encoding P. aeruginosa, strongly linked to hospital-wastewater, rose with the length of hospital stay, which prompts to continuously rethink hospital-built environments and further optimize all precautions according to risk factors and the spectrum of expected pathogens.

18 FELIX SOMMER

MICROBIAL REGULATION OF HEXOKINASE 2 LINKS MITOCHONDRIAL METABOLISM AND CELL DEATH IN COLITIS

Finn Hinrichsen[1#] & Jacob Hamm[1#], Magdalena Westermann[1], Lena Schröder[1], Kensuke Shima[2], Neha Mishra[1], Alesia Walker[3], Nina Sommer[1], Kenneth Klischies[1], Daniela Prasse[4], Johannes Zimmermann[5], Sina Kaiser[1], Dora Bordoni[1], Antonella Fazio[1], Georgios Marinos[5], Georg Laue[1], Valentina Tremaroli[6], Marijana Basic[7], Robert Häsler[1,8], Ruth A. Schmitz[4], Stefan Krautwald[9], Andrea Wolf[10], Bärbel Stecher[11,12], Philippe Schmitt-Kopplin[3], Christoph Kaleta[5], Jan Rupp[2], Fredrik Bäckhed[6,13,14], Philip Rosenstiel[1] & Felix Sommer[1]

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Abstract: Hexokinases (HK) catalyze the first step of glycolysis and thereby limit its pace. HK2 is highly expressed in the gut epithelium, plays a role in immune responses and is upregulated in inflammation and ulcerative colitis. Here, we examined the microbial regulation of HK2 and its impact on intestinal inflammation by generating mice lacking HK2 specifically in intestinal epithelial cells (Hk2ΔIEC). Hk2ΔIEC mice were less susceptible to acute intestinal inflammation upon challenge with dextran sodium sulfate (DSS). Analyzing the epithelial transcriptome from Hk2ΔIEC mice during acute colitis revealed downregulation of cell death signaling and mitochondrial dysfunction dependent on loss of HK2. Using intestinal organoids derived from Hk2ΔIEC mice and Caco-2 cells lacking HK2, we identified peptidyl-prolyl cis-trans isomerase (PPIF) as a key target of HK2-mediated regulation of mitochondrial permeability and repression of cell-death during intestinal inflammation. The microbiota strongly regulated HK2 expression and activity. The microbially-derived short-chain fatty acid (SCFA) butyrate repressed HK2 expression and oral supplementation protected wildtype but not Hk2ΔIEC mice from DSS colitis. Our findings define a novel mechanism how butyrate may act as a protective factor for intestinal barrier homeostasis and suggest targeted HK2 inhibition as a promising therapeutic avenue in intestinal inflammation. Available as pre-print at DOI: 10.1101/2020.12.22.423953v1.

Keywords: hexokinase / inflammation / microbiota / intestinal epithelial cell / immunometabolism

19 IRIS STOLZER

INTESTINAL EPITHELIAL NECROSIS INFLUENCES TRYPTOPHAN METABOLISM AND BONE MASS

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Up to 50% of patients with inflammatory bowel dieses (IBD) develop extraintestinal manifestations. These manifestations are prevalent in both, Crohn's disease (CD) and ulcerative colitis (UC), and can affect almost every organ including the hepato-pancreato-biliary, dermatologic and musculoskeletal system. Accordingly it has been demonstrated that intestinal inflammation and dysbiosis can affect homeostasis of joints and bones, and patients suffer from arthritis, osteopenia or osteoporosis. Although this gut-bone/joint axis has been described in various preclinical and clinical studies, the underlying mechanisms remain incompletely understood. Here, we used a well-established mouse model (*Casp8*$^{\Delta IEC}$ mice) that mimics several key features of Crohn's disease to better delineate the impact of intestinal inflammation on bone homeostasis. We uncovered that *Casp8*$^{\Delta IEC}$ mice display a trabecular bone loss and alterations of the bone marrow in the paws. Alterations in systemic bone mass was associated with an increase in the number of osteoclast and accompanied by an altered tryptophan metabolism as evident by increased serotonin levels and increased intestinal AhR signaling. Of note tryptophan metabolites can be produced by gut microorganism (microbial-derived tryptophan metabolites) as well as host tissues. Interestingly, germ-free *Casp8*$^{\Delta IEC}$ mice display strongly ameliorated intestinal inflammation, whereas bone loss was only slightly improved. Our results suggest that microbiota dependent and independent mechanisms foster the pathogenesis of extraintestinal manifestations.

Poster Session 5

18^{20}-18^{40}

20 JAMIE AFGHANI

ENHANCED ACCESS TO THE HEALTH-RELATED SKIN METABOLOME BY FAST, REPRODUCIBLE AND NON-INVASIVE WET PREP SAMPLING

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Our skin influences our physical and mental health, and its chemical composition can reflect environmental and disease conditions. Through sampling the skin metabolome, we can provide a promising window into the mechanisms of body. But the broad application of skin metabolomics has recently been hampered by a lack of easy and widely applicable sampling methods. Here, we present a novel rapid, simple, and most importantly, pain-less, non-invasive sampling technique suitable for clinical studies of fragile or weakened skin. The method is called WET PREP and is simply a lavage of the skin which focuses on capturing the metabolome separately from the (phospho)lipidome. We systematically evaluate WET PREPs in comparison with the non-invasive method of choice in skin metabolomics, swab collection, using LC-MS² on two complementary chromatographic columns (RP and HILIC). We also integrate targeted key metabolites of skin relevance. Overall, WET PREP provides a strikingly more stable shared metabolome across sampled individuals, while also being able to capture unique individual metabolites with a high consistency in intra-individual reproducibility. With the exception of lipidomics studies, we therefore recommend WET-PREPs as the preferred

skin metabolome sampling technique due to the quick preparation time, low-cost, and our results.

21 OLIVIA COLEMAN
MUCOSA-ADHERENT MUCISPIRILLUM SPP. DRIVE ATF6-DEPENDENT COLORECTAL TUMORIGENESIS IN MURINE MODELS

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Introduction: Colorectal cancer (CRC) is a leading cause of cancer-related deaths worldwide. Chronic intestinal inflammation increases cancer risk, potentially leading to colitis-associated cancer (CAC). Alterations in intestinal microbiota are associated with CRC and CAC, placing particular importance on host-microbe interactions in tumorigenesis. Our transgenic murine model of intestinal epithelial-specific overexpression of the activated form of activating transcription factor 6 (nATF6^{IEC}) presents a novel, human relevant model of spontaneous microbiota-dependent CRC. To assess the role of inflammation and the microbiota in this context, we crossed nATF6^{IEC} mice with IL-10-deficient mice (nATF6^{IEC}xIL10$^{-/-}$) under specific pathogen-free (SPF) and germfree (GF) conditions.

Aims & Methods: We aimed to characterize and compare tumorigenic phenotypes, and identify tumor-associated bacteria, in nATF6^{IEC} and nATF6^{IEC}xIL10$^{-/-}$ mice. Tumor development (incidence and frequency) and inflammation were determined via histological scoring and qPCR analysis. Host mucus alterations were assessed through a combination of FISH, PAS/AB staining and qPCR. To identify tumor-driving taxa we characterized colonic-mucosal microbiota using 16S rRNA profiling and identified tumor-predictive ASVs using machine-learning. To characterise microbiota associated with tumor location, we established a protocol to determine spatial structure of mucosal microbiota at millimetre resolution along the colonic longitudinal axis.

Results: IL10-deficiency increased tumor susceptibility, with otherwise tumor-free monoallelic nATF6^{IEC} mice developing tumors in the proximal to mid colon with an incidence of ~70%, and biallelic mice displaying an exacerbated phenotype and greatly reduced lifespan. Goblet cell loss and enhanced microbial penetration into the mucus layer were key characteristics of tumor-susceptible nATF6^{IEC} and nATF6^{IEC}xIL10$^{-/-}$ mice. Importantly, GF nATF6^{IEC}xIL-10$^{-/-}$ mice remained tumor-free. Mucosal microbiota was significantly altered in tumor-bearing mice and outperformed luminal microbiota at discriminating phenotype (luminal AUC = 0.80,

tissue AUC = 0.87). To identify microbial drivers, we applied the passenger-driver model, identifying several putative drivers, including ASVs classified as *Mucispirillum,* Lachnospiraceae and *Bacteroides*. Validation in our spatially resolved dataset showed depletion of driver taxa in tumours regardless of location. As *Mucispirillum* spp. were identified as drivers in both models, we characterised their spatial distribution demonstrating an increase in abundance and prevalence in tumor-susceptible proximal colonic regions, compared to non-susceptible distal regions. Notably, *Mucispirillum* abundance was reduced in monoallelic nATF6^{IEC}xIL10$^{-/-}$ mice that remained tumor-free.

Conclusion: Here we introduce a novel mouse model of CAC driven by the tumorigenic transcription factor nATF6, and identify a shared pathobiont driving tumorigenesis. Despite being a common commensal, *Mucispirillum schaedleri,* the sole representative of the genus *Mucispirillum*, has been shown to possess virulence factors and is enriched in mucosal samples. Loss of the stratified mucus barrier in our mouse models may provide *Mucispirillum* with unrestricted access to the epithelial layer and the possibility to modify host cell function. To investigate their causal role in driving tumorigenesis in our models, we are currently conducting *M. schaedleri* monocolonizations in GF mice.

22 TINA EISMANN

CORRELATION OF INTESTINAL MICROBIOME COMPOSITION, METABOLITE PROFILES AND CLINICAL PARAMETERS IN PATIENTS SUFFERING FROM HEMATOLOGICAL MALIGNANCIES: RECOVERY OF INTESTINAL MICROBIOME AFTER HEMATOPOIETIC 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). But this treatment is associated with risks such as vulnerability to opportunistic infections and acute graft-versus-host disease (GvHD). The human intestine is one of the organs most severely affected by 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. We are especially interested in whether there is a recovery of intestinal microbiome 56 days after allo-HSCT in comparison to microbiome at baseline which is not modified by any medication. We want to evaluate whether microbiome reverts to baseline, adjusts to a new baseline or continues to be dysbiotic and how different variables such as antibiotic therapy or complications such as GvHD and infections could contribute to these various outcomes. We aim to correlate the extent of microbiome recovery with day 100 survival as well as with the occurrence of GvHD or infectious complications during the course of transplantation. Our hypothesis is that patients who did not suffer from infectious complications or GvHD during/after allo-HSCT, could have better recovery of microbiome and thus better survival than those suffering from complications.

Methods: We perform translational analysis based on the ongoing prospective, observational clinical study “Metabolites and Microbiome in Acute Leukemia (M&M-AL)”. Here, patients with initial diagnosis of acute leukemia or with inpatient admission for allo-HSCT are enrolled. We obtain stool and blood samples longitudinally starting from the day of recruitment. From the day of transplantation samples are obtained weekly until dischargement from hospital. We

established an additional time point 56 (+/- 7) days after allo-HSCT as follow up care allowing us to analyze the extent of microbiome recovery. We will compare metabolite profiles and microbiome compositions, profiled by 16S rRNA gene sequencing, of stool samples obtained at day 56 with those profiles of samples obtained at initial diagnosis, that means before receiving any microbiome-modifying medication. Clinical parameters (e.g. infections, GVHD, survival) are assessed simultaneously with bio samples and will be correlated with these.

Preliminary results and outlook: The characteristics of our study patients (e.g. age, initial diagnosis, conditioning protocol) are comparable with those from other established cohorts such as MSK New York and Regensburg [1]. In these cohorts there was observed a slight recovery of microbiome after the transplantation period. We want to verify this phenomenon with our own study and will investigate the relevance of microbiome recovery as a potential prognostic marker for survival after allo-HSCT.

[1]Peled et al., Microbiota as Predictor of Mortality in Allogeneic Hematopoietic-Cell Transplantation. NEJM, 2020 Feb 27;382(9):822-834.

23 JULIA NOTTER

MICROBIOME-MEDIATED METABOLISM OF ANTIRETROVIRAL DRUGS IN HIV INFECTED PATIENTS WITH TOXICITY – A PILOT STUDY

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Background: Antiretroviral drugs can induce a wide range of adverse effects ranging from acute events to long-term toxicity. With treatment predicted to last lifelong, choosing an antiretroviral drug with the lowest possible toxicity is crucial; for many drugs, however, toxicity cannot be reliably predicted, as underlying mechanisms are poorly understood. Increasing evidence suggests that the gut microbiota contributes to drug metabolism (both intestinally and systemically), influencing drug bioavailability, metabolism and metabolite absorption, and thus has the potential to influence interpersonal variation in drug efficacy and toxicity. Therefore, a better understanding of interactions between the gut microbiome and antiretroviral drugs is needed to improve the quality of patient care. Tenofovir disoproxil fumarate (TDF) combined with Emtricitabine (FTC) is frequently used as a backbone in antiretroviral treatment (ART) regimens, and is known to induce nephrotoxicity in up to 15% of treated patients. This pilot study compared the plasma metabolome of TDF-treated patients with and without adverse drug effects and controls to identify key metabolites involved in TDF toxicity and potential microbiota derived toxicity biomarkers.

Methods: We retrospectively evaluated plasma samples collected in the Swiss HIV Cohort Study (SHCS) biobank. We used samples taken before starting TDF (T1) and at the time toxicity developed (T2). Metabolomics analysis of plasma samples was performed using liquid chromatography coupled mass spectrometry (LC-MS). Targeted integration of the signals for drug and known drug metabolites was performed to reveal patients' plasma levels of drug and drug metabolites. Furthermore, differential analysis of untargeted metabolomics data was performed (including correction for multiple hypothesis testing) to identify metabolites that are different between patient groups (with and without drug-related side effects).

Results: Plasma samples of 18 patients on TDF exhibiting well-known toxicity (proximal tubular dysfunction) were compared to 36 control patients (matched for gender, age, HIV viral load and duration of TDF therapy). Principal component analysis highlighted a possible source of bias in sample collection time, not relatable to age, gender, co-medication or toxicity. However, we were able to identify TDF/FTC, its metabolites and four other metabolites altered between the toxicity and control group at T2. In all cases, samples showing toxiciy have a significantly higher level of these components in the serum metabolites. Not all identified metabolites could be annotated and therefore their putative origin (host/microbiome) or role in toxicity remains to be discovered.

Discussion: Using prospectively collected plasma samples, the detection of HIV antiretroviral drugs and their putative metabolite is possible. However, the method used here shows several limitations: 1) due to non-availablility of stool samples, a direct link to the gut microbiome is impossible and 2) longer sample storage of the samples may have influenced the reliability of the results. To forego these issues, we are planning a prospective study collecting plasma and stool samples over a short period of time.

24 STEFANIE WAGNER
IMPROVING MICROBIOME DATA READOUT BY INTEGRATION OF TOTAL CELL NUMBERS

Stefanie Wagner, Thilo M. Fuchs

Institute of Molecular Pathogenesis, Federal Research Institute for Animal Health, Naumburger Str. 96a, D-07743 Jena

The influence of antibiotics on the intestinal microbiome is of major impact for the health of humans and animals, since they are not only effective against pathogenic bacteria. They also affect the commensal gut microbiome and thus the functionality of the gut. However, a major drawback of studies in the field is that they provide compositional data only in relative abundances, thus not allowing to determine the effect of antibiotics on the extent and/or directionality of changes in the microbial composition.

Here, we focused on the macrolide antibiotic tylosin applied in veterinary medicine and its impact on the gut microbiome in swine used as a large animal model. We analyzed the composition of the intestinal microbiome via sequencing of the bacterial 16S rRNA genes of fecal samples. Then, we clustered the sequencing reads to amplicon sequence variants (ASVs) and mapped them to rRNA gene databases. Additionally, we performed a copy number correction of these genes on the basis of a phylogenetic similarity prediction of the sequences. In order to investigate the effect of the antibiotic substance in more detail, we wanted to improve the readout of the mapped and corrected sequencing data. Therefore, we complemented the analysis with a flow cytometric cell counting of the same samples and integrated the total bacterial load into the ASV's relative abundancies. After that, we performed statistical testing of the resulting absolute abundancies of ASVs.

We observed individual and time-dependent variations of the bacterial cell numbers within all samples. The integration of these cell counting data revealed noticeably more significant changes of genera abundancies (17 changes) than 16S rRNA gene sequencing data alone (11 changes). In conclusion, we found that corrected and quantified microbiome data allow a more precise interpretation of the influence of the antibiotic substance on the microbiome composition, in particular with respect to the extent of shifts of the microbial composition.

CONFERENCE PROGRAM

Saturday, July 3rd

Session 5
10^{00}-11^{15}

EARLY LIFE MICROBIOME ASSEMBLY: A WINDOW OF OPPORTUNITY

Debby Bogaert
Edinburgh Medical School, UK

Over the past decades, researchers have begun to unravel the causes and consequences of variation within microbial communities, and how these might influence pathogenesis of a broad range of diseases. In the first decade, the gut has been the niche of focus for human microbiome research, but recent studies have revealed an unexpected diversity of bacteria in other body niches too, and more importantly, with clear associations between microbial community composition and health outcome for those niches as well.
In early life, the assembly of the complete human microbial ecosystem occur at a very rapid pace, strongly driven by host and environmental drivers. In my presentation, I will discuss the current body of evidence regarding initial seeding and development of the human microbiome in early life, the importance of specific environmental and lifestyle factors, and focus on how these ultimately appear linked with immune development and infection susceptibility. Finally, I will provide some ideas and examples of potential next phase studies, possibilities for adapted diagnostic tools, and therapeutic interventions.

INVITED ABSTRACT NO. 9:
THE ROLE OF MATERNAL MICROBIOTA IN DURABLY SHAPING INTESTINAL IMMUNITY AND GENE EXPRESSION IN THE OFFSPRING THROUGH EPIGENETIC MECHANISMS

Cristina Kalbermatter, Nerea Fernandez Trigo, Sandro Christensen, Anna S. Wenning, Mercedes Gomez de Agüero, Andrew J. Macpherson and Stephanie C. Ganal-Vonarburg

Gastroenterology and Mucosal Immunology, Department for BioMedical Research, University of Bern, Switzerland

Birth represents a disruption from the sheltered environment in utero to the microbial-rich world. This change is encompassed with the colonization of all our body surfaces, including the gastrointestinal tract. To protect the newborn from potential infections the innate immune system, but also breast milk provide crucial defense elements. However, the starting point of gathering our immune strategies takes place already in utero. Herein, the maternal microbiota places an important role in priming fetal immune development through the transfer of microbial metabolites via the placenta. Using an auxotrophic E. coli strain, it is possible to reversibly colonize pregnant dams during gestation and hence distinguish between the contribution of the maternal microbiota from the influence of postnatal colonization. Microbial signals during pregnancy were able to durably affect gene expression in the neonatal small intestine and particularly involved genes relevant for epithelial cell homeostasis. Since this effect in mice remained until adulthood, we hypothesize that microbe-host interactions during gestation shape neonatal immunity and epithelial maturation by altering its epigenetic landscape. Epigenetic remodeling regulates gene accessibility and is directed by histone modifications and DNA methylation, the latter being the major factor early in life. E. coli colonization of the dam during gestation only triggered an intermediate DNA methylation level in the offspring's intestine positioned between germ-free and SPF pups. Moreover, genes with enhanced H3K4me3 marks were shared between germ-free and gestation-only colonized mice compared to SPF. Nevertheless, offspring of E. coli treated mice exhibited additional genes with H3K4me3 enrichment, indicating that the maternal microbiota is able to alter gene expression through DNA methylation and H3K4me3. Whether these changes in the epigenome also have functional consequences is currently being investigated. Our research will reveal essential insights into epigenetic mechanisms as a result of interactions between

the maternal microbiota, the embryo, and the neonate. It will increase the knowledge about the importance of maternal colonization during pregnancy for neonatal health and display its durable consequences.

INVITED ABSTRACT NO. 10:
PARALLELISM OF THE INTESTINAL SECRETORY IGA RESPONSE MODULATES FUNCTIONAL MICROBIAL FITNESS

Tim Rollenske[1], Sophie Burkhalter[1], Lukas Muerner[2], Stephan von Gunten[2], Hedda Wardemann[3,†] and Andrew J. Macpherson[1,†]

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† These authors made an equal contribution.

IgA secretion across mucous membranes accounts for most antibody production in mammals. Although toxin neutralisation or microbial aggregation can protect against pathogens, the spectrum of functional effects on non-pathogenic microbes and how far these are mediated by specific binding or polyreactive secretory (S)IgA remains unclear. By fine dissection of the intestinal plasma cell response to microbial colonisation with a single microbe in mice, we identify a range of antigen-specific SIgA species targeting defined surface and non-surface membrane antigens. *In vivo* secretion of individual monoclonal dimeric IgAs with different binding characteristics showed distinct alterations of intestinal bacterial function and metabolism, largely through specific binding. Fine specificity of monoclonal SIgA on the same microbial target molecule determined specific microbial metabolic alterations, whereas SIgA bacterial surface coating generically reduced motility and abrogated bile acid toxicity. Parallel components of the overall intestinal IgA response to a single microbe therefore have distinct effects on microbial carbon-source uptake, phage susceptibility, motility and membrane integrity.

Hot Topic 1
11^{30}-12^{00}

IDENTIFYING MICROBIOME CONTRIBUTIONS TO DRUG METABOLISM AND TOXICITY

Michael Zimmermann

European Molecular Biology Laboratory (EMBL), Structural and Computational Biology Unit, Germany

Individuals vary widely in their drug responses, which can be dangerous and expensive due to significant treatment delays and adverse effects. Growing evidence implicates the gut microbiome in this variability, however the molecular mechanisms remain mostly unknown. Using antiviral nucleoside analogues as examples, we reported experimental and computational approaches to separate host and gut microbiota contributions to drug metabolism. The resulting pharmacokinetic models identified measurable physiological, microbial and chemical parameters that dictate host and microbiome contributions to the metabolism of xenobiotics. To systematically map the drug metabolizing capacity of the gut microbiota and assess its potential contribution to drug metabolism, we further measured the ability of 76 diverse human gut bacteria to metabolize each of 271 oral drugs. We found that two thirds of these drugs are chemically modified by at least one of the tested microbes. Through combination of high-throughput bacterial genetics with mass spectrometry, we systematically identified drug-metabolizing microbial gene products. These gene products better explain the drug-metabolizing capacity of bacterial strains than their phylogenetic classification. We further demonstrate that the abundance of homologs of these gene products predict the capacity of complete human gut communities to metabolize the targeted drugs. These causal links between microbiota gene content and metabolic activities connect inter-individual microbiome variability to interpersonal differences in drug metabolism, which has translatable potential on medical therapy and drug development across multiple disease indications.

webpage: www.embl.org/zimmermann

Hot Topic 2
12^{00}-12^{30}

MATERNAL MICROBIOTA AND NEONATAL BARRIER FUNCTION

Mercedes Gomez de Agüero
University Würzburg, Germany

Neonates rely on competent tissue barriers at birth to deal with environmental challenges. As most of the body surfaces, the skin develops in two steps. While the ontogeny occurs during the gestation, the maturation happens postnatally. Several studies illustrated the key role played by the microbiota in the postnatal maturation of the skin. However, the molecular actors driving the ontogeny of the skin remains to be elucidated. Metabolites derived from the maternal microbiota delivered to the offspring during the gestation and lactation have been previously associated with the development of other barrier tissues, such as the intestine, and systemic organs, such as the brain.

To investigate the potential contribution of the maternal microbiota on the perinatal development of the skin barrier function, a model of gestational colonisation based on intragastric delivery of the auxotrophic Escherichia coli HA107 to the pregnant germ-free mice was used. Sterile offspring was analysed embryonically and postnatally.

Single cell sequencing, flow cytometry and histological analysis illustrated an embryonic epidermis composed of a tightly organized stem cell monolayer, overlain with multiple layers of differentiated keratinocytes in the offspring of gestationally colonized dams. In the offspring of germ-free dams, the embryonic epidermis is constituted of several layers of undifferentiated and highly proliferative keratinocytes formed the embryonic epidermis and led to an undifferentiated architecture postnatally. Furthermore, maternal microbiota reprogramed the embryonic keratinocyte stem cell transcriptional profile, including increased expression of genes encoding for cell differentiation. Postnatal microbial colonization was not able to restore epidermal architecture of the offspring of germ-free dams. Both, host and microbial maternal derived metabolites cross the placenta and reach the embryonic skin. Metabolites involved in epidermal differentiation and skin barrier development such as vitamins, malic and citric acid, and pyrimidine- and purine-derivatives were enriched in the embryonic skin of offspring of

gestationally colonized dams. Embryonic skin of offspring of germ-free dams accumulated metabolites associated with skin atrophy such as hydrocortisol and glutathione. Trans-epidermal water loss and dye penetration, hallmarks of abnormalities in permeability barrier, was reduced in neonates of gestationally colonized dams.

Our results show that maternal microbiota drives epidermal ontogeny and sets up skin barrier by the time of birth.

Invited Speakers + Steering Committee 2021

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