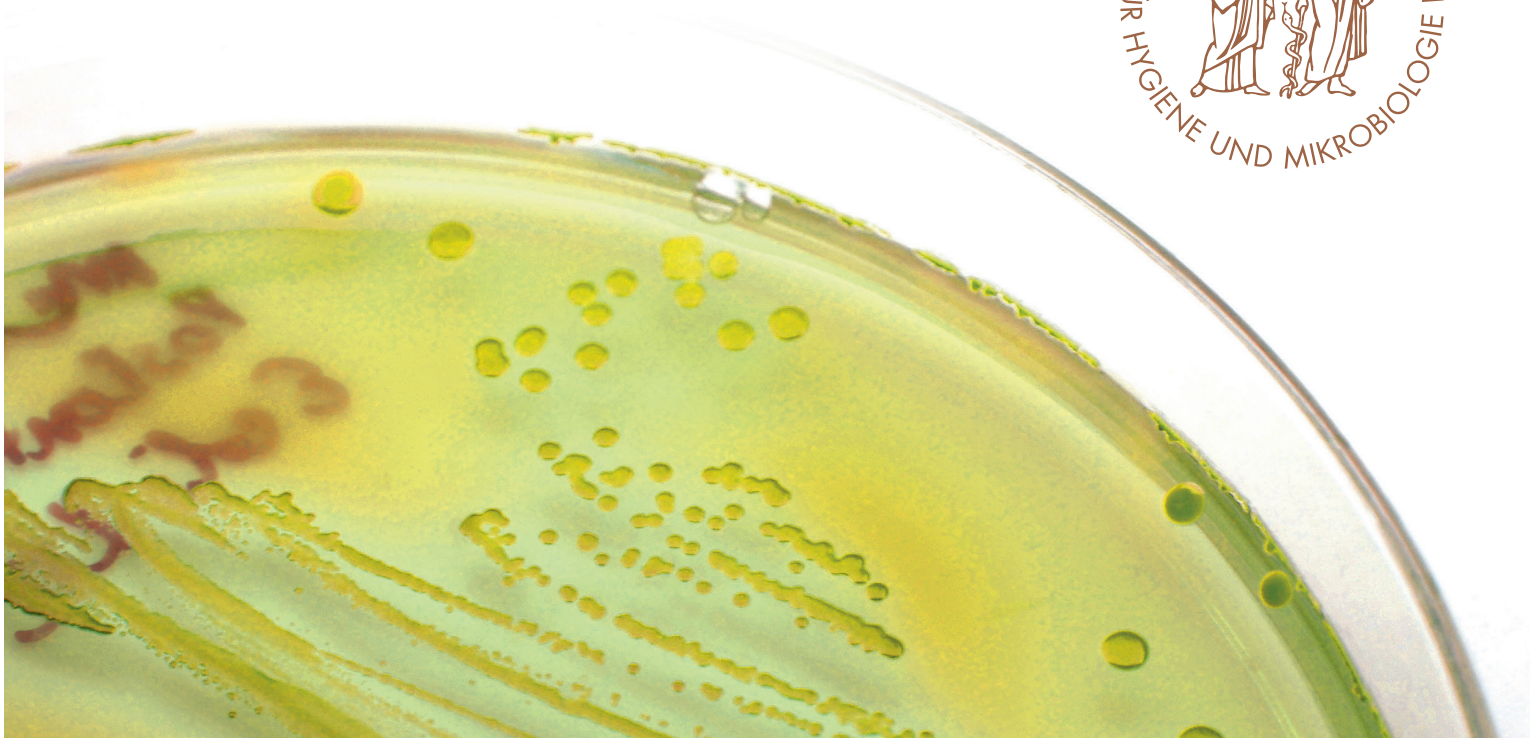# INTESTINAL
MICROBIOTA

8th
Seeon Conference and Science Camp

SPP 1656 „From sequencing to function"

10 to 12 July 2015, Conference Center Monastery Seeon

Abstractband

July 10, 2015

Dear Participant,

This is an exciting moment for us to welcome you at the 8th Seeon Conference organized for the first time as joint meeting of the German Society of Hygiene and Microbiology (DGHM) and the Priority Program SPP1656 of the German Research Foundation (DFG).

Back in 2008 the DGHM section "Microbiota, Probiota and Host" was established with the vision to build and shape an active community of researchers across Germany dedicated to generate a better understanding of microbe-host interactions in health and disease. These activities layed the foundation and represent the scientific spirit of the DFG Priority Program "MICROBIOTA – a Microbial Ecosystem at the Edge between Immune Homeostasis and Inflammation" (SPP1656).

The gut microbial ecosystem serves as a new paradigm in biomedical research with the promise to impact our understanding for the prevention of health and to affect therapeutic strategies in the treatment of diseases. The "Seeon Conference" has become a vital platform to facilitate this ambition and integrates various disciplines in basic and clinical sciences unified by the aim to understand the human microbiome.

We are looking forward to fruitful discussions and good science … let's all sharpen our desire to know!

Prof. Dr. Julia Frick and Prof. Dr. Dirk Haller

Prof. Dr. Julia-Stefanie Frick
University Tübingen
Medical Microbiology + Hygiene
Elfriede-Aulhorn-Str. 6
72076 Tübingen
Tel.: +49-(0)7071-29-82352
Fax: +49-(0)7071-29-5440
julia-stefanie.frick@med.uni-tuebingen.de

Prof. Dr. Dirk Haller
TU München
Ernährung und Immunologie
Gregor-Mendel-Str.2
85350 Freising
Tel.: +49-(0)8161-71-2026
dirk.haller@tum.de

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PROGRAM Friday, July 10

14^{00} - 14^{15} Welcome

SESSION 1: MICROBES AND CHRONIC INFLAMMATION

Chair: **Dirk Haller**, Nutrition and Immunology, Technische Universität München

14^{15} – 14^{50} **1st Keynote Lecture**: **Herbert Tilg**, Universitätsklinik für Innere Medizin I, Innsbruck
"NAFLD and microbiota"

14^{50}– 15^{50} Selected Abstracts

K. Koller, Mikrobiologisches Institut - Klinische Mikrobiologie, Immunologie und Hygiene, Universitätsklinikum Erlangen, Friedrich-Alexander Universität Erlangen-Nürnberg
"CD101 inhibits intestinal inflammation"

M. Hefele, Medical Clinic 1, Friedrich Alexander University, Erlangen
"The role of epithelial caspase-8 and the microflora during infection with Salmonella typhimurium"

S. Menz, Institute of Medical Microbiology & Hygiene, University of Tuebingen and German Center of Infection Research (DZIF)
"FliC of E. coli Nissle protects specific against DSS- induced colitis by activation of CD11c$^+$ intestinal lamina propria cells"

15^{50}-16^{30} Coffee Break

PROGRAM Friday, July 10

16^{30}-17^{05} **2nd Keynote Lecture: Hans-Christian Reinecker**, Genetics, Genomics and Molecular Biology Care, Harvard Medical School, Boston
"Control of mucosal immunity by microbial nucleic acid sensing"

17^{05}– 18^{05} Selected Abstracts

M. Schaubeck, Chair of Nutrition and Immunology, Technische Universität München
"Dysbiotic gut microbiota causes transmissive Crohn´s disease-like ileitis"

F. Schmidt, Charité - Universitätsmedizin Berlin, Medizinische Klinik m.S. Gastroenterologie, Infektiologie, Rheumatologie, Berlin
"Impact of microbiota on immune cell composition and barrier function in the gut"

F. Rost, Division of Gastroenterology and Hepatology, University Hospital Zurich
"Impact of Nlrp3 x gut microbiota interactions on experimental colitis and insulin sensitivity"

18^{15}-19^{45} Dinner + Break

19^{45}-20^{20} **3rd Keynote Lecture: Nadine Cerf-Bensussan**, Intestinal Immunity, Institute Imagine and Université Paris Descartès-Sorbonne
"Segmented filamentous bacterium: an unusual symbiont that instructs the development of the gut barrier"

20^{20}– 21^{20} Selected Abstracts

S. Thiemann, Microbial Immune Regulation, Helmholtz Centre for Infection Research, Braunschweig
"Isogenic and isobiotic mouse lines reveal microbiota-mediated indirect colonization resistance against Salmonella infection"

S. Stebe, Department of Hepatology, Gastroenterology and Infectiology, University Hospital, Tuebingen
"HDAC related epigenetic control and the antimicrobial barrier in inflammatory bowel diseases"

S. Suerbaum, Institute of Medical Microbiology and Hospital Epidemiology, Hannover Medical School
"Mutual interactions between the pathobiont Helicobacter hepaticus and the mouse intestinal microbiota: ecology, mechanisms and relevance to the induction of IBD"

PROGRAM Saturday, July 11

SESSION 2: MICROBES AND GUT HOMEOSTASIS

Chair: **Julia Frick**, Medical Microbiology + Hygiene, University Tübingen

$08^{30} - 09^{05}$ **4th Keynote Lecture: Elke Cario**, Experimental Gastroenterology, Universitätsklinikum Essen
"Intestinal efflux transporters and multi-drug resistance in inflammation"

$09^{05} - 10^{05}$ Selected Abstracts

M. Hornef, Department of Microbiology, Veterinary University Hannover
"Early postnatal selection determines long-term microbiota composition"

M. Zaiss, École Polytechnique Fédérale de Lausanne (EPFL)
"Intestinal helminths modulate allergic inflammation indirectly by altering bacterial metabolism"

A. Woting, Department of Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal
"Why is Clostridium ramosum obesogenic? Hypotheses driven by metaproteomics and metabolomics"

$10^{05} - 10^{30}$ Coffee Break

PROGRAM Saturday, July 11

10^{30} – 11^{05} **5th Keynote Lecture: Alan Walker**, Microbiology group, Rowett Institute of Nutrition and Health, University of Aberdeen
"New insights into links between the gut microbiota and diarrhoeal pathogenicity in infants from low income countries"

11^{05} – 12^{05} Selected Abstracts

A. Sünderhauf, Molecular Gastroenterology, Department of Medicine 1, University of Lübeck
"Mitochondrial ATP8 gene polymorphism alters the gut microbiota and confers to the risk of experimental colitis"

A. Legatzki, LMU Munich, University Children's Hospital
"Gut microbiota in the first year of life and association with atopic dermatitis"

D. Huson, Center for Bioinformatics, University of Tübingen
"New algorithms and software for microbiome analysis"

12^{15} – 14^{30} Lunch + Break (Social Program)

14^{30} – 15^{15} **Georg Munz**, DFG, Bonn
„DFG funding opportunities - insights and entrances"

15^{15} – 16^{00} Coffee Break + Poster

PROGRAM Saturday, July 11

SESSION 3: MICROBES AND INFECTION

Chair: **Bärbel Stecher**, Max von Pettenkofer-Institut, Bakteriologie, München

$16^{00} – 16^{35}$ **6th Keynote Lecture: Manuela Raffatellu**, Dep. of Microbiology and Molecular Genetics, University of California Irvine
"Guts, germs and steel: microbes and metal in the inflamed gut"

$16^{35} – 17^{35}$ Selected Abstracts

C. Josenhans, Institute for Medical Microbiology and Hospital Epidemiology, Hannover Medical School
"Novel flagellin-like protein FlaC in unsheathed intestinal Helicobacter and Campylobacter species modulates the immune response and microbiota"

A. Suwandi, Institute for Experimental Medicine, Christian-Albrechts-University, Kiel; Hannover Medical School
"Intestinal Expression of the blood group gene b4galnt2 influences susceptibility to intestinal inflammation"

S. Wirtz, Medical Department 1, Friedrich-Alexander University Erlangen Nuremberg
"T6SS-mediated shaping of intestinal bacterial communities confers effective Citrobacter rodentium infection"

$17^{35} – 18^{10}$ **7th Keynote Lecture: Eric Pamer**, Immunology, Memorial Sloan Kettering Cancer Center, New York
"Microbiota-mediated defense against intestinal infections"

$18^{20} – 19^{15}$ Dinner

$19^{15} – 19^{45}$ SPP Member Meeting

$19^{45} –$ open   Poster Slam with Beer and Wine

PROGRAM Sunday, July 12

SESSION 4: MICROBIOME ANALYSIS

Chair: **Dirk Haller**, Nutrition and Immunology, Technische Universität München

$08^{30} – 09^{15}$ **SPP Consortium:**
John Baines, Evolutionary Genomics, Max-Planck-Institute for Evolutionary Biology, Plön
André Gessner, Institut für Med. Mikrobiologie und Hygiene, Universität Regensburg
"Variability and standardization of 16S-based analysis and mouse husbandry"

$09^{15} – 09^{55}$ Selected Abstracts

T. Clavel, ZIEL-Institute for Food and Health, Technische Universität München
"imNGS – the integrated microbial next generation sequencing platform for global assessment of 16S diversity in microbial ecology"

M. Beutler, Max-von-Pettenkofer Institut, LMU München
"Using a gnotobiotic mouse model to investigate the mechanisms of Salmonella-microbiota interaction in inflammation-induced pathogen blooms"

$09^{55} – 10^{20}$ Coffee Break

PROGRAM Sunday, July 12

Chair: **Thomas Clavel**, ZIEL-Institute for Food and Health, Technische Universität München

$10^{20} – 10^{55}$ **8th Keynote Lecture: Jens Walter**, Nutrition, Microbes and Gastrointestinal Health, University of Alberta
"Therapeutic modulation of the gut microbiota – an ecological perspective"

$10^{55}– 11^{55}$ Selected Abstracts

F. Peirera, Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, Faculty of Life Sciences, University of Vienna
"Tracking heavy water incorporation to identify and sort active cells of the gut microbiota"

A. Walker, Research Unit Analytical BioGeoChemistry, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg
"Bacteroidales species produce novel sulfonolipids in the murine gastrointestinal tract"

S. Haange, UFZ-Helmholtz Centre for Environmental Research, Department of Proteomics, Leipzig
"Revealing the response of the active mucosal microbiota to a change in diet: an experimental study in rats"

$11^{55}– 12^{30}$ Poster Award

12^{30} Lunch and Departure

PROGRAM
Friday, July 10

MICROBES AND CHRONIC INFLAMMATION

14^{15} – 14^{50} **1st Keynote Lecture**: **Herbert Tilg**, Universitätsklinik für Innere Medizin I, Innsbruck
"NAFLD and microbiota"

14^{50}– 15^{50} Selected Abstracts

K. Koller, Mikrobiologisches Institut - Klinische Mikrobiologie, Immunologie und Hygiene, Universitätsklinikum Erlangen, Friedrich-Alexander Universität Erlangen-Nürnberg
"CD101 inhibits intestinal inflammation"

M. Hefele, Medical Clinic 1, Friedrich Alexander University, Erlangen
"The role of epithelial caspase-8 and the microflora during infection with Salmonella typhimurium"

S. Menz, Institute of Medical Microbiology & Hygiene, University of Tuebingen and German Center of Infection Research (DZIF)
"FliC of E. coli Nissle protects specific against DSS- induced colitis by activation of CD11c^{+} intestinal lamina propria cells"

NAFLD AND MICROBIOTA

Herbert Tilg

Department of Internal Medicine I and Christian Doppler Research Laboratory for Gut Inflammation, Medical University Innsbruck, Innsbruck, Austria, Phone: +43 512 504 23539, Fax: +43 512 504 23538; E-mail: herbert.tilg@i-med.ac.at

Non-alcoholic fatty liver disease (NAFLD) has emerged as a major health problem throughout the world. Whereas overnutrition and obesity are obviously involved in the development of a simple fatty liver, it remains unclear why approximately 10-20% of all affected individuals develop non-alcoholic steatohepatitis (NASH). An association between the microbiota and development of obesity and its metabolic consequences including NAFLD is becoming increasingly recognized. Most data so far with respect to microbiota and obesity/NAFLD have focused on animal studies. Independent of obesity, insulin sensitivity could be directed by enhanced gastrointestinal permeability, allowing bacterial products to enter circulation. Metabolic infection of various distal tissues such as adipose tissue and liver may occur suggesting that indeed the constituents of the microbiota may evolve as major pathogenetic forces in NAFLD/NASH. First clinical studies are suggesting that microbiotal factors are driving forces of hepatic steatosis and inflammation involving certain toll-like receptors and pro-inflammatory cytokines such as tumor necrosis factor-alpha. Despite the attraction of such a concept and the overwhelming evidence from preclinical models about the potential relevance of the microbiota in NAFLD/NASH many questions remain open. Human data into this direction are still in its infancy and clinical research has to speed up to fill this gap generated by exciting basic science findings in the last years.

CD101 INHIBITS INTESTINAL INFLAMMATION

M. Purtak[1], J. Baier[1], K. Koller[1], R. Schey[1], P. Seeberger[2], R. Atreya[3], J. Mattner[1]

[1]Mikrobiologisches Institut - Klinische Mikrobiologie, Immunologie und Hygiene, Universitäts-klinikum Erlangen and Friedrich-Alexander Universität Erlangen-Nürnberg, 91054 Erlangen

[2]Max Planck Institute of Colloids and Interfaces, 14424 Potsdam, Germany and Freie Universität Berlin, 14195 Berlin

[3]Department of Medicine, Universitätsklinikum Erlangen and Friedrich-Alexander Universität (FAU) Erlangen-Nürnberg, D-91054 Erlangen.

CD101 exerts negative-costimulatory effects *in vitro*, but its function *in vivo* remains poorly defined. As CD101 is abundantly expressed in the gut, we assessed its impact on the course of inflammatory bowel disease (IBD). CD101 delayed the induction of colitis after an adoptive T cell transfer in lymphopenic recipient mice and CD101-expression inversely correlated with the severity of disease in IBD patients.

To elucidate the mechanisms regulating the function of CD101, we first compared the distribution of CD101-expression in naïve animals to dextran sodium sulfate (DSS)-and/or antibiotics-treated mice, partially reconstituted with distinct bacterial species. Predominantly enterobacteriaceae suppressed the expression of CD101 on myeloid cells, independently of inflammation. In contrast, bacteria-driven inflammation and/or bacterial antigens were required for the acquisition of CD101-expression on T cells. CD101-deletion, particularly on macrophages and granulocytes in DSS-treated or *Salmonella*-infected LysM Cre x CD101$^{fl/fl}$ mice enhanced the serum-reactivity towards bacterial oligosaccharides and the translocation of intestinal bacteria into mesenteric organs. Accordingly, a reduced CD101-expression on myeloid cells in intestinal tissue biopsies from IBD patients was associated with enhanced humoral immune responses to bacterial oligosaccharides in the sera.

CD101-expression was pivotal for the release of IL-10 and of metallomatrix proteases, particularly of MMP-9 and inhibited an infection of myeloid and intestinal epithelial cells with bacteria. Accordingly, *Salmonella*-infected CD101$^{+/+}$ animals cleared the infection faster than CD101$^{-/-}$ littermates and a reduced accumulation of enterobacteriaceae was associated with milder colitis in DSS-treated CD101$^{+/+}$ mice, as assessed by whole genome sequencing and conventional plating assays. As infected cells were predominantly CD101-negative, bacteria may disable CD101 to induce MMP-9-and IL-10-production. Thus, we concluded that CD101 maintains the integrity of the intestinal epithelial layer by controlling MMP-activation and IL-10-expression. Whether certain bacterial species (that might be missing in IBD patients) promote the expression of CD101, MMP-9 and IL-10 is part of our ongoing analyses.

THE ROLE OF EPITHELIAL CASPASE-8 AND THE MICROFLORA DURING INFECTION WITH *SALMONELLA TYPHIMURIUM*

M. Hefele[1], M.F. Neurath[1], S.Wirtz[1], C.Becker[1] C.Günther[1]

[1]Medical Clinic 1, Friedrich Alexander University, Erlangen, Germany

Mice with an intestinal epithelial cell specific deletion of the central apoptosis regulator caspase-8 (Casp8$^{\Delta IEC}$ mice) spontaneously develop a terminal ileitis which is driven by a Paneth cell depletion resulting in a diminished expression of AMP.

In patients with inflammatory bowel diseases, the pathogenesis of the diseases was associated with Paneth cell dysfunction and dysbiosis of the bacterial composition. The similar clinic picture between Crohn´s disease patients and Casp8$^{\Delta IEC}$ mice leads to the assumption, that these animals also harbour an altered composition of bacteria, which could promote inflammation. 16S RNA analysis of Casp8$^{\Delta IEC}$ mice showed an altered microbial composition including an increased amount of bacteria of the *Prevotella* genus compared to wild type mice.

To investigate, if the altered microbial composition of Casp8$^{\Delta IEC}$ mice drives intestinal inflammation, we performed faecal microbiota transplantation (FMT) experiments in which we reconstituted germ free wild type mice, with Casp8$^{\Delta IEC}$ and control microflora. Mice treated with Casp8$^{\Delta IEC}$ microflora showed a more elevated level of proinflammatory markers and antimicrobial peptides in the colon than mice that had received control microflora. In line with that, we identified that colonic lamina propria cells isolated from mice reconstituted with the Casp8$^{\Delta IEC}$ flora showed an increased inflammatory phenotype compared to control flora.

In order to investigate the role of caspase-8 during infectious colitis, we infected Casp8$^{\Delta IEC}$ and control animals with *Salmonella typhimurium*. Interestingly, Casp8$^{\Delta IEC}$ mice displayed severe weight loss, high lethality and severe tissue damage after infection compared to wild type mice. Barrier brake down caused by excessive cell death results in a systemic spread of *Salmonella typhimurium* and commensal bacteria, which finally results in a septic shock.

Since commensal bacteria are important in the defence against pathogens, we further investigated the contribution of the altered microbial composition in Casp8$^{\Delta IEC}$ mice. Therefore we depleted the microflora of C57BL/6 mice by antibiotic treatment, performed FMT with Casp8$^{\Delta IEC}$ and control microflora and infected the animals with *Salmonella*. Interestingly, mice reconstituted with caspase-8 flora, showed an increased susceptibility towards *Salmonella* infection.

In conclusion our results demonstrate an essential role for caspase-8 in maintaining the gut barrier in response to mucosal pathogens. Moreover we identified that tightly controlled regulation of the cell death machinery in the intestinal epithelium is critical for controlling the microbial composition.

FLIC OF *E. COLI* NISSLE PROTECTS SPECIFIC AGAINST DSS- INDUCED COLITIS BY ACTIVATION OF CD11C^{+} INTESTINAL LAMINA PROPRIA CELLS

S. Menz [1,6], K. Gronbach [1,6], A.- G. Korkmaz [1,6], A. Bender [1,6], S. Wagner [1,6], Mehari Tesfazgi-Mebrhatu [1,6], T. Ölschläger [2], A. Wieser [3,7], S. Schubert [3,7], D. Vöhringer [4], L. Alexopolou[5], I.B. Autenrieth [1,6], J.-S. Frick [1,6]

[1]*Institute of Medical Microbiology & Hygiene, University of Tuebingen, Elfriede- Aulhorn-Str. 6, 72076 Tübingen, sarah.menz@med.uni-tuebingen.de*
[2] *Institute of Molecular Infection Biology, University of Wuerzburg*
[3]*Max von Pettenkofer Institute, University of Munich*
[4]*Department of Infection Biology, University Hospital Erlangen*
[5]*Centre d'Immunologie de Marseille-Luminy, Marseille Cedex, France; [6]German Center of Infection Research (DZIF)*

Microbe-associated molecular patterns (MAMPs) induce host innate intestinal immunity resulting in inflammation or homeostasis. We showed that the flagella of *E. coli* Nissle (FliC[EcN]) protect mice against DSS- induced colitis. This effect was EcN specific as WT mice fed with the flagella of different *Salmonella* and *E. coli* strains were not protected against DSS induced colitis. Additionally FliC exchange mutants confirmed the protective role of FliC[EcN]. To identify the target cell addressed by EcN or the EcN flagella respectively we used bone marrow chimeric mice. WT or TLR5^{-/-} mice were irradiated and transplanted with bone marrow from WT or TLR5^{-/-} mice (TLR5^{-/-} => WT; WT => TLR5^{-/-}). Administration of EcN or EcNΔfliC, a flagella deficient mutant of EcN, and treatment with 3.5% DSS revealed that TLR5 expression on myeloid cells is essential for the EcN mediated protective anti-inflammatory effect. To identify the myeloid cell type facilitating the protection we irradiated WT mice and transplanted TLR5^{-/-} and ΔDC bone marrow (TLR5^{-/-} + ΔDC =>WT). Feeding of EcN did not longer protect from colitis, thus revealing that CD11c^{+} TLR5^{+} cells are crucial for the EcN mediated protective effect against DSS- induced colitis.

MICROBES AND CHRONIC INFLAMMATION

16^{30}-17^{05} **2nd Keynote Lecture: Hans-Christian Reinecker**, Genetics, Genomics and Molecular Biology Care, Harvard Medical School, Boston
"Control of mucosal immunity by microbial nucleic acid sensing"

17^{05}– 18^{05} Selected Abstracts

M. Schaubeck, Chair of Nutrition and Immunology, Technische Universität München
"Dysbiotic gut microbiota causes transmissive Crohn´s disease-like ileitis"

F. Schmidt, Charité - Universitätsmedizin Berlin, Medizinische Klinik m.S. Gastroenterologie, Infektiologie, Rheumatologie, Berlin
"Impact of microbiota on immune cell composition and barrier function in the gut"

F. Rost, Division of Gastroenterology and Hepatology, University Hospital Zurich
"Impact of Nlrp3 x gut microbiota interactions on experimental colitis and insulin sensitivity"

CONTROL OF MUCOSAL IMMUNITY BY MICROBIAL NUCLEIC ACID SENSING

Hans-Christian Reinecker

Associate Professor of Medicine, Harvard Medical School, Director, Genetics, Genomics and Molecular Biology Core, Center for the Study of Inflammatory Bowel Diseases.

The innate immune response in the intestine plays a critical role in the maintenance of intestinal homeostasis, protection from microbial damage and mucosal repair mechanisms that are disrupted in inflammatory bowel disease (IBD). Innate immunity relies on microbial pattern recognition receptors that bind a variety of microbial components. These pathogen-associated molecular patterns include RNA and DNA derived from the human intestinal microbiome as well as viruses that replicate in eukaryotic cells, plants, fungi or bacteria. Nucleic acids derived from the microbiota can be recognized by receptors in the cytoplasm or endosome of macrophages and dendritic cells. Nucleic acid binding to corresponding receptors subsequently activates signaling cascades leading to secretion of type I interferons and pro-inflammatory cytokines. Microbial nucleic acids can be sensed by teh endosomal toll-like receptors TLR7 and TLR9, by cytosolic RIG-I-like receptors that function as RNA sensors and DNA sensors that include DDX41, AIM2, IFI16 and cGAS. TLR7 and TLR9 detect bacterial single stranded RNA and unmethylated CpG-DNA, and recruit the common adaptor molecule MyD88 to activate signaling cascades that induce NF-κB dependent transcriptional programs. In contrast, intracellular recognition of RNA and DNA leads to interferon response factor 3 dependent induction of type I interferon driven host defenses that dependent on either mitochondria-localized antiviral protein MAVS (also known as VISA, IPS1 and CARDIF) or STING (stimulator of interferon genes, also known as TMEM173 MITA, MPYS and ERIS). Our work identified the guanine nucleotide exchange factor H1 (GEF-H1) encoded by the arhgef2 gene as the central component of host defense activation by viral and bacterial pathogens in both the MAVS and STING pathways of nucleic acid sensing. GEF-H1 is required for the STING dependent recognition of cyclic diguanylate monophosphate that plays critical regulatory roles in multiple species of bacteria that can be released into the cytosol of host cells during microbial infections. The generation of GEF-H1 deficient mice makes it now possible to determine how nucleic acid recognition controls intestinal innate and adaptive antiviral and antibacterial host defenses. We demonstrate that continuous recognition of commensal and pathogenic microbiota in the intestine includes innate immune activation through microbial nucleic acid signaling. Understanding the fundamental mechanisms of innate immune sensing of nucleic acids will guide strategies to target transcriptional programs that play a pivotal role in pathways that are linked to aberrant innate immune activation seen in IBD.

DYSBIOTIC GUT MICROBIOTA CAUSES TRANSMISSIVE CROHNS´S DISEASE-LIKE ILEITIS

Monika Schaubeck[1], Thomas Clavel[2], Jelena Calasan[1], Ilias Lagkouvardos[2], Ludovica Butto[1], Amira Metwaly[1], Sven Bastiaan Haange[3], Nico Jehmlich[3], Marijana Basic[6], Aline Dupont[7,8], Alesia Walker[9], Mathias Hornef[7,8], Martin von Bergen[3,4,5], André Bleich[8], Dirk Haller[1,2]

1Chair of Nutrition and Immunology, Technische Universität München, Freising-Weihenstephan, Germany; 2 ZIEL-Institute for Food and Health, Technische Universität München, Freising-Weihenstephan, Germany; 3Department of Proteomics, Helmholtz-Centre for Environmental Research—UFZ, Leipzig, Germany; 4UFZ, Department of Metabolomics, Helmholtz-Centre for Environmental Research, Leipzig, Germany; 5Department of Biotechnology, Chemistry and Environmental Engineering, University of Aalborg, Aalborg, Denmark; 6Institut for Medical Microbiology, RWTH University, Aachen, Germany; 7Institute of Medical Microbiology and Hospital Epidemiology, Hannover Medical School, Hannover, Germany; 8Institute for Laboratory Animal Science, Hannover Medical School, Hannover, Germany; 9Helmholtz-Centre for Health and Environment, Neuherberg, Germany

Dysbiosis of the intestinal microbiota is associated with Crohn´s disease (CD). Functional evidence for a causal role of bacteria in the development of small intestinal inflammation is lacking. Similar to human pathology, TNFdeltaARE/+ (ARE) mice develop a CD-like transmural inflammation with predominant ileal involvement.

GF-ARE mice were completely free of intestinal inflammation and antibiotic treatment of CONV-ARE mice induced remission of ileitis but not colitis, demonstrating that disease severity and location are microbiota dependent. Usage of different antibiotics showed beside shifts in the microbial community also changes in the metabolite profile and interesting associations of intestinal metabolites to some bacterial taxa were observed. In contrast to CONV housing, SPF-ARE mice were free of colitis but developed different ileitis phenotypes along with loss of Paneth cell function. 16S rRNA gene sequencing and LC-MS metaproteomic-analysis identified compositional and functional divergence of gut bacterial communities associated with disease phenotypes. Transfer of microbiota from SPF-ARE into GF-ARE recipient mice caused CD-like inflammation only with dysbiotic communities from inflamed SPF donors, while recipients of non-inflamed ARE microbiota showed no signs of intestinal inflammation. Neither microbiota induced inflammation in WT mice. Loss of Paneth cell function was associated with reduction of antimicrobial peptides, showing a concomitant but not preceding failure of Paneth cell function in ileitis development. The transfer of a functionally dysbiotic microbiota from inflamed AREs induced inflammation in GF mice, supporting a causal role of bacteria for CD-like pathologies in the susceptible host.

The monocolonization of GF-ARE mice with CD related strains or selected consortia, as well as humanization of mice with microbiota from CD patients during inflammation and remission are being performed. First results show the necessity of a complex, dysbiotic microbial structure for ileitis development. By using different mouse-models of intestinal inflammation we want to give insight into the characteristics of a dysbiotic microbiota and functional mechanisms which are involved in immune-activation and inflammation development.

IMPACT OF MICROBIOTA ON IMMUNE CELL COMPOSITION AND BARRIER FUNCTION IN THE GUT

Franziska Schmidt[1], Tassilo Kruis[1], Christiane Ring[2], Katja Dahlke[2], Gunnar Loh[2], Michael Schumann[1], Anja A. Kühl[1], Michael Blaut[2], Arvind Batra[1], Britta Siegmund[1]

[1]*Charité - Universitätsmedizin Berlin, Medizinische Klinik m.S. Gastroenterologie, Infektiologie, Rheumatologie, Berlin, Germany;* [2]*Deutsches Institut für Ernährungsforschung Potsdam-Rehbrücke, Abteilung für Gastrointestinale Mikrobiologie, Nuthetal, Germany*

Introduction: A striking increase in the incidence of inflammatory bowel diseases (IBD) has been observed since the Second World War possibly attributed to environmental changes. Hence the effect of defined bacteria early in live is detrimental for the development of the intestinal immune cell compartment, barrier function and the related susceptibility to intestinal inflammation.

Methods: To evaluate the impact of the presence or absence of bacteria early in life the immune cell phenotype in the lamina propria of germfree (GF), specific pathogen free (SPF) and GF-mice colonized with SPF-microbiota (COL) at the age of 5 weeks was analyzed at week 9 or 10. Immune cells were characterized by flow cytometry and immunohistology. Colitis was induced by dextran sodium sulfate (DSS). Barrier function of the colon was determined by electrophysiology. Local cytokine production was assessed in colon culture supernatants. To further determine the impact of bacteria on monocyte or macrophage phenotype, human monocytes isolated from the peripheral blood were subjected to *in vitro* polarization to macrophages. Monocytes or macrophages were stimulated with bacterial cells or supernatants and production of cytokines and surface marker expression were assessed by flow cytometry.

Results: Remarkably, in GF-mice the total colonic epithelial resistance was decreased accompanied by increased ^{3}H-mannitol- and decreased HRP-flux indicating a barrier dysfunction. Furthermore the subset of pro-inflammatory CD11c$^+$ macrophages was increased whereas the T-cells were decreased in the terminal ileum of these mice. Exposure to DSS resulted in colitis in SPF- as well as COL-mice, whereas GF-mice died following exposure. However the inflammation score and the expression of MCP-1 and IL-6 in the colon were higher in SPF- than in COL-mice suggesting a more potent defense in the SPF-group. In line, defined bacteria supernatants were able to modulate the phenotype of monocytes as well as the phenotype of descendant macrophages if present in the course of their maturation. In contrast, polarized macrophages were not affected by bacteria. Conclusion: The presence of microbiota is crucial for the development of the intestinal integrity. Defined bacteria can modify monocyte function and macrophage maturation and might hence serve as therapeutic option.

Impact of *Nlrp3* x Gut Microbiota Interactions on Experimental Colitis and Insulin Sensitivity

F. Rost[1], P. Pavlov[1], S. Wueest[2], K. Atrott[1], G. Rogler[1,2], D. Konrad[2,3], I. Frey-Wagner[1,2]

[1]*Division of Gastroenterology and Hepatology, University Hospital Zurich, Zurich, Switzerland*
[2]*Division of Endocrinology and Diabetology, University Children's Hospital Zurich, Zurich, Switzerland*
[3]*Zurich Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland*

Background: NOD-like receptor pyrin domain containing 3 (*Nlrp3*) genotype has been shown to affect experimental colitis and glucose metabolism. We here investigate whether Nlrp3 genotype-induced changes in gut microbiota composition are involved in the observed effects.

Methods: Female *Nlrp3*$^{-/-}$ and wild-type (WT) littermates underwent dextran sodium sulfate (DSS)-induced acute (7 days 1% DSS) or chronic colitis (4 x 7 days 1% DSS). Colitis severity was evaluated by body weight, colonoscopy, mRNA expression of pro-inflammatory cytokines and histology. Male Nlrp3$^{-/-}$ and WT littermates received a high fat diet (HFD) or normal chow for 6 weeks and glucose tolerance was assessed. Gut microbiota composition was studied by 16S rRNA amplicon sequencing before and after induction of colitis / HFD. Additionally, samples for the Swiss IBD cohort (SIBDC) will be analyzed to compare and extend the relevance of our findings to the human gut microbiome x genotype interactions.

Results: *Nlrp3*$^{-/-}$ mice showed a more severe phenotype in acute and chronic DSS colitis. HFD-fed *Nlrp3*$^{-/-}$ mice showed improved glucose tolerance compared to WT littermates. Notably, *Nlrp3*$^{-/-}$ and corresponding WT littermates differed strongly in their gut microbiota composition under basal conditions as well as after induction of colitis / HFD.

Conclusions: Gut microbiota composition, experimental colitis and glucose tolerance are affected by the Nlrp3$^{-/-}$ genotype. Further experiments with microbiota transfer as well as colitis models / HFD in animals with a limited defined flora will address a causal relationship between gut microbiota composition and colitis / glucose tolerance outcome.

MICROBES AND CHRONIC INFLAMMATION

19^{45}-20^{20} **3rd Keynote Lecture: Nadine Cerf-Bensussan**, Intestinal Immunity, Institute Imagine and Université Paris Descartès-Sorbonne
"Segmented filamentous bacterium: an unusual symbiont that instructs the development of the gut barrier"

20^{20}– 21^{20} Selected Abstracts

S. Thiemann, Microbial Immune Regulation, Helmholtz Centre for Infection Research, Braunschweig
"Isogenic and isobiotic mouse lines reveal microbiota-mediated indirect colonization resistance against Salmonella infection"

S. Stebe, Department of Hepatology, Gastroenterology and Infectiology, University Hospital, Tuebingen
"HDAC related epigenetic control and the antimicrobial barrier in inflammatory bowel diseases"

S. Suerbaum, Institute of Medical Microbiology and Hospital Epidemiology, Hannover Medical School
"Mutual interactions between the pathobiont Helicobacter hepaticus and the mouse intestinal microbiota: ecology, mechanisms and relevance to the induction of IBD"

SEGMENTED FILAMENTOUS BACTERIUM: AN UNUSUAL SYMBIONT THAT INSTRUCTS THE DEVELOPMENT OF THE GUT BARRIER

Nadine Cerf-Bensussan

Laboratory of Intestinal Immunity. Institut Imagine and Université Paris-Descartes-Sorbonne Paris Cité

To cope with the complex microbial community that settles in the distal part of our intestine after birth, hosts have evolved a spectrum of complementary innate and adaptive immune mechanisms. This highly dynamic barrier is programmed ante-natally but fully develops only after birth in response to signals from the microbiota. Studies using gnotobiotic mice unexpectedly revealed that a restricted number of unculturable and host-specific bacterial species, the prototype of which is Segmented Filamentous bacterium (SFB), are necessary for the complete maturation of the gut immune barrier. In contrast to other members of the microbiota, which live in the intestinal lumen entrapped within the mucus, this unusual symbiont develops host-specific adherence to the ileal mucosa. This property likely enables SFB to deliver signals which instruct the development of gut innate immune responses, stimulate the development of gut-associated lymphoid tissues and the activation of intestinal IgA and T cell adaptive immune responses. SFB is notably indispensable to develop homeostatic TH17 responses, either directed against SFB antigens but also likely against other antigens. SFB may the promote the development of immune responses against other members of the microbiota and, accordingly, the development of an efficient gut barrier. Our recent work has allowed to design conditions for *in vitro* culture or SFB that offer new perspectives to analyse SFB interactions with the host epithelium. More work is however needed to define whether the data obtained in mice can be translated to humans in whom the presence of a human SFB homologue is likely, at least at the level of 16SRNA.

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* Co-last authors

ISOGENIC AND ISOBIOTIC MOUSE LINES REVEAL MICROBIOTA-MEDIATED INDIRECT COLONIZATION RESISTANCE AGAINST *SALMONELLA* INFECTION

S. Thiemann[1], E. Galvez[1], T. Strowig[1]

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

The intestinal microbiota protects the host against invading enteropathogens by a process called colonization resistance: directly by producing inhibitory substances and competing for nutrients or indirectly, by priming protective immune responses. The highly variable composition of the microbiota in humans may contribute to varying susceptibilities to infections. Until now, little is known about the contributions of individual members of the intestinal microbiota to confer colonization resistance.

To understand how different intestinal microbial communities can influence the susceptibility to enteropathogens, we compared isogenic mouse lines with distinct microbiota compositions, which were challenged with *Salmonella* Typhimurium. Using the streptomycin model of *S.* Typhimurium infection, we identified microbial signatures, which correlate with a decreased disease severity characterized by reduced weight loss and bacterial organ burden. Transfer of bacteria from different microbiota settings into germfree mice was sufficient to reproduce the phenotype demonstrating that the protective effect was microbiota-dependent. Moreover, we could show through anaerobic culture techniques that bacteria conferring resistance are cultivable. We are now pursuing the isolation of protective bacteria species.

The amount of *S.* Typhimurium in the fecal content did not differ in the investigated microbiota settings at different time points indicating that the phenotype is likely not mediated by direct colonization resistance. Expression analysis during infection revealed a distinct cytokine expression profile suggesting different regulation of antibacterial immunity. By using isobiotic mice deficient in the adaptive immune system, we could show that B cells and T cells are dispensable. The influence of different arms of the innate immune system is currently under investigation.

Identifying the basic principles of immune regulation by individual members of the gut microbiota may allow harnessing their abilities to develop improved therapies for mucosal infections.

HDAC RELATED EPIGENETIC CONTROL AND THE ANTIMICROBIAL BARRIER IN INFLAMMATORY BOWEL DISEASES

S Stebe[1,2], MJ Ostaff[2], EF Stange[3], J Wehkamp[1]

[1]Department of Hepatology, Gastroenterology and Infectiology, University Hospital, Tuebingen, Germany; [2]Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart and University of Tübingen, Germany. [3] Department of Gastroenterology, Robert Bosch Hospital, Stuttgart, Germany

In inflammatory bowel diseases (IBD) the antimicrobial epithelial immunity is impaired. Colonic Crohn's Disease is, for instance, associated with a diminished upregulation of the inducible antimicrobial peptide human beta-defensin 2 (HBD2). HBD2 is normally highly upregulated during epithelial inflammation. Recently, a link between the function of the epigenetically active histone deacetylases (HDAC) and the control of beta-defensin expression has been reported. We therefore aimed at determining the expression profiles of class I HDACs in the intestinal epithelium of patients with IBD and to study their potential role in the transcriptional regulation of HBD2. Firstly, mRNA expression levels of HDACs 1, 2, 3, and 8 were measured in ileal and colonic tissue of Crohn's Disease (CD) and ulcerative colitis (UC) patients, and healthy controls. To evaluate the influence of HDACs on HBD2 regulation, colonic epithelial CaCo2 cells were stimulated by inflammatory mediators or bacteria while HDACs were inhibited by different compounds. HBD2 mRNA expression was subsequently analyzed. Furthermore, in a more *in vivo* like approach, we also stimulated freshly obtained colonic biopsies with probiotic E.*coli* Nissle, with or without HDAC inhibition. We found significantly lower HDAC 2 levels in ileal and colonic CD and in colonic tissue of UC patients. HDAC 1 was lower in the colon of CD and UC patients alike, while the latter also showed reduced HDAC 3 and 8. *In vitro* we found paramount enhancement of HBD2 induction when HDAC function was inhibited. In *ex vivo* treated biopsies we could show that while E.*coli* Nissle is indeed capable of inducing HBD2 in cultured tissue, however additional HDAC inhibition prevented this upregulation. Our work shows that class I HDACs are differentially expressed in intestinal tissue of IBD patients. They seem to play an important role in controlling HBD2 inducibility and thereby in intestinal antimicrobial defense. However, the influence of HDACs appears to be different in normal human biopsies versus cell lines.

Mutual interactions between the pathobiont *Helicobacter hepaticus* and the mouse intestinal microbiota: Ecology, mechanisms and relevance to the induction of IBD

S. Suerbaum[1,2], I. Yang[1,2], F. Kops[1,2], B. Brenneke[1,2], S. Woltemate[1,2], Z. Fiebig[1,2], A. Bleich[3], A. D. Gruber[4], J. G. Fox[5], C. Josenhans[1,2]

[1]*Institute of Medical Microbiology and Hospital Epidemiology, Hannover Medical School, Hannover Germany*
[2]*German Center for Infection Research, Hannover-Braunschweig Site, Hannover, Germany*
[3]*Institute of Laboratory Animal Science, Hannover Medical School, Hannover, Germany*
[4]*Institute of Veterinary Pathology, Free University Berlin, Berlin, Germany*
[5]*Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA, USA*

The prototype pathobiont, *Helicobacter hepaticus*, induces inflammatory bowel disease in susceptible immunocompromised mouse strains, and *H. hepaticus* infection has become a widely used model to investigate bacterial factors involved in IBD pathogenesis. Available data indicate that the pathology induced by *H. hepaticus* infection depends on both specific pathogenic mechanisms of the bacterium as well as the composition of the intestinal microbiota. We previously showed that C57BL/6 IL-10 ko mice reared at two different institutions (MHH and MIT) display very different susceptibilities to IBD after *H. hepaticus* infection. Microbiota analyses of these mice by deep sequencing of 16S rDNA amplicons identified multiple culturable and non-culturable bacterial species that are only present in one group of mice and therefore might be involved in augmenting or suppressing inflammation. Within the DFG priority programm SPP1656, we investigate the role of microbiota in determining the pathology associated with *H. hepaticus* infection. Germfree C57BL/6 IL-10 ko mice at MHH were cohoused with MIT mice and subsequently infected with *H. hepaticus* 3B1 to test the hypothesis that the differential susceptibility of MHH vs. MIT mice to *H. hepaticus*-induced pathology results from the different microbiota composition. Additional experiments are under way testing whether supplementation of MHH microbiota with individual components of MIT microbiota increases *H. hepaticus*-induced colitis. We also evaluate the influence of different diets used at MIT vs. MHH on microbiota composition and susceptibility to *H. hepaticus*-induced colitis.

PROGRAM
Saturday, July 11

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MICROBES AND GUT HOMEOSTASIS

$08^{30} – 09^{05}$ **4th Keynote Lecture: Elke Cario**, Experimental Gastroenterology, Universitätsklinikum Essen
"Intestinal efflux transport and multi-drug resistance in inflammation"

$09^{05} – 10^{05}$ Selected Abstracts

M. Hornef, Department of Microbiology, Veterinary University Hannover
"Early postnatal selection determines long-term microbiota composition"

M. Zaiss, École Polytechnique Fédérale de Lausanne (EPFL)
"Intestinal helminths modulate allergic inflammation indirectly by altering bacterial metabolism"

A. Woting, Department of Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal
"Why is Clostridium ramosum obesogenic? Hypotheses driven by metaproteomics and metabolomics"

INTESTINAL EFFLUX TRANSPORTERS AND MULTI-DRUG RESISTANCE IN INFLAMMATION

Elke Cario

Experimental Gastroenterology, Division of Gastroenterology and Hepatology, Universitätsklinikum Essen, Germany; email: elke.cario@uni-due.de

The gastrointestinal tract is constantly exposed to numerous xenobiotics which may be harmful to the body. Detoxification represents a key component of the intestinal mucosal barrier defense. Efflux transporters, such as ABCB1/MDR1 – encoded p-glycoprotein (p-gp), protect against xenobiotic accumulation and resulting toxicity. Emerging evidence suggests that p-gp interacts with the mucosal innate immune system and may thus be involved in the pathogenesis of gastrointestinal disorders (1, 2).

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Early postnatal selection determines long-term microbiota composition

Marcus Fulde, Felix Sommer, Michael Hensel, Marijana Basic, André Bleich, Frederik Bäckhed, <u>Mathias W. Hornef</u>

Department of Microbiology, Veterinary University Hannover; Hannover, Germany; Wallenberg Laboratories, Göteborg University, Göteborg, Sweden; Department of Microbiology, Osnabrück University, Osanbrück, Germany; Central Animal Laboratory, Hannover Medical School, Hannover, Germany, Institute of Microbiology, RWTH University Hospital, Aachen, Germany

Alterations of the enteric microbiota exert a major influence on the host's metabolism increasing the risk for metabolic diseases such as obesity and diabetes. Although exogenous modulators of the microbiota have been identified, endogenous mechanisms that drive the establishment of a beneficial microbiota have not been defined. Here we investigated the influence of the neonatal mucosal innate immune system on bacterial colonization. Epithelial expression of the flagellin receptor toll-like receptor (Tlr)5 was limited to the postnatal period. Tlr5 expression in the neonate restricted the colonization by flagellated bacteria via MyD88 signaling and expression of the c-type lectin RegIII?. As a consequence, flagellated bacteria were outcompeted by non-flagellated isogenic mutants. Also, bacteria of the frequently flagellated phylum Bacteroides were underrepresented in the microbiota. Although bacterial selection was observed in neonate but not adult animals, the modified microbiota persisted and prevented long-term changes in the glucose metabolism and body weight. Thus, consistent with a window of opportunity during infancy, early host-driven selection of the enteric microbiota influences long-term metabolic changes and the risk for metabolic disease.
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INTESTINAL HELMINTHS MODULATE ALLERGIC INFLAMMATION INDIRECTLY BY ALTERING BACTERIAL METABOLISM

Mario M. Zaiss[1], Alexis Rapin[1], Luc Lebon[1], Lalit Kumar Dubey[1], Ilaria Mosconi[1], Alessandra Piersigilli[1,2], Laure Menin[1], Alan W. Walker[3,4], Jacques Rougemont[5], Oonagh Paerewijck[6], Peter Geldhof[6], Andrew Macpherson[7], John Croese[8,9], Paul R. Giacomin[9], Alex Loukas[9], Tobias Junt[10], Benjamin J. Marsland[11], Nicola L. Harris[1*]

[1] *École Polytechnique Fédérale de Lausanne (EPFL), Switzerland*

[2] *University of Bern, Switzerland*

[3] *Wellcome Trust Sanger Institute, Hinxton, UK ;* [4] *University of Aberdeen, UK*

[5] *École Polytechnique Fédérale de Lausanne (EPFL) and Swiss Institute of Bioinformatics, Switzerland*

[6] *University of Ghent, Belgium*

[7] *University Hospital of Bern, Switzerland*

[8] *The Prince Charles Hospital, Brisbane, Australia*

[9] *James Cook University, Cairns, Australia*

[10] *Novartis Pharma AG, Basel, Switzerland*

[11] *Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland*

[*] *Corresponding author: Nicola L. Harris*

Intestinal helminths are potent regulators of their hosts immune system and can ameliorate inflammatory diseases such as allergic asthma. In the present study we assessed whether this anti-inflammaory activity was intrinsic to helminths, or whether it involves crosstalk with the local microbiota. We report that chronic infection with the murine helminth *Heligmosomoides polygyrus bakeri* (Hpb) alters the intestinal habitat allowing an enrichment of bacteria species belonging to the Bacteroidales and Clostridiales orders, and increasing short chain fatty acid (SCFA) production. Transfer of the Hpb modified microbiota alone was sufficient to mediate protection against allergic asthma and protection required the G protein - coupled receptor (GPR) -41 (also called free fatty acid receptor 3 or FFAR3). A similar alteration in the metabolic potential of intestinal bacterial communities was observed with diverse parasitic and host species also including humans, suggesting this represents an evolutionary conserved mechanism.

WHY IS *CLOSTRIDIUM RAMOSUM* OBESOGENIC? HYPOTHESES DRIVEN BY METAPROTEOMICS AND METABOLOMICS

A. Woting[1], G. Loh[1], S.B. Haange[2], N. Jehmlich[2], U. Rolle-Kampczyk[3], M. von Bergen[2,3], M. Blaut[1]

[1]Department of Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany
[2]Department of Proteomics, Helmholtz Centre for Environmental Research, Leipzig, Germany
[3]Department of Metabolomics, Helmholtz Centre for Environmental Research, Leipzig, Germany.

Background: *Clostridium ramosum* in the human intestine is associated with symptoms of metabolic disease. In gnotobiotic mice harbouring a simplified human intestinal microbiota the cell count of *C. ramosum* increases in response to a high fat diet (HFD). This proliferation is associated with increased body weight gain and body fat deposition in the mice. The absence of *C. ramosum* protects the gnotobiotic mice from severe symptoms of obesity.

Methods: To determine why *C. ramosum* proliferates under HFD feeding and how it promotes obesity development, we used mice monoassociated with this bacterium (Cra mice). The metaproteomes of *C. ramosum* and cecal mucosa as well as the plasma metabolome were compared between mice fed a low fat diet (LFD) and mice fed a HFD.

Results: Under HFD feeding, *C. ramosum* revealed higher protein levels in cell division and replication, which confirmed the observed proliferation of this bacterium under HFD conditions. Numerous bacterial proteins were only abundant in HFD-fed Cra mice, including leucyl aminopeptidase, dipeptide/oligopeptide ABC transporter permease, Hyaluronoglucosaminidase, *N*-acetylglucosamine-6-phosphate deacetylase, DHHA1 domain protein and chorismate mutase. The cecal mucosa metaproteome of HFD-fed Cra mice showed lower protein levels assigned to cell cycle control, cell division, cytoskeleton formation and cellular trafficking. Plasma concentrations of serotonin and iso-leucine were higher in Cra mice fed HFD than in Cra mice fed LFD.

Conclusion: HFD feeding might reduce metabolic flexibility of intestinal epithelial cells and thereby elevate their susceptibility to *C. ramosum*. Impaired epithelial cell function combined with the proliferation of *C. ramosum*, which produce obesogenic metabolites or virulence factors might promote metabolic disease in the host. Final conclusions can only be drawn when germfree mice have been analysed to distinguish between the effect of *C. ramosum* and that of the diet.

MICROBES AND GUT HOMEOSTASIS

$10^{30} - 11^{05}$ **5ᵗʰ Keynote Lecture: Alan Walker**, Microbiology group, Rowett Institute of Nutrition and Health, University of Aberdeen
"New insights into links between the gut microbiota and diarrhoeal pathogenicity in infants from low income countries"

$11^{05} - 12^{05}$ Selected Abstracts

A. Sünderhauf, Molecular Gastroenterology, Department of Medicine 1, University of Lübeck
"Mitochondrial ATP8 gene polymorphism alters the gut microbiota and confers to the risk of experimental colitis"

A. Legatzki, LMU Munich, University Children's Hospital
"Gut microbiota in the first year of life and association with atopic dermatitis"

D. Huson, Center for Bioinformatics, University of Tübingen
"New algorithms and software for microbiome analysis"

NEW INSIGHTS INTO LINKS BETWEEN THE GUT MICROBIOTA AND DIARRHOEAL PATHOGENICITY IN INFANTS FROM LOW INCOME COUNTRIES

Walker A.W., on behalf of the Global Enteric Multi-Center Study Consortium

Microbiology Group, Rowett Institute of Nutrition and health, University of Aberdeen, UK

Globally, there are nearly 1.7 billion cases of diarrhoeal disease every year, and it is the second most common cause of death in children under five years old. The impact of diarrhoeal disease is particularly severe in Africa and in Southeast Asia, where it also plays a major role in the development of infant malnutrition.

The intestinal microbiota plays a key role in preventing and suppressing disease caused by gastrointestinal pathogens. Indeed, pathogens such as *Salmonella* spp. and *Clostridium difficile* appear to have evolved distinct behavioural strategies in order to outcompete indigenous gut microbes, highlighting the tripartite nature of gastrointestinal infectious disease, involving the host, the pathogen and the microbiota. As such, there is a clear need to better understand the way in which alterations in the microbiota might impact disease outcome, in both beneficial and detrimental ways.

To begin to address these issues we carried out high-throughput bacterial DNA sequence analysis of faecal samples from around 1000 young children as part of the Bill and Melinda Gates Foundation-funded Global Enteric Multi-center Study (GEMS). This incorporated healthy control children as well as those suffering from moderate to severe diarrhoea (MSD) from three low-income countries in West and East Africa, and in Bangladesh.

Microbiota analysis confirmed that many known pathogens were associated with MSD, but also identified a number of novel, potentially detrimental agents such as *Streptococcus* and *Granulicatella* species. Diarrhoeal disease was commonly associated with distinct shifts in microbiota community structure, with reduced bacterial diversity and a greater dominance of facultative anaerobic lineages versus obligate anaerobes in diarrhoeal cases. The strongest anti-correlations were seen between *Prevotella* spp., which were dominant in healthy children, and *Escherichia coli*/*Shigella* spp., which were much more prominent in diarrhoeal disease. Microbiota diversity correlated with severity of disease, and when focusing specifically on *Shigella* spp./enteroinvasive *Escherichia coli* infection, sequence data suggested that four *Lactobacillus* taxa were associated with decreased disease risk.

Cumulatively, our data suggest therefore that the microbiota may have significant impacts on both risk of infants in low income countries developing diarrhoeal disease, and on subsequent severity and outcome of disease. This work provides a platform for detailed epidemiological and laboratory based studies, which may further elucidate the role of novel candidate pathogens and the impact that specific members of the microbiota may have in bolstering host resistance to infection.

Mail to: alan.walker@abdn.ac.uk   GEMS website: http://medschool.umaryland.edu/GEMS/

MITOCHONDRIAL ATP8 GENE POLYMORPHISM ALTERS THE GUT MICROBIOTA AND CONFERS TO THE RISK OF EXPERIMENTAL COLITIS

A. Sünderhauf[1], S. Jendrek[1], F. Bär[1], R. Pagel[1], S. Ibrahim[2], S. Derer[1], C. Sina[1]

[1]*Molecular Gastroenterology, Department of Medicine 1, University of Lübeck, Germany*
[2]*Lübeck Institute of Experimental Dermatology, University of Lübeck, Germany*

Mucosal ATP depletion as the consequence of reduced mitochondrial respiratory chain activity has been attributed to Ulcerative colitis (UC) pathogenesis more than 30 years ago. However, experimental evidence for this idea was missing for a long time due to the lack of appropriate animal models. Conplastic inbred mouse strains (CIS) now enable us to study the impact of mitochondrial gene polymorphisms on respiratory capacity in mice with an identical nuclear genetic background. Alterations of respiratory activity determine the susceptibility to experimental colitis. In this regards we already showed that CIS mice with increased mucosal ATP levels were protected against experimental colitis. However, the underlying mechanisms still remain elusive.

Conplastic B6-mt^{FVB} mice, carrying an aspartic acid to tyrosine substitution in the mitochondrial mt-Atp8 gene, were generated by backcrossing female FVB mice with C57.BL6/J male mice for more than 10 generations. *Ex vivo* testing of mitochondrial function was performed by Seahorse analysis. Intestines were evaluated histologically, and lamina propria mononuclear cells (LPMC) were characterized by flow cytometry. Microbiota analysis of faeces was performed by next generation sequencing using the MiSeq sequencing system. Experimental colitis was induced by cyclic administration of 2% (v/v) dextran sodium sulfate (DSS) to the drinking water and evaluated by disease activity index (DAI) and murine endoscopic index of colitis severity (MEICS) scores.

In B6-mt^{FVB} mice, reduced mucosal ATP levels and OXPHOS activity were associated with increased susceptibility to DSS colitis, characterized by elevated DAI, MEICS and histologic scores. The composition of intestinal epithelial cell and LPMC populations was similar between genotypes. However, the microbiota showed a clear shift in beta diversity for B6-mt^{FVB} mice as well as increased abundance of Firmicutes and Proteobacteria. In addition, preliminary data point to a reduced mucus layer thickness in B6-mt^{FVB} mice. We therefore hypothesize that mucus producing goblet cells are particularly sensitive to alterations in respiratory chain activity induced by mitochondrial gene polymorphisms. The resulting changes in mucus thickness and composition might be the reason for microbiota variations, together determining colitis susceptibility.

Gut microbiota in the first year of life and association with atopic dermatitis

M. Depner[1]*, P. V. Kirjavainen[2]*, A. Legatzki[1], M. J. Ege[1], K. M. Kalanetra[3], D. A. Mills[3], C. Braun-Fahrländer[4], J. Riedler[5], J. Genuneit[6], JC. Dalphin[7], J. Pekkanen[2,8], E. von Mutius[1], and the PASTURE Study Group

[1]LMU Munich, University Children's Hospital, Munich, Germany; [2]Department of Environmental Health, National Institute for Health and Welfare, Kuopio, Finland; Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland [3]Viticulture and Enology, University of California, Davis,USA; [4]Swiss Tropical and Public Health Institute, Basel, Switzerland; [5]Children and Young Adults' Medicine, Children's Hospital, Schwarzach, Austria; [6]The Institute of Epidemiology and Medical Biometry, Ulm University, Ulm, Germany; [7]The Department of Respiratory Disease, University Hospital, Université de Franche-Comté, Besancon, France; [8]Hjelt Institute, University of Helsinki, Helsinki, Finland
* these authors contributed equally

The incidence for atopic dermatitis (AD) is increasing worldwide. AD is a chronic inflammatory skin disease leading to skin barrier dysfunction as well as dysregulation of immune responses. Evidences exist that the early gut microbiota of children who later develop AD is modified regarding diversity and specific bacterial groups. The goal of this study was to investigate the bacterial community composition of the gut microbiota from infants at age 2 months and 1 year and to test whether specific associations of community patterns with AD exist. The V4 region of 16S rRNA genes of stool samples from infants of the PASTURE (Protection against Allergy–Study in Rural Environments) birth cohort (n_{month2} = 744; n_{year1} = 867) was analyzed by using the Illumina MiSEQ platform. Sequences were analyzed with QIIME and the R package phyloseq. *Bifidobacteriaceae* dominated the composition of the 2-months samples with 64%, followed by *Enterobacteriaceae* with 10%. In contrast, at 1 year the relative abundance of *Bifidobacteriaceae* decreased to 27%. The most common family was *Lachnospiroceae* with 39%. Associations of the relative family abundances with development of AD during the first 2 years of life were tested using logistic regression models. An inverse association of relative abundance of *Bifidobacteriaceae* in 2 months (Odds Ratio and 95% confidence interval for a 10% increase in relative abundance 0.53(0.28-1.01), p=0.056) but not in 1 year samples with AD was observed. These data suggest that an increased abundance of *Bifidobacteriaceae* in young infants could protect from AD development.

NEW ALGORITHMS AND SOFTWARE FOR MICROBIOME ANALYSIS

Daniel H. Huson

Center for Bioinformatics, University of Tübingen, Germany

Microbiome projects involve hundreds of samples, each containing many millions of DNA sequencing reads. Computational analysis of such datasets has become a major bottleneck. In this talk we present some recent advances that aim at significantly speeding up the primary analysis of microbiome shotgun sequencing data (DIAMOND, Buchfink et al, Nature Methods, 2015) and at simplifying the interactive exploration and analysis of microbiomes (MEGAN 6, Huson et al, in preparation).

MICROBES AND INFECTION

$16^{00} - 16^{35}$ **6th Keynote Lecture: Manuela Raffatellu**, Dep. of Microbiology and Molecular Genetics, University of California Irvine
"Guts, germs and steel: microbes and metal in the inflamed gut"

$16^{35} - 17^{35}$ Selected Abstracts

C. Josenhans, Institute for Medical Microbiology and Hospital Epidemiology, Hannover Medical School
"Novel flagellin-like protein FlaC in unsheathed intestinal Helicobacter and Campylobacter species modulates the immune response and microbiota"

A. Suwandi, Institute for Experimental Medicine, Christian-Albrechts-University, Kiel; Hannover Medical School
"Intestinal Expression of the blood group gene b4galnt2 influences susceptibility to intestinal inflammation"

S. Wirtz, Medical Department 1, Friedrich-Alexander University Erlangen Nuremberg
"T6SS-mediated shaping of intestinal bacterial communities confers effective Citrobacter rodentium infection"

$17^{35} - 18^{10}$ **7th Keynote Lecture: Eric Pamer**, Immunology, Memorial Sloan Kettering Cancer Center, New York
"Microbiota-mediated defense against intestinal infections"

GUT, GERMS AND STEEL: MICROBES AND METAL IN THE INFLAMED GUT

Manuela Raffatellu

Department of Microbiology and Molecular Genetics, University of California Irvine

Mucosal surfaces are often the first interface between the host, the commensal microbiota and pathogenic microorganisms. Among the most complex of these environments is the gut mucosa, where trillions of bacteria (the commensal microbiota) coexist with the host in a mutually beneficial equilibrium. Infection with enteric pathogens like *Salmonella* Typhimurium disrupts this equilibrium by causing intestinal inflammation, a response that suppresses the growth of the commensal microbiota and favors the growth of *S*. Typhimurium by several mechanisms. Infection with *S*. Typhimurium results in the upregulation of antimicrobial proteins that inhibit bacterial growth by limiting the availability of essential nutrients, including metal ions, in a process termed "nutritional immunity". My lab studies the mechanisms by which *S*. Typhimurium evades nutritional immunity and acquires metal ions in the inflamed gut, allowing this pathogen to successfully compete with the microbiota for these essential nutrients.

NOVEL FLAGELLIN-LIKE PROTEIN FLAC IN UNSHEATHED INTESTINAL *HELICOBACTER* AND *CAMPYLOBACTER* SPECIES MODULATES THE IMMUNE RESPONSE AND MICROBIOTA

Eugenia Leno[1], Eugenia Gripp[1], Sven Maurischat[2], Bernd Kaspers[3], Karsten Tedin[2], Sarah Menz[1], Sabrina Woltemate[1], Sebastian Suerbaum[1], Silke Rautenschlein[4], Christine Josenhans[1]

[1]*Institute for Medical Microbiology and Hospital Epidemiology, Hannover Medical School, Carl Neuberg Str. 1, 30625 Hannover, Germany;* [2]*Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany;* [3]*Institute for Animal Physiology, Ludwig Maximilians Universität Munich, Munich, Germany;* [4]*Clinics for Poultry, Veterinary Medical School Hannover, Hannover, Germany.*

Objectives: Bacterial microorganisms which colonize the intestinal tract have to deal with several unique characteristics specific for this habitat: a high density of resident microbiota of various species and an immunological environment primed by the resident microbiota. Little is known about how *Helicobacter* and *Campylobacter* ssp. interact with the innate immune systems of their hosts and with the major pattern recognition receptors (PRR) such as TLR and NLR receptors. *C. jejuni* and the closely related gastric pathogen *H. pylori* are restricted in their abilities to activate the innate immune system via TLR5 and also via TLR4.

Methods and Results: In addition to the classical flagellin molecules, we identified the unusual flagellin-like protein FlaC and potential orthologues to be conserved in nine different *Campylobacter*, three intestinal *Helicobacter* and one *Wolinella* species. FlaC is a secreted protein, not involved in motility. Its amino acid sequences appear to be chimeras with amino acid similarities to both, TLR5-stimulating and non-stimulating, flagellins. We hypothesized that FlaC might be involved in host immune modulation, either by TLR5 ligation or by other innate immune activation. For characterizing this hypothetical function, we exploited *Campylobacter* FlaC as a model. Coincubation experiments of highly purified FlaC with chicken and human cell lines were performed. FlaC was able to activate different cell-types, inducing the production of cytokine mRNA. Preincubation with FlaC reduced the responsiveness of chicken and human macrophages towards bacterial LPS. Additionally, FlaC was shown to directly interact with TLR5, and appeared to elicit an antibody response in chicken. Furthermore, a chicken infection experiment indicated that FlaC was able to modulate the intestinal microbiota of chicken and their immune response.

Conclusions: We conclude that a subgroup of intestinal pathogenic epsilonproteobacteria, including various *Helicobacter* and *Campylobacter* spp. which possess flagella without a sheath, have evolved the novel host-stimulatory chimeric flagellin-like molecule FlaC. FlaC specifically modulates host responses, particularly towards other bacterial PRR ligands. We propose that this protein acts predominantly as a homeostatic or tolerogenic signal in the intestinal tract in the presence of the resident microbiota and can modulate the intestinal microbiota composition.

INTESTINAL EXPRESSION OF THE BLOOD GROUP GENE *B4GALNT2* INFLUENCES SUSCEPTIBILITY TO INTESTINAL INFLAMMATION

Abdulhadi Suwandi[1,2], Natalie Steck[1], Philipp Rausch[1], Janin Braun[1], Marijana Basic[2], Andre Bleich[2], Jill M. Johnsen[3], John F. Baines[1] and Guntram A. Grassl[1,2]

[1]Institute for Experimental Medicine, Christian-Albrechts-University, Kiel, Germany; [2]Hannover Medical School, Hannover, Germany; [3]Research Institute, Puget Sound Blood Center, Seattle, WA, USA

Glycans play important roles in host-microbe interactions. The glycosyltransferase gene *b4galnt2* encodes a beta-1,4-*N*-acetylgalactosaminyltransferase known to catalyze the last step in the biosynthesis of the Sd(a) and Cad blood group antigens and is expressed in the GI tract of most mammals, including humans. Loss of B4galnt2 expression is associated with altered intestinal microbiota. We hypothesized that variation of B4galnt2 expression alters susceptibility to intestinal inflammation, induced by infection with *Salmonella* Typhimurium or in dextran sodium sulfate (DSS)-induced colitis.

Here, we found *B4galnt2* intestinal expression was strongly associated with increased susceptibility to *Salmonella* as evidenced by increased histopathological changes, intestinal inflammatory cytokines and infiltrating immune cells. Fecal transfer experiments demonstrated a crucial role of the *B4galnt2* dependent microbiota in conferring susceptibility to *Salmonella* infection. Interestingly, the loss of *B4galnt2* intestinal expression increased the susceptibility to chronic DSS treatment. A significantly enhanced pathological score was found in B4galnt2 deficient mice, although lower infiltration of CD68+ and CD3+ cells were found in those mice. These data support a critical role for *B4galnt2* in intestinal inflammation. We speculate that *B4galnt2*-specific differences in host susceptibility to intestinal inflammation underlie the strong signatures of balancing selection observed at the *B4galnt2* locus in wild mouse populations.

T6SS-MEDIATED SHAPING OF INTESTINAL BACTERIAL COMMUNITIES CONFERS EFFECTIVE *CITROBACTER RODENTIUM* INFECTION

R. Lakra[1], M. Kindermann[1], A. Ennser[2], M.F. Neurath[1], S. Wirtz[1]

[1]*Medical Department 1, Friedrich-Alexander University Erlangen Nuremberg, Germany*
[2]*Department of Virology, Friedrich-Alexander University Erlangen Nuremberg, Germany*

The enteric pathogen *Citrobacter rodentium* (*CR*) is widely used as rodent model for infections with EHEC/EPEC and inflammatory bowel diseases. While the importance of the type III secretion system encoded in the locus of enterocyte effacement (LEE) is well established, the functional role of the recently discovered type VI secretion system (T6SS) in the course of *CR* infection remains to be determined.

Based on our initial evidence that the T6SS actively secreted the hallmark protein hcp *in vitro*, we next disrupted several conserved T6SS genes in a *CR* reporter strain to determine the role of the T6SS for successful colonization of the murine gastrointestinal tract. Interestingly, whole body imaging clearly showed a significantly reduced capacity of these mutants to colonize the gut at different time points post infection as compared to wildtype *CR*. Furthermore, FISH analysis revealed that wildtype *CR* strongly occupied the epithelial layer lining the colonic lumen. By contrast, in the case of T6SS mutants such staining patterns were not observed suggesting that *CR* requires a functional T6SS to colonize the mouse intestine with optimal efficiency and to exhibit full inflammatory activity in mice.

In other bacterial species, T6SS were shown to contribute to bacterial pathogenesis by noxious effects on host cells or effects on other bacterial species. By competition experiments under aerobic and anaerobic conditions using freshly prepared stools or specific bacterial species as prey and *CR* strains as predator, we were able to demonstrate that the T6SS conferred strong interbacterial competition. Notably, this notion was further supported by infection experiments in germ-free mice, where T6SS mutant CR established similar infection loads and growth dynamics compared to the wildtype *CR*. A 16S-based sequencing strategy indicated that community wide changes in the microbial composition in the colonic and cecal microbiome in the course of *CR* infections largely depend on the presence of a functional T6SS.

Collectively, our results suggest that T6SS-dependent effects on other bacterial communities allow the model organism CR and potentially other T6SS harbouring enteric bacterial pathogens effective colonization of the complex ecosystem of the gastrointestinal tract.

MICROBIOTA-MEDIATED DEFENSE AGAINST INTESTINAL INFECTION

Eric G. Pamer

Infectious Diseases Service, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, New York 10065, USA

Infections caused by antibiotic-resistant bacteria generally begin with colonization of mucosal surfaces, in particular the intestinal epithelium. The intestinal microbiota provides resistance to infection with highly antibiotic-resistant bacteria, including Vancomycin Resistant Enterococcus (VRE) and *Clostridium difficile*, the major cause of hospitalization-associated diarrhea. Metagenomic sequencing of the murine and human microbiota following treatment with different antibiotics is beginning to identify bacterial taxa that are associated with resistance to VRE and *C. difficile* infection. We demonstrate that reintroduction of a diverse intestinal microbiota to densely VRE colonized mice eliminates VRE from the intestinal tract. While oxygen-tolerant members of the microbiota are ineffective at eliminating VRE, administration of obligate anaerobic commensal bacteria to mice results in a billion-fold reduction in the density of intestinal VRE colonization. Many antibiotics destroy intestinal microbial communities and disable the native microbiota's ability to inhibit *C. difficile* growth and toxin production. Which intestinal bacteria provide resistance to *C. difficile* infection and their *in vivo* inhibitory mechanisms remains unclear. By treating mice with different antibiotics that result in distinct microbiota changes and lead to varied susceptibility to *C. difficile*, we correlated loss of specific bacterial taxa with development of infection. Mathematical modeling augmented by microbiota analyses of hospitalized patients identified resistance-associated bacteria common to mice and humans. Using these platforms, we determined that *Clostridium scindens*, a bile acid 7-dehydroxylating intestinal bacterium, is associated with resistance to *C. difficile* infection and, upon administration, enhances resistance in a secondary bile acid-dependent fashion. Using a workflow involving mouse models, clinical studies, metagenomic analyses and mathematical modeling, we identified a probiotic candidate that corrects a clinically relevant microbiome deficiency. Our studies indicate that obligate anaerobic bacteria enable clearance of intestinal VRE colonization and may provide novel approaches to prevent the spread of highly antibiotic-resistant bacteria.

PROGRAM Sunday, July 12

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MICROBIOME ANALYSIS

08^{30} – 09^{15} **SPP consortium:**

John Baines, Evolutionary Genomics, Max-Planck-Institute for Evolutionary Biology, Plön

André Gessner, Institut für Med. Mikrobiologie und Hygiene, Universität Regensburg

"Variability and standardization of 16S-based analysis and mouse husbandry"

09^{15} – 09^{55} Selected Abstracts

T. Clavel, ZIEL-Institute for Food and Health, Technische Universität München
"imNGS – the integrated microbial next generation sequencing platform for global assessment of 16S diversity in microbial ecology"

M. Beutler, Max-von-Pettenkofer Institut, LMU München
"Using a gnotobiotic mouse model to investigate the mechanisms of Salmonella-microbiota interaction in inflammation-induced pathogen blooms"

SPP 1656 COLLABORATIVE C57BL6/J FECAL MICROBIOTA SURVEY

Philipp Rausch[1,2], Marijana Basic[3], André Bleich[3] and John Baines[1,2]

[1]Institute for Experimental Medicine, Christian-Albrechts-University of Kiel, Kiel, Germany
[2]Max Planck Institute for Evolutionary Biology, Plön, Germany
[3]Institute for Laboratory Animal Science, Hannover Medical School, Hannover, Germany

The intestinal microbiota are involved in many physiological processes, and it is increasingly recognized that differences in community composition can influence the outcome of a variety of murine models used in biomedical research. In an effort to describe- and account for the variation in intestinal microbiota composition across the animals facilities of Priority Program members, we performed a survey of C57BL6/J mice from 21 different mouse rooms/facilities located at 13 different institutions across Germany. Fresh feces was sampled from five mice per room/facility using standardized procedures, followed by extraction and 16S rRNA gene profiling (V1-V2 region, Illumina MiSeq) on both the DNA- and cDNA level. In order to determine the variables contributing to bacterial community differences, we collected detailed questionnaires of animal husbandry practices and incorporated this information into our analyses. We identified considerable variation in a number of descriptive aspects including the proportions of major phyla, alpha- and beta diversity. Further, we compiled a list of common shared- and unique taxa among facilities. Together, these results will help Priority Program members account for differences in murine models, choose potential fecal donors that may minimize- or maximize such differences, and provide an inventory of communities containing specific bacterial taxa of interest.

IMNGS – THE INTEGRATED MICROBIAL NEXT GENERATION SEQUENCING PLATFORM FOR GLOBAL ASSESSMENT OF 16S DIVERSITY IN MICROBIAL ECOLOGY

Ilias Lagkouvardos,[1] Martin Kapfhammer,[2] Dyvia Joseph,[2] Dirk Haller,[1,3] Matthias Horn,[4] Thomas Clavel[1,2]

[1]ZIEL-Institute for Food and Health, TU München, Freising, Germany, [2]Junior Research Group Intestinal Microbiome, ZIEL, TU München, Freising, Germany, [3]Chair of Nutrition and Immunology, TU München, Freising, Germany, [4]Department of Microbiology and Ecosystem Science, University of Vienna, Austria

Raw sequencing reads archives (e.g. SRA) serve as the primary depositories for an increasing stream of data available from Next Generation Sequencing technologies. They currently host more than 80 thousands of raw amplicon datasets. One half of these data derive from human, mice and other animal hosts samples, while the rest originates from various environmental sources. In order to utilize this accumulating pool of sequence knowledge, we developed IMNGS, a platform that uniformly and systematically screens for, retrieves, processes, and analyzes all available prokaryotic 16S rRNA gene amplicon datasets present in SRA and use them to build sample-specific sequence databases and OTU-based profiles. Using a web front, this massively integrated sequence resource can be queried and the retrieved information can be used to address questions of relevance in microbial ecology, for example with respect to the ecological prevalence of specific microorganisms in different hosts or ecosystems or to perform targeted diversity studies for selected taxonomic groups.

Using a gnotobiotic mouse model to investigate the mechanisms of *Salmonella*-microbiota interaction in inflammation-induced pathogen blooms

<u>Markus Beutler</u>[1], Sandrine Brugiroux[1], Simone Herp[1], Debora Garzetti[1], Patrick Schiller[1], Saib Hussain[1], Diana Ring[1], Kathy McCoy[2], Andrew J. Macpherson[2] and Bärbel Stecher[1][§]

[1]*Max-von-Pettenkofer Institut, LMU München, GERMANY*
[2]*Department for Clinical Research, University of Berne, SWITZERLAND*
[§] Corresponding author

Salmonella enterica serovar Typhimurium (*S*. Tm) infection induces acute gut inflammation, which is followed by dramatic changes in microbiota composition as well as by *Salmonella* overgrowth. These conditions are termed dysbiosis and pathogen "blooming", respectively. Recently, first insights into the underlying mechanisms have been obtained. On the one hand, anaerobic electron acceptors and iron are selectively consumed by the pathogen. On the other hand, the microbiota might experience collateral damage caused by leukocytes, which infiltrate the gut lumen in response to *Salmonella*-induced inflammation. Yet, it is still unclear which of these two mechanisms is more important for the induction of pathogen "blooming": the altered nutritional environment or differential killing by the inflammatory immune response. Furthermore, it has remained elusive how the environment of an inflamed gut impacts on the different members of a normal microbiota. To address these questions, we employ a novel gnotobiotic mouse model, the Oligo Mouse Microbiota (Oligo-MM). The Oligo-MM consists of 12 mouse-adapted strains representing 5 main Eubacterial phyla of the mammalian gut (*Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Verrucomicrobia* and *Proteobacteria*). Oligo-MM mice develop colitis after oral *S*. Tm infection and display pathogen "blooming" and dysbiosis at later infection stages. We studied the fate of each of the individual members of the Oligo-MM consortium using a strain-specific real-time PCR assay and FISH. In addition, we establish *in vitro* assays to analyze the contribution of defined environmental changes on dysbiosis and pathogen "blooming". Using this reductionist model we envision extending the current knowledge on gut inflammation-inflicted dysbiosis and, thereby, contribute to the development of new therapies to prevent pathogen "blooming" and collateral damage of the gut microbiota.

MICROBIOME ANALYSIS

$10^{20} – 10^{55}$ **8th Keynote Lecture: Jens Walter**, Nutrition, Microbes and Gastrointestinal Health, University of Alberta
"Therapeutic modulation of the gut microbiota – an ecological perspective"

$10^{55} – 11^{55}$ Selected Abstracts

F. Peirera, Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, Faculty of Life Sciences, University of Vienna
"Tracking heavy water incorporation to identify and sort active cells of the gut microbiota"

A. Walker, Research Unit Analytical BioGeoChemistry, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg
"Bacteroidales species produce novel sulfonolipids in the murine gastrointestinal tract"

S. Haange, UFZ-Helmholtz Centre for Environmental Research, Department of Proteomics, Leipzig
"Revealing the response of the active mucosal microbiota to a change in diet: an experimental study in rats"

THERAPEUTIC MODULATION OF THE GUT MICROBIOTA – AN ECOLOGICAL PERSPECTIVE

Jens Walter

Nutrition, Chair for Nutrition, Microbes, and Gastrointestinal Health, Department of Agricultural, Food & Nutritional Science/Department of Biological Sciences, University of Alberta, 4-126A Li Ka Shing Centre for Health Research Innovation and 7-142 Katz Group Center, Edmonton, Alberta, T6G 2E1, Email: jwalter1@ualberta.ca

Diverse strategies have been used for several decades to improve human and animal health through the modulation of the gut microbiota, spanning from the administration of defined probiotic strains (or Live Biotherapeutics), whole microbial consortia (e.g. fecal bacteriotherapy), to the provision of bacterial growth substrates (prebiotics and dietary fiber). However, we still lack a conceptual understanding on how the gut microbiota can be modulated. In this presentation I will summarize how ecological theory can provide a framework by which to understand characteristics of the human gut microbiota and the impact of microbiome-modulating strategies. I will present some of our own studies that investigate basic ecological questions regarding the factors that shape the gut microbiome and how it can be manipulated. The methodological toolset that is now available (e.g. through next-generation sequencing) provides an unprecedented opportunity to obtain phylogenetic, compositional, and functional information of microbial communities. When analyzed in the light of ecological theory, this has the potential to elucidate the factors and ecological processes that determine and potentially predict the response of the gut microbiota to therapeutic modulations.

TRACKING HEAVY WATER INCORPORATION TO IDENTIFY AND SORT ACTIVE CELLS OF THE GUT MICROBIOTA

F. Pereira[1], B. Sziranyi[1], A. Loy[1], M. Wagner[1], D. Berry[1]

[1]*Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, Faculty of Life Sciences, University of Vienna, A-1090 Vienna, Austria*

Certain pathogenic microorganisms as well as members of the commensal intestinal microbiota can forage on glycans present in the secreted mucus layer covering the mammalian gastrointestinal tract. However, the identification of these organisms constitutes a major challenge due to the difficulty in identifying the activity of microbial cells under natural conditions in the complex intestinal ecosystem. To overcome this problem, we have developed a new method to identify and sort active microbes on the single-cell level in complex samples using stable isotope probing with heavy water (D_2O) combined with Raman microspectroscopy. Microbial communities were amended with heavy water (D_2O), a treatment that does not change the available substrate pool. Incorporation of D_2O-derived D into the biomass of physiologically active cells could be unambiguously detected via the C-D signature peak region in single-cell Raman spectra. For functional analyses of microbial communities, the detection of D incorporation from D_2O in individual microbial cells via Raman microspectroscopy can be directly combined with FISH for the identification of active microbes. Applying this approach to mouse cecal microbiota revealed that the host-compound foragers *Akkermansia muciniphila* and *Bacteroides acidifaciens* exhibited distinctive response patterns to amendments of mucin and sugars. By Raman-based cell sorting of active (deuterated) cells with optical tweezers and subsequent multiple displacement amplification and DNA sequencing, novel cecal microbes stimulated by mucin and/ or glucosamine were identified, demonstrating the potential of the non-destructive D_2O Raman approach for targeted sorting of microbial cells with defined functional properties for single-cell genomics. This approach is now being applied to identify members of the gut microbiota that can forage other mucosal sugars such as sialic acid, fucose and N-acetyl-glucosamine. These monosaccharides are used by certain pathogens to expand and colonize the gut. Commensal organisms identified by this approach may be able to outcompete pathogens for key nutrients and thereby eradicate them from the gut, and therefore might represent promising candidates for bacterial-based therapies.

BACTEROIDALES SPECIES PRODUCE NOVEL SULFONOLIPIDS IN THE MURINE GASTROINTESTINAL TRACT

Alesia Walker[1], Barbara Pfitzner[2], Mourad Harir[1], Monika Schaubeck[3], Jelena Calasan[3], Thomas Clavel[4], Dirk Haller[3], David Endesfelder[5], Wolfgang zu Castell[5], Michael Schmid[2], Anton Hartmann[2], Philippe Schmitt-Kopplin[1,6]

[1]*Research Unit Analytical BioGeoChemistry, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany*

[2]*Research Unit Microbe-Plant Interactions, Research Group Molecular Microbial Ecology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany*

[3]*Chair of Nutrition and Immunology, Technische Universität München, Freising-Weihenstephan, Germany*

[4]*Research Group Intestinal Microbiome, ZIEL Institute for Food and Health, TU München, Freising, Germany*

[5]*Scientific Computing Research Unit, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany*

[6]*Chair of Analytical Food Chemistry, Technische Universität München, Freising-Weihenstephan,Germany*

Sphingolipids represent a class of lipids, which was assumed to be specific to eukaryotes. Bacteria belonging to the phylum *Bacteroidetes* are dominant colonizers of the mammalian intestine and were also found to produce sphingolipids, especially members of the genus *Bacteroides.* By using high resolution mass spectrometry techniques, we identified a novel and unusual class of sphingolipids in the murine intestine, namely the sulfonolipids (SLs). SLs were previously reported in *Cytophaga* species. High resolution mass spectrometry based analysis allowed us to identify 18 different SLs in cecal content of C57BL/6N mice. Moreover, we observed that the occurrence of the novel SLs in the mouse cecum was altered after feeding high-fat diets for 3 weeks. Lipids are major constituents of membranes and can be used as chemotaxonomic markers for the >500 bacterial species that are members of the gut microbiome. We used a combination of metagenomics and culture-dependent SLs analyses and found two candidate species within the order *Bacteroidales* capable of producing SLs. In future, SLs will be used as metabolic markers which reflect directly the composition of *Bacteroidales* in the complex gut microbiome environment.

REVEALING THE RESPONSE OF THE ACTIVE MUCOSAL MICROBIOTA TO A CHANGE IN DIET: AN EXPERIMENTAL STUDY IN RATS

Sven-Bastiaan Haange[1], Andreas Oberbach[2, 3], Nadine Schlichting[2, 3], Marco Heinrich[2], Floor Hugenholtz[4] Hauke Smidt[4], Holger Till[5], Jana Seifert[6], Nico Jehmlich[1], Martin von Bergen[1,7,8]

[1] *UFZ-Helmholtz Centre for Environmental Research, Department of Proteomics, Leipzig, Germany*

[2] *University of Leipzig, Leipzig Heart Center, Department of Cardiac Surgery, Leipzig, Germany*

[3] *Leipzig University Medical Centre, Integrated Research and Treatment Center (IFB) Adiposity Diseases, Leipzig, Germany*

[4] *Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands*

[5] *Department of Paediatric and Adolescent Surgery, Medical University of Graz, Austria*

[6] *Institute for Animal Nutrition, University of Hohenheim, Stuttgart, Germany*

[7] *UFZ-Helmholtz Centre for Environmental Research, Department of Metabolomics, Leipzig, Germany*

[8] *Department of Biotechnology, Chemistry and Environmental Engineering, University of Aalborg, Aalborg, Denmark*

The focus of this experimental study in rats being fed either high fat diet (HFD) or chow diet CD) was to observe the response of the mucosal microbiota after switching their diet.

To assess the microbiome16S rRNA gene pyrosequencing and LC-MS/MS metaproteomic analysis hyphenated with protein-based stable isotope probing (protein-SIP, ^{15}N-fully labelled diet) was performed. As a result, we were able to decipher the mucosal colon microbiota community structure in regard to taxonomy, enzymatic functionalities and active taxa related to nitrogen utilisation from the feed over a three day period. Microbial active taxa in regard to nitrogen utilisation belonged to the abundant phyla like *Firmicutes*, *Proteobacteria* and *Bacteroidetes* as well as those from low abundant phyla like *Spirochaetes*, *Deinococcus-Thermi* and *Planctomycetes*. In addition, we observed rapid changes in the community composition including a decline of *Enterobacteriaceae* and *Streptococcaceae* whereas an increase was seen for *Desulfovibrionacea*. Assigning identified proteins to functional categories we detected an overrepresentation of replication, transcription, signal transduction as well as carbohydrate and amino acid metabolism.

This integrated data analysis opens the path to elucidate the complex gut microbiota in more detail using protein-SIP for identification of active taxa and specific substrate utilisation.

1 SHAPING THE INFANT FECAL MICROBIOTA BY MODE OF DELIVERY AND TYPE OF FEEDING

Monika Bazanella[1], Tanja Maier[2], Thomas Clavel[3], Ilias Lagkouvardos[3], Philippe Schmitt-Kopplin[2], Dirk Haller[1,3]

[1]*Technische Universität München, Chair of Nutrition and Immunology, 85350 Freising-Weihenstephan, Germany*
[2]*Helmholtz Zentrum München, Research Unit Analytical BioGeoChemistry (BGC), Germany*
[3]*Technische Universität München, ZIEL – Institute for Food and Health, Germany*

Early life probiotic intervention endeavors to resemble the gut microbial composition of formula-feds to that of breast-feds. Probiotic effects in infancy were studied intensely, however, primarily in association with gut and immunologically related disease prevention or alleviation. This double blinded, randomized study is the first to describe the effects of four *Bifidobacterium* spp. on the fecal microbiota of healthy infants from birth until the first year of life with special emphasis on the comparison of fecal microbial composition in exclusively probiotic/non-probiotic formula fed to breast fed infants and a combination thereof. 106 infants were randomized in two groups (group A and B) receiving infant formula substituted with four *Bifidobacterium* spp., or a placebo without *Bifidobacterium* spp. 9 infants did not get any formula. Monthly fecal samples were sequenced and metabolic profiles were established.

16S Illumina sequencing of fecal samples showed significant differences in microbial consortia three months after birth between infants born by caesarean section and vaginal delivery. Caesarean section infants showed significantly elevated levels of *Firmicutes* (p=0.00093), in particular *Enterococcaceae* (p=0.015) and *Veillonellaceae* (p=0.0079), and *Proteobacteria* (p=0.021), in particular *Enterobacteriaceae* (p=0.017). Formula fed infants generally showed significantly higher species diversity with higher abundance in *Actinobacteria*, and lower counts in *Firmicutes* and *Proteobacteria* when compared to breast fed infants three months after birth. Metabolomic studies showed distinct clusters of breast fed to formula fed infants, independent of the type of formula. Clustering can be seen until ninth month of life, when both groups start to converge. No significant differences in metabolite profiles could be observed in probiotic vs. non-probiotic throughout first year of life.

In conclusion, metabolite profiles of fecal samples did not differ between infants fed with probiotic or non-probiotic formula. However, significant shifts in microbial numbers due to mode of delivery and type of feeding (breast vs. formula) were observed.

2 GENOMIC AND POST-GENOMICS INSIGHTS INTO THE MUCUS-DWELLING BACTERIUM *MUCISPIRILLUM SCHAEDLERI*

David Berry[1], Carina Pfann[1], Buck Hanson[1], Michaela Steinberger[1], Sandrine Brugiroux[2], Clarissa Schwab[3], Tim Urich[3], Thomas Rattei[4], Bärbel Stecher[2], Alexander Loy[1]

[1]*Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, University of Vienna, Austria*
[2]*Max von Pettenkofer Institute of Hygiene and Medical Microbiology, Ludwig-Maximilians-University of Munich, Munich, Germany*
[3]*Archaea Biology and Ecogenomics Division, Department of Ecogenomics and Systems Biology, University of Vienna, Austria*
[4]*Division of Computational Systems Biology, Department of Microbiology and Ecosystem Science, University of Vienna, Austria*

Mucispirillum schaedleri inhabits the intestinal mucus layer of several species of animals, where it is thought to degrade mucus. To gain insights into its lifestyle, we sequenced the genome of *M. schaedleri* ASF457 using a combination of platforms, including the state-of-the-art Oxford Nanopore MinION. Selected predicted traits were confirmed with physiological experiments. Surprisingly, the *M. schaedleri* genome encoded a limited repertoire of enzymes for degrading host-derived mucus or other complex compounds. Metabolism seems to rather rely on the utilization of small compounds such as peptides, amino acids, glycerol, and short chain fatty acids. It's ability to reduce nitrate and the presence of oxygen and reactive oxygen species scavenging systems accounts for its survival close to the mucosal tissue blooms during inflammation. *M. schaedleri* has a type VI secretion system (T6SS) as well as several proteins with eukaryotic domains, which may be involved in interacting with the host and may play a role in inflammation. Putative interaction genes are also expressed *in vivo*. Phylogenetic analysis suggests extensive lateral gene transfer, primarily from intestinal Epsilon- and Deltaproteobacteria. Laterally acquired pathways from gut organisms (e.g. T6SS and glycerol-P utilization) are likely important for its survival, suggesting that lateral gene transfer may have facilitated its establishment in the gut ecosystem.

3 INTERACTION BETWEEN CDCS1 DETERMINED COLITIS SUSCEPTIBILITY AND T CELL RESPONSES

I. Bruesch[1], M. Buettner[1], A. Bleich[1]

[1]Institute of Laboratory Animal Science, MHH, Carl-Neuberg Str 1, 30625 Hannover

The interleukin-10-deficient mouse ($Il10^{-/-}$) model is a suitable model for human inflammatory bowel disease research. It is known that the colitis severity in this model is modified by the background strain. In a quantitative trait loci (QTL) analysis between B6.129P2/JZtm-$Il10^{tm1Cgn}$ (B6-$Il10^{-/-}$) and C3Bir.129P2/JZtm-$Il10^{tm1Cgn}$ (C3Bir-$Il10^{-/-}$) animals, 10 QTL called *Cdcs1* to *10,* were identified. By breeding congenic and subcongenic strains, *Cdcs1* derived from C3Bir-$Il10^{-/-}$ was identified as the major genetic modifier and the cause for an adaptive hyperresponsiveness.

Here we point out that naive CD4$^+$ T cell subsets have different colitiogenic properties depending on the *Cdcs1* locus

In an adoptive transfer of naïve T cell subsets characterized by different markers (CD4$^+$CD62L$^+$, CD4$^+$CD45^{rbhigh}, CD4$^+$CD25$^-$), cells with parts of *Cdcs1* derived from C3Bir-$Il10^{-/-}$ were able to induce a stronger colitis compared to cells derived from B6-$Il10^{-/-}$. There was no difference between the used subsets and an influence of IL-10 producing regulatory T cells could be excluded. Moreover, we showed that the delay until the onset of colitis depends on the number of transferred cells.

In a microarray analysis of naive T cells 47 differently expressed probes were identified by comparison of resistant and susceptible strains.

The combination of an adoptive transfer model and a microarray analysis is a powerful approach to identify causative genes for colitis susceptibility within *Cdcs1*.

4 Cd14 PLAYS A PROTECTIVE ROLE IN EXPERIMENTAL MODELS OF COLITIS BY ENHANCING THE INTESTINAL BARRIER FUNCTION

S. Buchheister[1], M. Buettner[1], A. Siebert[1], A. Smoczek[1], A. Bleich[1]

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

Inflammatory bowel disease is characterised by relapsing inflammation of the gut. The pathogenesis of disease is still unknown, however intestinal barrier disruption could likely play a key role in IBD development. In a mouse model based on Interleukin-10 (*Il10*) deficiency *Cd14* was suggested as a genetic modifier of colitis susceptibility. To determine the role of *Cd14* for colitis development its influence in a chronical model of intestinal inflammation based on *Il10*-deficiency was investigated as well as in a Dextran-Sodium-Sulfate (DSS) induced model of acute colitis. Therefore barrier function was analysed in B6-$Il10^{-/-}Cd14^{-/-}$ mice and in DSS treated B6-$Cd14^{-/-}$ mice. Intestinal permeability was investigated *in vivo* by intestinal FITC-dextran uptake and *in vitro* by qRT-PCR of Tight-Junction-Proteins (TJ) and immunohistological staining of Occludin, Ki67 and TUNEL apoptosis assay. Severity of intestinal inflammation was evaluated histologically and TNFα and IFNγ gene expressions were quantified by qRT-PCR. Untreated B6-$Cd14^{-/-}$ mice showed no differences in this experimental setup compared to wildtype controls. However, FITC-dextran uptake was increased and TJ expression was decreased in B6-$Il10^{-/-}Cd14^{-/-}$ mice compared to *Il10*-deficient mice. Likewise the immunohistological stainigs indicated a barrier disruption in the B6-$Il10^{-/-}Cd14^{-/-}$ mice. Histology and inflammatory cytokine expression revealed increased intestinal inflammation in B6-$Il10^{-/-}Cd14^{-/-}$ mice. In addition to this DSS treated *Cd14*-deficient mice showed an increased intestinal barrier disruption in the acute DSS colitis model compared to the wildtype controls referring to FITC Dextran uptake, mRNA expression of TJ proteins and immunohistology. Histological Scoring and inflammatory cytokine expression were likewise higher in the DSS treated *Cd14*-deficient mice compared to the wildtype controls. *Cd14* deficiency seems to have no influence on epithelial tightness under steady state conditions but it untightens the intestinal barrier under inflammatory conditions. Thus *Cd14* plays a protective role in IBD development by enhancing the intestinal barrier function.

5 ELUCIDATING THE MECHANISM OF SHIGA TOXIN REDUCTION IN ENTEROHEMORRHAGIC *E. COLI* BY THE PROBIOTIC *E. COLI* STRAIN NISSLE 1917

S. Bury[1], S. Rund[1], T.A. Oelschlaeger[1]

[1]*Institute for molecular infection biology, University of Wuerzburg, Germany*

Enterohemorrhagic *E. coli* (EHEC), which are transmitted by contaminated food, have become a significant threat for humansas these pathogens can lead to the development of severe gastrointestinal disease and life threatening complications such as HUS. Since the large outbreak in Germany in 2011 a lot of research addressed pathogenicity of EHEC and the development of new treatment strategies. The most important EHEC virulence factor is Shiga toxin (Stx), an AB_5 exotoxin. Once secreted this toxin can bind with itsB subunits to the globotriaosylceramide receptors (Gb3) ofenterocytes and enter the cells by endocytosis. The A subunit has a specific N-glycosidase activity and cleaves an adenine base from the 28S rRNA of the ribosome by which the proteinsynthesis is blocked and the cells dye due to apoptosis. Treatment of patients with antibiotics is not recommended as this is linked to an increase of released Stx [1]. Previous studies with probiotics showed*E. coli*Nissle 1917 (EcN) to inhibit both growth of and Stxproduction by EHEC strains,which canonly be traced back in part to the production of antibacterial operatingmicrocins [2, 3].Co-cultivation studies by Stefan Rund could show that EcN has strong Stx reducing effects on EHEC strains[3]. The next objective is to elucidate the underlying mechanism by examination of the cultivation media for substances secreted by EcN. Up to now the Stx production of EHEC strains was always analyzed in complex medium, which contains too many different substances for analysis. Therefore, we positively tested whether EHEC strains grow and produce Stx in a defined minimal medium. We could also verify that the microcin negative EcN mutant SK22D shows Stx reducing effects being co-cultivated with EHEC in minimal medium. In a next approach we will investigate whether EHEC needs to be present in order to induce the production of Stx-reducing substancesby EcN. Therefore EHEC will be incubated in EcN spent culture minimal medium and examined for the Stx production via a Stx-ELISA. This will be followed by a mass spectrometric analysis of the spent culture media. These approaches could help to get a better understanding about the Stx regulation in EHEC by EcN.

1.   DGI *EHEC und Antibiotikabehandlung*. 2011. 3.
2.   Reissbrodt, R., et al., *Inhibition of growth of Shiga toxin-producing Escherichia coli by nonpathogenic Escherichia coli.* FEMS Microbiology Letters, 2009. **290**(1): p. 62-69.
3.   Rund, S.A., et al., *Antagonistic effects of probiotic Escherichia coli Nissle 1917 on EHEC strains of serotype O104:H4 and O157:H7.* International Journal of Medical Microbiology, 2013. **303**(1): p. 1-8.

6 MiBC - The Mouse Intestinal Bacterial Collection: Host-Specific Insights Into Cultivable Diversity and Genomic Novelty of the Mouse Gut Microbiome

Ilias Lagkouvardos,[1] Birte Abt,[2] Rüdiger Pukall,[2] Sandrine Brugiroux,[3] Caroline Ziegler,[4] Floor Hugenholtz,[5] Thi Phuong Nam Bui,[5] Caroline M Plugge,[5] Dirk Haller,[6] Daniel Peterson,[7] Hauke Smidt,[5] Bärbel Stecher,[3] Thomas Clavel[1,4]

[1]ZIEL Institute for Food and Health, TU München, Freising, Germany, [2]DSMZ - German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany, [3]Max von Pettenkofer-Institute, Ludwig-Maximilians-Universität München, Germany, [4]Junior Research Group Intestinal Microbiome, ZIEL, Technische Universität München, Freising, Germany, [5]Department of Microbiology, Wageningen University, Wageningen, The Netherlands, [6]Chair of Nutrition and Immunology, Technische Universität München, Freising, Germany, [7]Immunology laboratory, Johns Hopkins University School of Medicine, Baltimore, USA.

Molecular techniques have generated major breakthroughs in microbial ecology of the mammalian gut. However, it became clear in recent years that isolation, thorough characterization and archiving of gut bacteria is urgently needed for the description of novel diversity, for the amendment of databases to improve the interpretation of omics datasets, and for the sake of gnotobiotic studies. By establishing the Mouse intestinal Bacterial Collection (MiBC), we aimed at providing the first state-of-the-art repository of bacterial strains and associated genomes from the mouse intestine. We isolated aerobes and strict anaerobes from various gut location, mouse strains and facilities and selected 100 bacteria, including strains from wild and newborn mice, representing 74 species across 26 families that cover the majority of known phylogenetic diversity in the mouse gut. A total of 13 species that are most prevalent in or specific to the mouse intestine were identified. Genome sequences from these and additional members of the collection were obtained, thereby providing new mouse-derived bacterial genomic information. Novel diversity was also described via taxonomic characterization of 16 new bacteria within the *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria*, including novel butyrate- and secondary bile acid-producing species. Via analysis of SRA-derived 16S rRNA gene datasets from the mammalian gut, we demonstrated that some specific species are characteristic of mice. In summary, cultivable bacteria represent a substantial part of the mouse gut ecosystem and we provide via MiBC a unique tool to the scientific community for the purpose of omics and colonization studies.

7 ORAL VERSUS INTRAVENOUS IRON REPLACEMENT THERAPY DISTINCTLY ALTERS THE GUT MICROBIOTA AND METABOLOME IN PATIENTS WITH INFLAMMATORY BOWEL DISEASES

Thomas Lee,[1,*] Thomas Clavel,[2,*] Kirill Smirnov,[3,*] Annemarie Schmidt,[4] Ilias Lagkouvardos,[2] Alesia Walker,[3] Marianna Lucio,[3] Bernhard Michalke,[3] Philippe Schmitt-Kopplin,[2,3] Richard Fedorak,[1,#] Dirk Haller[2,4,#]

[1] *Division of Gastroenterology, department of Medicine, University of Alberta, Edmonton, Canada*
[2] *ZIEL Institute for Food and Health, Technische Universität München, Freising, Germany*
[3] *Research Unit Analytical BioGeoChemistry, German Research Center for Environmental Health, Neuherberg, Germany*
[4] *Chair of Nutrition and Immunology, Technische Universität München, Freising, Germany*

Objective Iron deficiency is a common complication in patients with inflammatory bowel diseases (IBD) and oral iron therapy is suggested to exacerbate IBD symptoms. We performed an open-labelled clinical trial to compare the effects of oral (PO) *vs.* intravenous (IV) iron replacement therapy (IRT).

Design The study population included patients with Crohn's disease (CD; N = 31), ulcerative colitis (UC; N = 22) and control subjects with iron deficiency (NI = 19). After randomization, participants received iron sulphate (PO) or iron sucrose (IV) over 3 months. Clinical parameters, fecal bacterial communities and metabolomes were assessed before and after intervention.

Results Both PO and IV treatments ameliorated iron deficiency, but higher ferritin levels were observed with IV. Changes in disease activity were independent of iron treatment types. Fecal samples in IBD were characterized by marked inter-individual differences, lower phylotype richness and proportions of *Clostridiales*. Metabolite analysis also showed separation of both UC and CD from control anemic participants. Major shifts in bacterial diversity occurred in approximately half of all participants after IRT, but CD patients were most susceptible. Despite individual-specific changes in phylotypes due to IRT, PO treatment was associated with decreased abundances of OTUs assigned to the species *Faecalibacterium prausnitzii*, *Ruminococcus bromii*, *Dorea* sp. and *Collinsella aerofaciens*. Clear IV- and PO-specific fingerprints were evident at the level of metabolomes, with changes affecting cholesterol-derived host substrates.

Conclusion Shifts in gut bacterial diversity and composition associated with iron treatment are pronounced in IBD participants. Despite similar clinical outcome, oral administration differentially affects bacterial phylotypes and fecal metabolites compared with IV therapy.

8 HELMINTH PARASITE MODULATES MURINE IL-17 EXPRESSION VIA TYPE 2 IMMUNITY-DEPENDENT REDUCTION OF SEGMENTED FILAMENTOUS BACTERIA

W. Florian Fricke[1,2], Yang Song[2], An-Jiang Wang[2], Allen Smith[3], Viktoriya Grinchuk[2], Chenlin Pei[2], Bing Ma[2], Joseph F. Urban, Jr.[3], Terez Shea-Donohue[2], Aiping Zhao[1].

[1]University of Hohenheim, Stuttgart, Germany; [2]University of Maryland Baltimore, USA; [3]U.S. Department of Agriculture, Beltsville, Maryland, USA

Gastrointestinal immune homeostasis depends on dynamic interactions between the host and the various inhabitants of the gastrointestinal tract. Helminthic parasites modulate the host immune response, resulting in increased susceptibility to pathogen infection on the one hand and protection against autoimmune disease on the other. The underlying mechanisms of this process remain largely unknown.

We infected mice with the parasitic nematode *Nippostrongylus brasiliensis* and characterized the intestinal microbiota of infected mice by 16S rRNA amplicon sequencing and quantitative real-time PCR. Expression of mucins, antimicrobial peptides (AMP), and components of the host immune response were quantified by RT-PCR.

We showed that the type 2 immune response to *N. brasiliensis* infection in mice was associated with altered ileal and jejunal mucin and AMP expression profiles, as well as shifts in the microbiota composition. Most strikingly, intestinal concentrations of segmented filamentous bacteria (SFB), known inducers of T helper type 17 cells, were reduced in infected mice, as well as IL-17-associated gene expression. SFB-negative Jackson mice did not alter IL-17 gene expression in response to *N. brasiliensis* infection. Infected mice deficient in either IL-13 or STAT6 did not reduce levels of SFB or IL-17, whereas administration of IL-25 reduced SFB and IL-17 in uninfected mice.

Our data suggest that parasitic nematodes decrease intestinal SFB and expression of IL-17-associated genes through host type 2 immunity in SFB-positive mice, providing a potential mechanistic explanation for the reported immune modulating effects of parasite infection.
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9 Role of virulence factors, immune system and microbiota derived colonization resistance in *Yersinia enterocolitica* infection

Janina Geißert[1], Sina Beier[2], Erwin Bohn[1], Monika Schütz[1], Daniel Huson[2], Ingo B. Autenrieth[1]

[1] *Interfaculty Institute for Microbiology and Infection Medicine, University Clinics Tübingen, Elfriede-Aulhorn-Straße 6, Tübingen, D-72076, Germany*
[2] *Centre for Bioinformatics, University of Tübingen, Sand 14, Tübingen, D-72076, Germany*

Yersinia enterocolitica (Ye) expresses a number of virulence factors like the adhesin Yad A and a type-III-secretion system that both contribute to effective colonization, invasion and abscess formation in lymphoid tissues after orogastral infection.

The gastrointestinal (GI) tract harbors a dense and complex microbial community which is important for the maturation of the host immune system and may confer colonization resistance (CR) against enteric pathogens like Ye.

The aim of our project is to investigate the trilateral interactions between Ye, intestinal microbiota and host immune response. We want to shed light on the alterations of microbial composition in the murine GI tract during Ye infection and the consequences for further pathogen colonization, overgrowth of certain bacterial species and the shaping of host immune response. Furthermore, we want to find out which constituents of the commensal microbiota or metabolites contribute to CR against Ye and to evaluate the role of Ye virulence and fitness factors in this interplay. In first experiments we could show that mutant strains of Ye, lacking certain virulence factors, are unable to establish intestinal colonization in the presence of a commensal microbiota, but are highly virulent in germfree (GF) mice. It is not clear whether these effects are due to direct interactions with intestinal commensals or due to host inflammatory response. Co-infection experiments via the orogastric route using 1:1 mixtures of Ye wildtype and mutant strains will allow us to investigate the role of virulence factors in the presence of the commensal microbiota and under GF conditions. Microbiome and metagenome analyses, followed by computation of statistical correlations between present taxa will enable us to predict possible relations and interactions between Ye and commensal bacteria. Furthermore, we will try to shed light on the involved immune mechanisms by performing oral infection experiments with normally colonized or GF immunodeficient mouse strains.

10 IL28 INFLUENCES PANETH CELL HOMEOSTASIS BY TRANSCRIPTIONAL REGULATION OF MLKL

C. Günther[1], G. He[2], M. Hefele[1], B. Buchen[1], H. Neumann[1], M. Basic[2], A. Bleich[2], M. Blaut[3], A. Woting[3], M.F. Neurath[1], S.Wirtz[1], C.Becker[1]

[1]Medical Clinic 1, Friedrich Alexander University, Erlangen, Germany
[2]Institute for Laboratory Animal Science and Central Animal Facility, Hannover Medical School, Hannover, Germany
[3]Department of Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Berlin, Germany

Homeostasis of the intestine depends on a complex interplay between the gut microbiota, the intestinal epithelium and immune cells. Paneth cells (PC) are an important factor in controlling the balance between these factors. Recently we have identified that PC undergo a novel form of programmed cell death (necroptosis) leading to or supporting intestinal inflammation. Thus PC necroptosis emerges as a potential regulator of intestinal immune homeostasis and might be involved in the pathogenesis of inflammatory bowel disease. However the impact of PC necroptosis on the microbiome and how the microbiome can influence different forms of programmed cell death in the gut is still unknown.

Initially, we have evaluated the expression of various cell death executor molecules in the terminal ileum of germfree animals (GF) and mice which has been reconstituted with SPF flora. Interestingly we could observe that MLKL, a key mediator of the necroptosis, was significantly upregulated during colonization with SPF flora. Interestingly MLKL upregulation could not be observed by colonization with a defined flora, like the altered Schädler Flora or a simplified human flora. We identified that IL28, a cytokine that was also transcriptionally activated during colonization mediates MLKL upregulation in intestinal epithelial cells via the STAT1 pathway. In order to investigate the consequence of activated MLKL gene transcription, we overexpressed IL28 in wildtype animals. Surprisingly we could observe that overexpression of IL28 causes necrotic PC death. PC depletion results in reduced expression of antimicrobial peptides, which further sensitizes these mice to *Salmonella Typhimurium* infection. Interestingly IL28 mediated PC depletion was not dependent on type I IFNs, since overexpression of IL28 in type I IFN receptor deficient mice also causes PC depletion. In order to investigate if PC undergo necroptosis in response to IFNs, we next overexpressed IL28 in MLKL-/- and RipK3-/- mice. Interestingly, MLKL-/- mice but not RipK3-/- animals showed significantly more PC compared to wildtype animals that had received IL28. These data were unexpected since the current model of necroptosis predicts that RipK3 is an essential mediator of necrosis signalling upstream of MLKL. In a translational approach, we identified a strong induction of MLKL and IL28 gene transcription in intestinal epithelial cells of CD patients, suggesting a potential contribution of these factors in the pathogenesis of CD. In summary, our data uncover a novel form of programmed necrotic Paneth cell death that is mediated by MLKL and triggered by IL28. Moreover our data suggest that MLKL levels have to be tightly controlled during postnatal colonization in order to maintain Paneth cell homeostasis.

11 Tracing the in vivo fate of colonic glucose with ^{13}C glucose ureide: microbiota, metabolites and host metabolism

Buck T. Hanson, Orest Kuzyk, Thomas Decker, Andreas Richter, Wolfgang Wanek, Douglas Morrison, David Berry, Alexander Loy

Department of Microbiology and Ecosystem Science, Division of Microbial Ecology, University of Vienna, Althanstraße 14, 1090 Vienna, Austria.

The manifestation of many dietary-induced diseases emanates from a dynamic, metabolite-mediated dialog between the gut microbiota, host physiology, and dietary composition. Microbial degradation and fermentation of complex polysaccharides in the colon produces short-chain fatty acids, which have far-reaching, often beneficial effects on host physiology. In contrast, dietary overabundance of simple sugars such as glucose can have harmful effects on host health and energy homeostasis. To explore the connections between dietary glucose and the microbial ecology of colonic glucose metabolism, we are establishing glucose ureide as a model substrate for targeted delivery of ^{13}C-labeled glucose to distal gut microbial populations and *in vivo* stable isotope probing. Glucose ureide, a substrate protected from host degradation, is specifically degraded by distal gut microbiota. When ^{13}C-glucose ureide is given orally to mice we observe a peak in ^{13}C-labeling of CO_2, bulk cecal biomass, and cecal DNA between 6 and 9 hours after dosing. ^{13}C-labeling was also detected in host blood plasma, indicating that there was host absorption of microbially-derived metabolites. ^{13}C-glucose ureide shows great promise as an *in vivo* stable isotope probing approach for revealing the metabolic path of dietary glucose, the fate of metabolic products, the interactions between microbial populations, and contributions to host health and physiology.

12 Generation of gnotobiotic mice to investigate the role of the intestinal microbiota in *Salmonella enterica* spp. I serovar Typhimurium colitis in AGR2-deficient mice

Sandrine Brugiroux[1], Diana Ring[1], Debora Garzetti[1], <u>Simone Herp</u>[1], Markus Beutler[1], Saib Hussain[1], Hans-Joachim Ruscheweyh[2], Daniel Huson[2], Eva Rath[3], Dirk Haller[3], Mark Boekschoten[4], Mikael Sellin[5], Marijana Basic[6], André Bleich[6] and Bärbel Stecher[1§]

[1]*Max-von-Pettenkofer Institut, LMU Munich, GERMANY*

[2] *Chair of Algorithms in Bioinformatics, University of Tübingen, GERMANY*

[3] *ZIEL-Research Center for Nutrition and Food Science, TUM Munich, GERMANY*

[4] *Agrotechnology & Food Sciences, Wageningen University, NETHERLANDS*

[5]*Institute of Microbiology, ETH Zurich, SWITZERLAND*

[6] *Institute of Laboratory Animal Science, Hannover Medical School, GERMANY*

[§] Corresponding author

The intestinal microbiota plays a pivotal role in protection against the enteric *Salmonella* serovar Typhimurium (*S.* Tm) infection. In mice, treatment with the antibiotic streptomycin transiently disrupts the microbiota and enables *S.* Tm colonization of the gut, tissue invasion and induction of intestinal inflammation. Besides the microbiota, the mucosal barrier significantly contributes to immediate immune defence against *S.* Tm. So far, the interplay between the microbiota and the intestinal mucus barrier is poorly understood. We have analyzed *S.* Tm infection in mice deficient in anterior gradient 2 (AGR2) gene, which have severe mucosal barrier defects. Yet, in opposition to our prediction, AGR2^{-/-} mice were somewhat protected against *S.* Tm-induced gut inflammation, in contrast to AGR2^{+/-} littermate controls. We showed that AGR2^{-/-} mice exhibit altered microbiota composition compared to AGR2^{+/-} cage- or littermates. Thus, imbalanced mucosal homeostasis in AGR2^{-/-} mice may lead to microbiota changes and altered resistance against *S.* Tm infection. In this project, we have performed epithelial gene expression profiling to characterize the antimicrobial mucosal defences in AGR2^{-/-} mice. Furthermore, we have generated germfree mice to test the dependence of the microbiota on susceptibility of AGR2^{-/-} mice to enteric *S.* Tm infection. Finally, we have employed a gnotobiotic mouse model based on a defined consortium of mouse-adapted strains, termed "Oligo Mouse Microbiota", to study the role of the mucus layer in microbial interaction and colonization resistance at bacterial strain level.

13 PRECLINICAL C57BL/6 MOUSE MODEL OF *PSEUDOMONAS AERUGINOSA* COLONIZATION AND INFECTION IN THE IMMUNOCOMPROMISED HOST

F. Hölzl[1], H. Hinkov[1], M. Willmann[1], S. Peter[1], K. Gronbach[1], I. B. Autenrieth[1], J.-S. Frick[1]

[1]*Institute for Medical Microbiology and Hygiene, University Hospital Tübingen, Germany*

The gastrointestinal tract contains the largest and most complex bacterial community in the human body, which protects its host in concert with the immune system from colonization and infection by both pathogenic and opportunistic organisms. Especially patients currently under immunosuppression are constantly threatened by nosocomial infections, including multi-resistant pathogens such as extensively drug-resistant (XDR) *Pseudomonas aeruginosa*. Colonization with XDR *Pseudomonas aeruginosa* is favored by prophylactic broad spectrum antibiotic therapy, which disrupts the microbiota-mediated colonization and infection resistance. It is as yet unclear, whether immunodeficiency, mucositis, an altered microbial pattern, the specific virulence/fitness factors of the pathogens themselves or a combination of these factors account for the increased susceptibility towards colonization and even bloodstream infection with germs such as XDR *Pseudomonas aeruginosa*.

This project aims to clarify the role of the immune system as well as the microbiota using a C57BL/6 mouse model for preclinical evaluation of interventions targeting the GI microbiota. Conventional and germfree C57BL/6 mice are orally exposed to *Pseudomonas aeruginosa*, including XDR strains isolated from hemato-oncological patients suffering from septicemia. Immunodeficiency and alterations in the microbiota are induced through cyclophosphamide injection, antibiotic treatment and/or fecal transplantation. Longitudinal changes in the microbial pattern are analyzed before and after treatment/exposure. The animals' susceptibility to colonization and infection are evaluated. Sequencing of the microbiota allows for the selection of bacterial candidates for further intervention trials.

14 EVALUATION OF SUPPRESSIVE AND PRO-RESOLVING EFFECTS OF EPA AND DHA IN HUMAN PRIMARY MONOCYTES AND T-HELPER CELLS

A. Jaudszus[1], M. Gruen[2], C. Ness[2], B. Watzl[1], G. Jahreis[2]

[1]Dept. of Physiology and Biochemistry of Nutrition, Max Rubner-Institut, Karlsruhe
[2]Institute of Nutrition, Nutritional Physiology, Friedrich-Schiller-University, Jena

Besides their health beneficial anti-inflammatory properties, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may increase the infection risk at high doses, likely by generating an immune-depressed state. To assess the contribution of different immune cell populations to the immunomodulatory fatty acid effect, we comparatively investigated several aspects of inflammation in human T-helper (Th) cells and monocytes.

Both fatty acids, but DHA to a lesser extent compared to EPA, selectively and dose-dependently reduced the percentage of cytokine expressing Th cells in a PPAR-gamma-dependent fashion, whereas the expression of the cell surface marker CD69 was unaltered on activated T cells. In monocytes, both EPA and DHA increased IL-10 without affecting TNF-α and IL-6. Cellular incorporation of EPA and DHA occurred mainly at the expense of arachidonic acid. Concomitantly, TXB2 and LTB4 in supernatants decreased, while levels of TXB3 and LTB5 increased. This increase was independent of activation and in accordance with cyclooxygenases expression patterns in monocytes. Moreover, EPA and DHA gave rise to a variety of mono- and trihydroxy derivatives of highly anti-inflammatory potential, such as resolvins and their precursors.

Our results suggest that EPA and DHA do not generally affect immune cell functions in an inhibitory manner but rather promote pro-resolving responses.

15 DENDRITIC CELL MATURATION: A PROTEOMICS APPROACH

G. Korkmaz[1], Marius Codrea[2], Sven Nahnsen[2], Boris Macek[3], Martin von Bergen[4], J-S. Frick[1]

[1]Institute of Medical Microbiology and Hygiene, University of Tübingen, Germany
[2]Quantitative Biology Center, University of Tübingen, Germany
[3]Proteome Center, University of Tübingen, Germany
[4]Helmholtz Centre for Environmental Research - UFZ Leipzig, Germany

Dendritic cells are integral components of the mammalian immune system, which take part in orchestrating and regulating the delicate balance of immune response. Dendritic cells (DCs) are potent activators of destructive responses of the immune system, at the same time, dendritic cells also take part in activating regulatory T-cells and dampening overly-destructive immune responses, as well as mediating immune tolerance. As can be expected, the multifaceted and sometimes contradictory functions of DCs are, at least in part, brought about by the phenotypical differences in dendritic cells that regulate the respective immune response. As an example, we have previously reported that feeding of *B. vulgatus* to IL-2$^{-/-}$ mice leads to production of semi-mature dendritic cells and prevents colitis, whereas feeding the pathogenic *E. coli* to IL2$^{-/-}$ mice leads to fully mature DCs and severe intestinal inflammation. Therefore, we believe that phenotypical differences in dendritic cells as seen in semi-mature and mature DCs, have an important effect on disease manifestation/progression in colitis. However, the intracellular factors and processes regulating dendritic cell maturation are not fully understood. In our project we aim to provide a closer look at the intracellular signalling pathways and processes that underlie dendritic cell maturation. Using dendritic cells generated *in vitro* from cultured mouse bone marrow, we induced semi-maturation by *B.vulgatus* stimulation and complete maturation by *E. coli* stimulation. The resulting cells are harvested and lysed for proteomics analysis. We performed total proteomics to analyze proteins that differ in their expression levels in different samples, and we also performed shotgun phosphoproteomics to detect proteins that are differentially phosphorylated. Thereby we aim to define proteins/processes/signalling pathways that "define" semi-mature and mature dendritic cells (expression analysis), and the factors that "lead" the cells towards different maturation states (phoshoproteomics analysis). In our preliminary analysis we have identified differentially regulated proteins that play important roles in inflammatory pathways, oxidative stress response, cytoskeletal remodelling and antigen processing/presentation. Next, we annotated the identified proteins into distinct functional classes using DAVID software, and plotted the observed expression level differences onto Voronoi treemaps to provide an overlook. We then employed the bioinformatical payhway annotation tool IPA, to have a deeper look in the major pathways in functional clusters (for example, differential regulation of proteins in Ras pathway with regard to immunologically relevant functions). Next we aim to finalize our results and analyze a selected set of pathways that are highly significant to maturation and immune response further by qPCR, FACS, ELISAs, Western Blotting and various assays for intracellular pathways, both on samples taken *in vitro* and *in vivo*. At the end of our project, we hope to provide a more systemic and comprehensive information on factors governing different states of dendritic cell maturation, as well as the effects of commensal and pathogenic bacteria on dendritic cell mediated immune regulation.

16 FINE-SCALE SPATIAL ARCHITECTURE OF INTESTINAL MICROBIAL COMMUNITIES IN MICE

O. Kuzyk[1], T. Decker[2], A. Loy[1], D. Berry[1]

[1]*Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, University of Vienna,*
[2]*Max F. Perutz Laboratories, University of Vienna, Austria*

The intestine is a multi-compartment organ that acts in consort with a complex microbiome to enable digestion and uptake of diet-derived nutrients. Most knowledge about this microbiome comes from stool samples, which represent a composite picture of intestinal microbial diversity. In the present study, we establish an approach to study fine-scale spatial structure of the intestinal microbiome in situ using sequencing technology and apply it to mice on different diets to examine the effect of dietary polysaccharides on microbiome composition. We cryopreserved regions of native intestinal tissue and contents with minimal disturbance, prepared longitudinal and cross-sectional samples and used laser micro-dissection to isolate small areas containing microbiota (~50x200x10 μm). We chose areas proximal and distal to the mucosal boundary from different intestinal compartments, extracted DNA, and applied bacterial 16S rRNA gene-targeted amplicon sequencing using Illumina MiSeq technology. From these microisolates we were able to succesfully investigate dominant bacterial families in different mouse intestinal tract locations. We have identified different bacterial families present in control vs. polysaccharide-free diet, have determined that the polysaccharide-free diet leads to decresed species richness in distal colon and leads to decreased fecal starch degrading activity, indicating the functional changes in the gut microbiota. The knowledge gained in this study shows the utility of the fine-scale approach and proves to be valuable for future studies of the functional significance of intestinal microniches.

17 BACTERIAL SURVIVAL STRATEGIES DURING INFLAMMATION

Anna Lange[1], Sina Beier[2], Daniel Huson[2], Stefan Henz[3], Klaus Hantke[4], Christa Lanz[3], Ingo B. Autenrieth[1] and Julia-Stefanie Frick[1]

[1]Interfacultary Institute for Microbiology and Infection Medicine Tübingen (IMIT), Germany
[2]Center for Bioinformatics, University of Tübingen, Germany
[3]Max Planck Institute for Developmental Biology, Tübingen, Germany
[4]University of Tübingen, Germany

During IBD the mostly well-balanced interplay between resident intestinal microbes is disturbed. It is well studied that the prevalence of certain Enterobacteriaceae is increased during inflammation whereas the amount of Bacteroidetes and Firmicutes is decreased.

We are interested in the mechanisms that lead to accumulation and blooming of Enterobacteriaceae in an inflamed gut. There are some hints on how enteropathogens like Salmonella benefit from inflammation but which survival strategies are used by commensal E. coli is not well investigated. We suppose that commensals might use similar strategies. Therefore we performed whole genome sequencing of our two model strains – an E. coli and a B. vulgatus isolate - to identify certain strain-specific survival strategies.

In E. coli we identified an ethanolamine and 1,2-propanediol utilization cluster which can also be found in Salmonella Typhimurium and may therefore reflect its competitive character during inflammation. In turn in B. vulgatus we found gene clusters that contribute to 1,2-propanediol production. This might reflect a certain microbe-microbe interaction. Additionally B. vulgatus contains an extensive "mobilome" which could also influence its ability to "adapt" to the environment..

18 PATHOGENICITY MECHANISMS OF *ENTEROCOCCUS FAECALIS* IN CHRONIC INTESTINAL INFLAMMATION

I. Lengfelder[1*], I. Sava[1*], S. Ocvirk[1], JJ. Hansen[2], RB. Sartor[2], D. Haller[1]

[1]*Chair of Nutrition and Immunology, 85350 Freising-Weihenstephan, ZIEL – Institute for Food and Health, Technische Universität München, Germany (www.nutrition-immunology.de)*
[2] *Division of Gastroenterology and Hepatology, Department of Medicine, University of North Carolina, USA*

Background: *Enterococcus faecalis* is a commensal of the human intestinal core microbiota harboring several putative virulence factors, which highlight its role as opportunistic pathogen. This dualistic character is supported by recent evidence linking *Enterococcus* spp. to the pathogenesis of inflammatory bowel diseases (IBD). Using IL10-/- mice, a well established mouse model of chronic colitis, monoassociated with wild-type *E. faecalis* and deletion mutants lacking different virulence-related genes, we have recently demonstrated that enterococcal polysaccharide antigen impacts biofilm formation and *in vivo* mucus penetration partially contributing to disease development, while functional *E. faecalis* lipoproteins involved in TLR2-mediated activation and recruitment of innate immune cells are required as prime bacterial structures responsible for the colitogenic activity of *E. faecalis* (PLOS Pathogens 2015).

Results: Next-generation RNA sequencing of *E. faecalis* identified 98 up- and 142 down-regulated genes under conditions of colonic inflammation. Surprisingly, the expression levels of most known *E. faecalis* virulence genes did not undergo substantial alterations, whereas several genes relevant for bacterial fitness were differentially expressed in the inflamed environment of the gut. Among the highest upregulated genes, a gene encoding for a major facilitator superfamily (MFS) transporter involved in antibiotic resistance mechanisms and several genes of the *Eut* locus controlling ethanolamine utilization were identified. In contrast to the cell wall components, *E. faecalis* OG1RFΔ*eut vw* mutant did not affect experimental colitis in IL10-/- mouse. Finally, *E. faecalis* adaption to the intestine of the germ-free mouse is associated with the occurrence of two different colony morphologies. Opaque and translucent colony variants were sequenced and functionally characterized with regard to shifts in bacterial virulence.

Conclusion: Innate immune activation mediated by *E. faecalis*-derived lipoproteins is the crucial step in the development of colitis in IL-10^{-/-} mice. Transcriptional profiling of the colitogenic bacteria identified additional disease-associated bacterial genes relevant for the fitness in an inflammatory milieu. Colonization of germ-free mice with *E. faecalis* OG1RF results in two different colony morphologies showing differences in growth characteristics and biofilm formation.

19 ACTIVATING TRANSCRIPTION FACTOR 6 LINKS UNRESOLVED UNFOLDED PROTEIN RESPONSE TO INTESTINAL DYSBIOSIS AND TUMORIGENESIS

Lobner, Elena[1]; Kober, Olivia[1]; Berger, Emanuel[1]; Clavel, Thomas[2]; Lagkouvardos, Ilias[2]; Weber, Achim[3]; Haller, Dirk[1, 2]

[1]*Technische Universität München, Chair of Nutrition and Immunology, Freising-Weihenstephan, Germany,*
[2]*Technische Universität München, ZIEL Institute for Food and Health, Freising-Weihenstephan, Germany,*
[3]*University Hospital Zurich, Institute of Surgical Pathology, Zurich, Switzerland*

Deregulation of the endoplasmic reticulum unfolded protein response (erUPR) at the epithelial level is involved in the onset and promotion of intestinal inflammation and several UPR-related risk polymorphisms are described for inflammatory bowel disease (IBD). IBD patients have an increased risk to develop colitis-associated cancer, suggesting a link between erUPR activation and oncogenic transformation.

To address the role of activating transcription factor (ATF)6-mediated erUPR signaling in intestinal epithelial cells (IEC), we generated Villin-Cre-driven IEC-specific transgenic mice overexpressing the activated form of ATF6 (nATF6[IEC]).

Intriguingly, homozygous nATF6[IEC-tg/tg] mice spontaneously developed colonic adenomas independent of major inflammatory processes at tumor onset, with an incidence of 100% at 12 weeks of age. In contrast, heterozygous nATF6[IEC-wt/tg] mice showed no spontaneous abnormalities and no tumor formation. However, heterozygous mice respond with the development of large intestinal tumors if combined with colitis susceptible IL-10 deficient mice or challenged with dextran sodium-sulfate.

Induction of erUPR-related gene expression and increased proliferation of IEC preceded tumor formation in nATF6[IEC-tg/tg] mice. In addition, loss of mucin-filled goblet cells was associated with increased microbial penetration of the mucus barrier. High-throughput 16S rRNA gene sequencing of cecal microbiota revealed a clear separation of bacterial communities according to genotype associated with reduced bacterial diversity. Finally, antibiotic treatment and germfree housing was shown to alleviate or even prevent tumor formation.

In conclusion, the novel nATF6[IEC] mouse model demonstrates for the first time a causal contribution of altered erUPR signaling to colonic tumorigenesis linked to changes in the gut microbiota. Unresolved activation of ATF6-related UPR signaling in IBD patients may increase their risk to develop colorectal cancer.

20 GENETIC STABILITY OF THE PROBIOTIC ESCHERICHIA COLI STRAIN NISSLE 1917 (EcN) – YET ANOTHER SAFETY ASPECT OF MUTAFLOR

Stefan Rund, Kathrin Stelzner, Tobias A. Oelschlaeger

Institut für Molekulare Infektionsbiologie, University of Wuerzburg, Germany

EcN, which has GRAS status, interferes in vitro with adhesion, replication and Shiga-toxin (Stx) production in EHEC strains (Rund et al. 2013; Reissbrodt et al. 2009). Since *stx* genes are usually encoded by λ-prophages and antibiotic resistant genes are present on a conjugative pESBL-plasmid in EAHEC strains from the 2011 outbreak in Germany, we tested EcN for becoming a host of λ-phages and of the pESBL-plasmid.

For that purpose Stx-phages from various EHEC/EAHEC (EDL933 O157:H7, 1530/99 O26:H11, TY3456 O104:H4, 4392/97 O145:H25) were isolated and mixed with either EcN or *E. coli* K-12 strains (MG1655, DH5α, HB101), plated in soft agar and phage plaques subsequently counted. In contrast to *E. coli* K-12 strains, there were never any phage plaques observed on EcN, neither was DNA of the corresponding λ-phage in or Stx-production by EcN detectable. The outer membrane protein LamB is the most important receptor for λ-phages. The role of the different C-terminal LamB sequence in EcN and the K-12 strains for the observed λ-phages-resistance of EcN is under investigation.

Similarly, after coincubation with EcN or the *E. coli* K-12 strains and the 2011 EAHEC strain TY3730 transconjugants were observed at a high ratio for *E. coli* K-12 strains harbouring plasmid pESBL but up to 4×10^6 – fold lower numbers of transconjugants for EcN. Interestingly, the isogenic microcin-negative EcN mutant SK22D showed a 180-fold higher conjugation rate than the EcN wild type. Obviously, the production of the two microcins H47 and M are only partly responsible for the very low transmission rate of the pESBL plasmid from EAHEC strain TY3730 into EcN. Also the conjugal transfer of the kanamycin-resistance mediating plasmid R1drd16 into EcN was 347- to 1729-fold less efficient compared with *E. coli* K-12 strains.

These results stress the fact, that EcN deserves GRAS status, also because of its high genetic stability.

Rund et al. (2013) Antagonistic effects of probiotic *Escherichia coli* Nissle 1917 on EHEC strains of serotype O104:H4 and O157:H7. Int J Med Microbiol 303: 1-8.

Reissbrodt et al. (2009) Inhibition of growth of Shiga toxin-producing *Escherichia coli* by nonpathogenic *Escherichia coli*. FEMS Microbiol Lett 290: 62-69.

21 DISTURBED WNT SIGNALLING CROSSTALK AND STEM CELL HOMEOSTASIS AT THE EPITHELIAL BARRIER

Maureen J. Ostaff[1], Lioba F. Courth[1,2], Sabrina R.D. Stebe[1,2] Eduard F. Stange[3], Jan Wehkamp[2]

[1] Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart and University of Tübingen, Germany [2] Department of Hepatology, Gastroenterology and Infectiology, University Hospital, Tuebingen, Germany [3] Department of Gastroenterology, Robert Bosch Hospital, Stuttgart, Germany

Small intestinal Crohn's Disease (CD), a debilitating chronic inflammation has been linked to Paneth cell impairments and distinct aberrations in the Wnt pathway. Paneth cells are crucial players in managing pathogenic threats and the homeostasis towards resident commensals. They critically depend on Wnt, which is also indispensable for stem cell maintenance and epithelial regeneration. We could previously associate the observed patient related Wnt impairments to irregularities in Paneth cell antimicrobial function. If they however also promote aberration in the epithelial regenerative homeostasis has not yet been studied. In addition, the effect of inflammation itself on ileal Wnt activity and Paneth cell antimicrobials is largely unknown. Using a tissue culture model we herein show that activated peripheral blood mononuclear cell (PBMC) derived immune cells can mediate Wnt pathway activation in small intestinal tissue via generation of Wnt ligands. This immune cell – epithelial tissue crosstalk is however disturbed in ileal CD. Furthermore we uncovered distinct expression differences in specific stem cell relevant genes with important functions in epithelial proliferation by analyzing mRNA levels and performing immunohistochemistry (IHC) of leucine-rich repeat G protein-coupled receptors (LGRs) and R-Spondin (RSPO) ligands. Interestingly, in cultured biopsies we could furthermore show that inflammatory conditions differently influence expression levels of these factors. The Proliferation marker Ki67 was also studied via real-time PCR and IHC, revealing a complicated picture with some patients displaying decreased and others increased levels. The presented results will contribute to the understanding of ileal CD pathogenesis, highlighting a potential role of a disturbed epithelial regenerative homeostasis and underscore an important role of Wnt signaling in the disease.

22 *T CELL DEFICIENCY RESULTS IN BLUNTED INFLAMMATORY RESPONSE DURING DYSBIOSIS-TRIGGERED COLITIS*

Urmi Roy[1], Eric Galvez[1], Till Strowig[1]

[1]*Microbial Immune Regulation, Helmholtz Centre for Infection Research (HZI), Inhoffenstraße 7, 38124, Braunschweig*

In healthy individuals the intestinal microbiota, a complex ecosystem comprising thousands of bacterial species, and the host maintain a mutually beneficial homeostasis termed eubiosis. However, changes in the host's health state, antibiotic treatments as well as other environmental factors may disrupt homeostasis of host and microbiota, resulting in dysbiosis. Notably, in many animal models of human diseases dysbiotic communities can directly modulate disease severity.

Our aim is to understand the role of specific components of the immune system that interplay with the dysbiotic microbiota to trigger pathologic changes. To do so we study the impact of a dysbiotic community (DysM) resulting from a deficiency of the Nlrp6 inflammasome on the host resulting in increased colitis severity. Important tools are isobiotic gene-deficient mouse lines in which the microbiota can be manipulated at will giving us a valuable opportunity to study the functionalities of immune pathways in eubiosis and dysbiosis.

In this model, DysM-exacerbated dextran sulfate sodium induced colitis severity was drastically decreased in T cell deficient mice (Tcrbd$^{-/-}$) compared to control mice suggesting a strong involvement of T cells. Furthermore, MHC class II-restricted TCR-transgenic and CD4-deficient mice were protected supporting that antigen-specific, potentially microbiota specific, CD4+ T cells are required in this model. Along these lines we observed significant increases in CD45+CD4+ T cells in DysM mice including both inflammatory IL17+ and regulatory Foxp3+ T cells further supported by multiplex immunoassay revealing local increases in proinflammatory cytokines IL-6 and IL-17.

Future investigations of the functions of intestinal CD4 T cells in DysM mice will be required to improve the understanding how a dysbiotic intestinal community results in alteration of disease severity.

23 THE ZYMOGEN GRANULE MEMBRANE GLYCOPROTEIN 2 (GP2) IN INFECTION AND INFLAMMATION

P. Schierack[1], S. Rödiger[1], D.C. Baumgart[2], D. Roggenbuck[1]

[1] *Faculty of Natural Sciences, Brandenburg University of Technology, Germany*
[2] *Division of Gastroenterology and Hepatology, Charité Medical School, Humboldt-University of Berlin, Germany*

The pathogenesis of Crohn's disease (CrD) and ulcerative colitis (UC), the two major inflammatory bowel diseases (IBD), remains poorly understood. Autoimmunity is considered to be involved in the triggering and perpetuation of IBD leading to overt disease. Approximately 30% of CrD patients and less than 8% of UC patients show evidence of humoral autoimmunity to exocrine pancreas. Pancreatic autoantibodies (PAB) were described for the first time in 1984, but the main autoantigenic target of PABs - the zymogen granule membrane glycoprotein 2 (GP2) - was identified only in 2009 (1). The expression of GP2 has been demonstrated at the site of intestinal inflammation in CrD. Novel ELISAs have confirmed the presence of IgA and IgG anti-GP2 PABs in CrD patients and revealed an association of antibody levels with a specific clinical phenotype and severity in CrD. Antibodies against GP2 were also recently found in other intestinal inflammatory disease like celiac disease.

GP2 is a homologue of the urinary Tamm-Horsfall protein (THP) which blocks bacterial adherence to urinary tract epithelial cells and inhibits infection. Similarly to THP, GP2 was demonstrated to be a specific bacterial receptor on microfold (M) cells of intestinal Peyer's patches, which are considered to be the original site of inflammation in CrD. Bacteria adhered in a species- and pathotype-specific manner to GP2 and bacterial adherence induced bacterial transcytosis (2, 3). Interestingly, bacteria isolated from CrD patients were less opsonized with GP2 than bacteria from healthy controls which emphasizes involvement of GP2 in inflammatory bowel disease (4). Finally, GP2 was defined as endogenous immunomodulator of innate as well as adaptive immune responses (5).

This contribution discusses the role of GP2 in intestinal homeostasis and in inflammation and infection.

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24 EFFECT OF HYPOXIA AND BED REST ON THE HUMAN GUT MICROBIOTA

N.S. Treichel [1], B. Stres [2], A. Schöler [1], R. Šket [2], T. Debevec [3], O. Eiken [4], I. B. Mekjavic [3], M. Schloter [1]

[1] Helmholtz Zentrum München, Research Unit Environmental Genomics, Neuherberg, Germany

[2] Department of Animal Science, University of Ljubljana, Ljubljana, Slovenia

[3] Department of Automation, Biocybernetics and Robotics, Jozef Stefan Institute, Ljubljana, Slovenia

[4] Department of Environmental Physiology, Swedish Aerospace Physiology Centre, Royal Institute of Technology, Stockholm, Sweden

The microbiota of the gut has great influence on the human physiology. It is involved in processing food, stimulating the immune system and defending its host against pathogens. Besides these important functions of the microbiome for health, changes in the microbiome have been observed with many human conditions. An imbalance in the microbial community is for instance supposed to lead to obesity, infection and inflammation.

Immobilized patients for example are a group of people who are prone to these conditions. The existing PlanHab project - initially designed to investigate physical inactivity and hypoxia induced changes in human physiological systems for life in space planetary habitats - was adopted to investigate the influence of hypoxia and immobilization on the microbiome of the human intestinal tract. Samples were collected from healthy male subjects during normoxic bed rest, hypoxic bed rest or hypoxic ambulatory over a time period of 21 days. Investigation of the microbiome was performed by paired-end next generation sequencing. To detect the most responsive groups the sequences were taxonomically affiliated and analyzed at 97% sequence identity with QIIME using the Greengenes database.

The gut microbial community of subjects under hypoxic treatment showed a significantly different composition compared to that of subjects under normoxic conditions. The hypoxic samples contained a lower abundance of bacteria belonging to the families of Prevotellaceae, Veillonellaceae, Ruminococcaceae and the Lachnospiraceae compared to the normoxic samples, while the amount of bacteria belonging to the Bacteroidaceae was increased.

To get a more detailed understanding of these changes and their influence on health, further experiments will be needed.

25 ROLE OF EPITHELIAL EXPRESSION OF THE GLYCOSYLTRANSFERASE ß-1,4-N-ACETYLGALACTOSAMINYLTRANSFERASE IN HOST-PATHOGEN INTERACTIONS

Janice A. Seidel*, Natalie Steck, Philipp Rausch, John F. Baines and Guntram A. Grassl

Institute for Experimental Medicine, Christian-Albrechts-University, Kiel, Germany and Hannover Medical School, Hannover, Germany
* Presenter, contact: seidel.janice@gmail.com

Glycans take part in regulating host-microbe interactions, e.g. by serving as attachment sites. The blood group glycosyltransferase β-1,4-N-acetylgalactosaminyl-transferase, which is encoded by the gene *B4galnt2,* is expressed variably in the intestine of mice. Alongside a loss of intestinal (epithelial) expression goes an accompanying vascular (endothelial) expression of *B4galnt2,* which leads to altered glycosylation of the clotting factor vWF (von Willebrand Factor) resulting in a higher clearance of vWF from circulation. Despite the resulting bleeding tendency this phenotype shows maintenance in the mouse population for several million years. Thus a protective role in host-pathogen interactions is to be assumed. Additionally it was observed that the loss of epithelial *B4galnt2* expression is associated with altered intestinal microbiota. Consequently we hypothesized that variation in *B4galnt2* expression leads to an alteration of susceptibility to intestinal pathogens. In order to specify the direct influence of intestinal *B4galnt2* expression on susceptibility to intestinal pathogens we performed in vitro experiments in order to investigate adhesion, invasion and activation of cell signaling. We used siRNA-mediated knockdown of *B4galnt2* in the intestinal epithelial cell line Mode-K and subsequent infection with *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) and *Citrobacter rodentium*. Adherent and invaded bacteria were determined using gentamicin-killing assays and inside-outside staining.

We found no difference in adhesion of *Salmonella* to *B4galnt2*-expressing or *B4galnt2*-knockdown cells. However, invasion of *S.* Typhimurium into *B4galnt2*-expressing cells is significantly higher. B4galnt2 had no influence on the adhesion or invasion of C. rodentium into Mode-K cells.

In conclusion, our data show that expression of *B4galnt2* facilitates invasion of *Salmonella* into epithelial cells.

26 Non-Targeted Metabolomics Approach to Study Impact of Whole Grain Diet on Human Gut Microbiome

K. Smirnov[1], I. Martinez[2], A. Walker[1], S. Forcisi[1], M. Lucio[1], J. Walter[2], P. Schmitt-Kopplin[1, 3]

[1]Research Unit Analytical BioGeoChemistry, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Germany
[2]Chair of Nutrition, Microbes and Gastrointestinal Health, University of Alberta, Canada
[3]Chair of Analytical Food Chemistry, Technische Universität München, 85354 Freising Weihenstephan, Germany.

Big efforts in current research have being put to connect alternations in human gut microbiota composition to host phenotypes. The present work is concentrated on three tasks: 1) to investigate how whole grain diet affects metabolome profiles of human fecal and plasma samples when analysed by non-targeted metabolomics; 2) to investigate the effect of diet on human fecal microbiome composition; 3) to link microbiome and metabolome information to describe possible interactions in host-microbiome-metabolome axis.

In a nutrition study a group of 28 healthy individuals were subjected to a crossover study design involving three kinds of whole grain diet (barley, brown rice, and combined diet) each of which lasted for four weeks followed by two-week wash-out period. Fecal and plasma samples were taken at baseline time point and at the end of every four-week diet regime. Metabolome profiling was performed by analysing fecal and plasma samples via ultrahigh resolution mass spectrometry (direct infusion electrospray Fourier ion cyclotron resonance mass spectrometry, 12 T). Fecal microbiota composition was characterized by pyrosequencing of 16S rRNA gene tags.

Evaluation of metabolomics datasets as well as microbiome dataset revealed features that are more prominent for certain diet types. In addition, unsupervised examination of the data revealed that fecal metabolomes and microbiomes clustered stronger by individuality than by dietary treatment showing that inter-individual differences in fecal microbiome composition and in fecal metabolome out-way the impact of diet. These individual differences is likely to lead to non-uniform responses of individuals treated by the same diet, and further analysis is concentrated on identifying individualized microbiome and metabolome signatures that determine how humans respond to a dietary treatment which can serve as a basis for personalized nutrition.

27 THE GUT MICROBIOTA MODULATES ENERGY METABOLISM IN THE HIBERNATING BROWN BEAR (*URSUS ARCTOS*)

Felix Sommer[1], Marcus Ståhlman[1], Olga Ilkayeva[2], Jon M. Arnemo[3,4], Jonas Kindberg[4], Johan Josefsson[5], Christopher Newgard[2], Ole Fröbert[5] and Fredrik Bäckhed[1,6,*]

[1]*Department of Molecular and Clinical Medicine, University of Gothenburg, Gothenburg, Sweden*
[2]*Duke Molecular Physiology Institute, Duke University Medical Center, Durham, USA*
[3]*Faculty of Forestry and Wildlife Management, Hedmark University College, Elverum, Norway*
[4]*Department of Wildlife, Fish, and Environmental Studies, Swedish University of Agricultural Sciences, Umeå, Sweden*
[5]*Department of Cardiology, Örebro University, Örebro, Sweden*
[6]*Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark*

Hibernation is an adaptation that helps many animals to conserve energy during food shortage in winter. Brown bears double their fat depots during summer and use these stored lipids during hibernation. Although bears seasonally become obese, they remain metabolically healthy. We analyzed the microbiota of free-ranging brown bears during their active phase and hibernation. Compared with the active phase, the hibernation microbiota had reduced diversity, reduced levels of Firmicutes and Actinobacteria, and increased levels of Bacteroidetes. Several metabolites involved in lipid metabolism including triglycerides, cholesterol and bile acids were also affected by hibernation. Transplantation of the bear microbiota from summer and winter to germ-free mice transferred some of the seasonal metabolic features and demonstrated that the summer microbiota promoted adiposity without impairing glucose tolerance, suggesting that seasonal variation in the microbiota contributes to host energy metabolism in the hibernating brown bear.

Keywords: microbiota / obesity / metabolism / hibernation / brown bear

28 ESTIMATING ABSOLUTE ABUNDANCES OF BACTERIA IN THE INTESTINAL MICROBIOME

F. Stämmler[1], J. Gläsner[2], A. Hiergeist[2], E. Holler[3], U. Reischl[2], A. Gessner[2], R. Spang[1]

[1]Institute of Functional Genomics, University of Regensburg, Regensburg, Germany
[2]Institute of Clinical Microbiology and Hygiene, University Medical Center, Regensburg, Germany
[3]Department of Hematology and Oncology, Internal Medicine III, University Medical Center, Regensburg, Germany

Microbial dysbiosis in the intestinal tract is associated with several diseases and metabolic disorders, like inflammatory bowel disease, diabetes and obesity. Sequencing of the 16S-rRNA gene is commonly used to profile microbial communities in the intestinal tract. Current studies compare relative abundances of organisms in different samples. However, fold changes of relative abundances can be hard to interpret. If the relative abundance of a bacterium increases, this can be either a consequence of an absolute increase of this bacterium or a decrease of the background consisting of all other bacteria. Here we suggest to use exogenous spike-in bacteria to estimate absolute bacteria abundances. These spike-in bacteria, rather than spike-in DNAs, offer control for variability in lysis and DNA amplification. We employ a spike-in normalization and validate it in a series of controlled experiments. For these experiments we vary the absolute abundances of certain bacteria and that of the background bacteria. Recovery of the expected fold changes is used as validation. The observed fold changes are consistent with those expected. Our protocol allows for an unbiased quantification of fold changes of absolute abundances that can differ significantly from fold changes of relative abundances. Finally we apply our method on a clinical data set of patients receiving allogenic stem cell transplantion, where we show overgrowth of Enterococcus after depletion of the patients gut microbiome, which can not be concluded by relative quantification alone.

29 INFLUENCE OF DIETARY COMPONENTS ON THE INTESTINAL MUCOSAL BARRIER AND TOOLS FOR ASSESSMENT OF THE INTESTINAL PERMEABILITY

Valentina Volynets, Stephan C. Bischoff

Dept. of Nutritional Medicine, University of Hohenheim, Stuttgart, Germany

Background: Numerous studies in mice and in humans indicated an influence of dietary components such as fats, sugars or fibers on the intestinal microbiota composition and the mucosal barrier function. However, most studies did not focus on diet primarily, and very different barrier assessment tools were used which limits the comparison of results from different studies.

Methods: To study the dietary impact of diet on the intestinal barrier, we first assessed and compared multiple means to measure barrier functions in healthy mice (FITC-Dextran 4000 (FITC), ovalbumin (OVA), and lipopolysaccharide (LPS) in plasma; polyethylene glycol (PEG) 400 /4000 and lactulose/ mannitol (Lac/Man) in urine), and then measured such parameters in mice exposed to different diets for 12 weeks (control diet (CD), CD plus drinking water supplemented with 30% fructose (CD+F), western style diet (WSD) or WSD plus fructose (WSD+F)).

Results: According to our results, FITC, LPS, PEG 400 and 4000 were best suitable to assess intestinal barrier functions in mice. These parameters were used in the feed experiments. An additional feeding of mice with fructose in addition to CD or WSD caused only minor weight gain within the given time compared to the respective control group. However, in both groups, fructose induced a significantly higher liver weight (CD: 0.88±0.03 g, CD+F: 1.20±0.03 g; WSD: 1.41±0.08 g; WSD+F: 1.55±0.10 g; all $p<0.001$) and liver to body weight ratio (CD: 4.26±0.2 %, CD+F: 5.39±0.1 %; WSD: 5.1±0.2 %; WSD+F: 5.7±0.1 %; all $p<0.05$). Plasma concentrations of FITC were enhanced by fructose (CD vs. CD+F, 1.56 ± 0.21 vs. 1.91 ± 0.46; WSD vs. WSD+F, 1.47 ± 0.16 vs 3.11 ± 0.76 µg/ml, $p <0.05$). LPS concentrations in portal vein plasma doubled in mice receiving fructose in addition to CD or WSD, respectively ($p<0.05$). Only feeding with 30% fructose resulted in an increased intestinal permeability based on PEG4000 recovery, but not PEG400 recovery.

Conclusion: Our data show that – provided suitable means to assess intestinal barrier functions are used – hypercaloric WSD and in particular fructose-enriched CD and WSD diet cause intestinal barrier disturbance largely independent of changes in body weight.

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30 STABLE ISOTOPE LABELING BY AMINO ACIDS IN CELL CULTURE BASED PROTEOMICS REVEALS DIFFERENCES IN PROTEIN ABUNDANCES BETWEEN SPIRAL AND COCCOID FORMS OF THE GASTRIC PATHOGEN *HELICOBACTER PYLORI*

Müller SA, Pernitzsch SR, Haange SB, Uetz P, Sharma CM, Kalkhof S, von Bergen M

Helicobacter pylori (H. pylori) is a ε-proteobacterium that colonizes the stomach of about half of the world's population. Persistent infections have been associated with several gastric diseases. Mainly rod- or spiral shaped but also coccoid H. pylori forms have been isolated from mucus layer biopsies of patients. It is still being debated whether the coccoid form can be transformed back into the spiral form or whether this morphology is a result of bacterial cell death or persistence. We established stable isotope labeling by amino acids in cell culture (SILAC) for quantitative proteomics of H. pylori and applied it to investigate differences between the spiral and the coccoid morphology. We detected 72% and were able to relatively quantify 47% of the H. pylori proteome. Proteins involved in cell division and transcriptional and translational processes showed a lower abundance in coccoid cells. Additionally, proteins related to host colonization, including CagA, the arginase RocF, and the TNF-α inducing protein were down-regulated. The fact that outer membrane proteins were observed at higher abundances might represent a mechanism for immune evasion but also preserves adherence to host cells. The established protocol for relative protein quantification of H. pylori samples offers new possibilities for research on H. pylori.

Our study shows that SILAC can be employed to study protein abundance changes in H. pylori. We have chosen to establish SILAC for H. pylori because it facilitates fractionation on both, protein and peptide level and thus enables deep proteome coverage. Furthermore, SILAC allows robust and highly accurate protein quantification. The manuscript includes a detailed description of the applied method, suggestions for further improvement as well as a practical application. The investigation of differences between the coccoid and infectious spiral morphology of H. pylori with SILAC revealed the regulation of proteins that are involved in host colonization, motility, cell division as well as transcriptional and translational processes. The data will help molecular biologist to focus on relevant pathways that were found to be regulated in response to morphological changes. Furthermore, the application of SILAC offers new possibilities to study the biology of H. pylori. It enables to monitor protein abundance changes in response to certain stimuli such as oxygen stress or antibiotics. Moreover, SILAC raises the possibility to study co-cultures of host cells and H. pylori on protein level. Additionally, pulsed SILAC experiments enable the quantification of protein turnover.

31 DIFFERENT GASTRIC MICROBIOTA COMPOSITIONS IN TWO POPULATIONS WITH HIGH AND LOW GASTRIC CANCER RISK IN COLOMBIA

Ines Yang[1,2], Sabrina Woltemate[1,2], Sandra Nell[1,2], M. Blanca Piazuelo[3], Alberto Delgado[3], Pelayo Correa[3], Christine Josenhans[1,2], James G. Fox[4*], Sebastian Suerbaum[1,2*]

[1] Institute of Medical Microbiology and Hospital Epidemiology, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany

[2] German Center for Infection Research, Hannover-Braunschweig Site, Carl-Neuberg-Str. 1, 30625 Hannover

[3] Departments of Cancer Biology and Medicine, Vanderbilt University, Nashville, TN, USA

[4] Division of Comparative Medicine, MIT, Cambridge, MA, USA

Among the inhabitants of the Colombian state of Nariño, stomach cancer rates in the Andean region around Túquerres are strikingly higher than in the coastal region around Tumaco. This is in contrast to the very similar levels of *H. pylori* infection, but is associated with differences in human and bacterial ancestries. In order to investigate whether bacteria other than *H. pylori* contribute to the differences in susceptibility between the inhabitants of the two regions, we analysed the composition of the gastric microbiota of individuals from both regions (n=20 each). In spite of very high within-population variability, we found significant differences in stomach microbiota between the two populations. We identified operative taxonomic units (OTUs) and phylogenetic clades with significant abundance differences between the two towns. This included two OTUs significantly more abundant in Túquerres, which were identified as *Leptotrichia wadei* and as a member of the genus *Veillonella*, respectively, and 16 OTUs significantly more abundant in Tumaco. Tumaco-specific OTUs included an OTU identified as a member of the genus *Staphylococcus* which was found in 35% of the Tumaco samples. Additionally, we identified OTUs correlated with patient characteristics such as diagnosis of intestinal metaplasia of the stomach epithelium. We also tested for correlation of the microbiota composition with the population, ancestry and *cag*PAI status of the infecting *H. plyori* strains.

Follow-up studies to test candidate bacterial strains for their accelerating or protective effect on the development of *H. pylori*-induced preneoplastic lesions in animal models are under way.

Participants

PARTICIPANTS 2015

Prof. John Baines
Max-Planck-Institute for Evolutionary Biology
Evolutionary Genomics
August-Thienemann-Str. 2
24306 Plön
baines@evolbio.mpg.de

Ms. Monika Bazanella
TU München
Lehrstuhl für Ernährung und Immunologie
Gregor-Mendel-Str.2
85354 Freising
Monika.Bazanella@tum.de

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

Prof. Stephan C. Bischoff
Universität Hohenheim
Ernährungsmedizin
Fruwirthstr. 12
70599 Stuttgart
kerstin.sommer@uni-hohenheim.de

Prof. André Bleich
MHH
Institute for Laboratory Animal Science
Carl-Neuberg-Str. 1
30625 Hannover
bleich.andre@mh-hannover.de

Prof. Susanne Brix
Technical University of Denmark
Department for Systems Biology
Soltofts Plads, Buildn 221
DK-2800 Kgs. Lyngby
sbp@bio.dtu.dk

Ms. Stephanie Buchheister
MHH
Institute for Laboratory Animal Science
Carl-Neuberg-Str. 1
30625 Hannover
buchheister.stephanie@mh-hannover.de

Dr. Marijana Basic
MHH
Institute of Laboratory Animal Science
Carl-Neuberg-Str.1
30625 Hannover
basic.marijana@mh-hannover.de

Prof. Christoph Becker
University of Erlangen-Nuremberg
Dept. of Medicine 1
Hartmannstrasse 14
91052 Erlangen
christoph.becker@uk-erlangen.de

Mr. Markus Beutler
LMU Muenchen
Max von Pettenkofer-Institut
Pettenkoferstr. 9a
80336 Muenchen
Beutler@mvp.uni-muenchen.de

Prof. Michael Blaut
DIfE
Gastrointestinal Microbiology
Arthur-Scheunert-Allee 114 - 116
14558 Nuthetal
blaut@dife.de

Dr. Erwin Bohn
Universitätsklinikum Tübingen
Medical Microbiology
Elfriede Aulhornstrasse 6
72070 Tübingen
erwin.bohn@med.uni-tuebingen.de

Mrs. Inga Brüsch
MHH
Institute for Laboratory Animal Science
Carl-Neuberg-Str1
30625 Hannover
bruesch.inga@mh-hannover.de

Mrs. Susanne Bury
Institute for Molecular Infection Biology
Infection Biology
Josef-Schneider-Str. 2/D15
97080 Würzburg
susibury@hotmail.de

Dr. Ludovica Butto
TUM
Nutrition and Immunology
Gregor-Mendel-Str. 2
85350 Freising
ludovica.butto@tum.de

Prof. Nadine Cerf-Bensussan
Université Paris Descartes-Sorbonne Paris
Cité
Institut IMAGINE-INSERM 1163; Laboratory
of Intestinal Immunity
24, boulevard du Montparnasse
F-75015 Paris
nadine.cerf-bensussan@inserm.fr

Dr. Christoph Cichon
Center for Molecular Biology of Inflammation
Institute of Infectiology
Von-Esmarch-Strasse 56
48149 Münster
cichon@uni-muenster.de

Prof. Andreas Diefenbach
University of Mainz
Research Centre Immunology and Institute
of Medical Microbiology
Obere Zahlbacher Strasse 67
55131 Mainz
diefenbach@uni-mainz.de

Prof. W. Florian Fricke
Universität Hohenheim
Nutrigenomics
Fruwirthstr. 12
70597 Stuttgart
w.florian.fricke@uni-hohenheim.de

Prof. André Gessner
Institute of Clinical Microbiology and Hygiene
University of Regensburg
Franz-Josef-Strauss-Allee 11
93053 Regensburg
andre.gessner@ukr.de

Prof. Guntram Grassl
University of Kiel
Inst. for Experimental Medicine
Parkallee 30
23485 Borstel
ggrassl@fz-borstel.de

Prof. Elke Cario
Universitätsklinikum Essen
Klinik für Gastroenterologie und Hepatologie
Hufelandstr. 55
45122 Essen
elke.cario@uni-due.de

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

Dr. Thomas Clavel
TUM
ZIEL
Gregor-Mendel-Str 2
85354 Freising
thomas.clavel@tum.de

Prof. Julia Frick
University of Tübingen
Medical Microbiology
Elfriede-Aulhorn-Strasse 6
72076 Tübingen
julia-stefanie.frick@med.uni-tuebingen.de

Ms. Janina Geißert
Interfaculty Institute for Microbiology and
Infection Medicine
University Clinics Tübingen
Elfriede-Aulhorn-Str. 6
72076 Tübingen
janina.geissert@med.uni-tuebingen.de

Dr. Georg Gradl
Eurofins Genomics
NGS Sales
Anzinger Str. 7a
85560 Ebersberg
georggradl@eurofins.com

Dr. Hans-Dieter Grimmecke
Laves Arzneimittel GmbH
R&D
Laves Strasse
CH-6247 Schötz
d.grimmecke@laves-pharma.ch

Dr. Claudia Günther
Universitätsklinikum Erlangen
Medizinische Klinik1
Hartmannstrasse 14
91052 Erlangen
c.guenther@uk-erlangen.de

Prof. Dirk Haller
Technische Universität München
Chair of Nutrition and Immunology
Gregor-Mendel-Str. 2
85354 Freising
dirk.haller@tum.de

Prof. Anton Hartmann
Helmholtz Zentrum München
Dep. for Environmental Sciences, Research
Unit Microbe-Plant Interactions
Ingolstaedter Landstrasse 1
85764 Neuherberg
anton.hartmann@helmholtz-muenchen.de

Dr. Markus Heimesaat
Charité-University Medicine Berlin
Institute for Microbiology and Hygiene
Garystr. 5
14195 Berlin
markus.heimesaat@charite.de

Mr. Florian Hölzl
Universitätsklinikum Tübingen
Institut für Medizinische Mikrobiologie und
Hygiene
Elfriede-Aulhorn-Straße 6
72076 Tübingen
florian.hoelzl@med.uni-tuebingen.de

Prof. Daniel Huson
University of Tuebingen
Center for Bioinformatics
Sand 14
72076 Tuebingen
daniel.huson@uni-tuebingen.de

Dr. Anke Jaudszus
Max Rubner-Institute
Physiology and Biochemistry of Nutrition
Haid-und-Neu-Str. 9
76131 Karlsruhe
anke.jaudszus@mri.bund.de

Mr. Sven Haange
Helmholtz Centre for Environmental
Research-UFZ
Department of Proteomics
Permoserstrasse 15
4318 Leipzig
sven.haange@ufz.de

Dr. Buck Hanson
University of Vienna
Department of Microbiology and Ecosystem
Science
Althanstrasse 14
A-1090 Wien
hanson@microbial-ecology.net

Ms. Manuela Hefele
Universitätsklinikum Erlangen
Medizin 1
Hartmannstraße 14
91052 Erlangen
Manuela.hefele@uk-erlangen.de

Ms. Simone Herp
LMU Muenchen
Max von Pettenkofer-Institut
Pettenkoferstr. 9a
80336 Muenchen
herp@mvp.uni-muenchen.de

Prof. Mathias Hornef
RWTH Aachen
Med. Microbiology
Pauwelsstr. 30
52074 Aachen
mhornef@ukaachen.de

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

Dr. Nico Jehmlich
Helmholtz-Zentrum für Umweltforschung
GmbH - UFZ
Proteomik
Permoser Straße 15
4318 Leipzig
nico.jehmlich@ufz.de

Prof. Christine Josenhans
Medizinische Hochschule Hannover
Institut für Medizinische Mikrobiologie und
Krankenhaushygiene
Carl-Neuberg-Str. 1
30625 Hannover
josenhans.christine@mh-hannover.de

Ms. Anna Katharina Koller
FAU Erlangen
Klinische Mikrobiologie
Wassertrumstraße 3/5
91054 Erlangen
Anna.Koller@uk-erlangen.de

Prof. Karsten Kristiansen
University of Copenhagen
Biology
Universitetsparken 13
DK-2100 Copenhagen
kk@bio.ku.dk

Ms. Anna Lange
University of Tuebingen
Institute for Medical Microbiology & Hygiene
Elfriede-Aulhorn-Straße 6
72076 Tuebingen
anna.lange@med.uni-tuebingen.de

Ms. Isabella Lengfelder
Technische Universität München
Ernährung und Immunologie
Gregor-Mendel-Str. 2
85350 Freising
isabella.lengfelder@tum.de

Dr. Gunnar Loh
Max Rubner-Institute
Physiology and Biochemistry of Nutrition
Haid-und-Neu-Str. 9
76131 Karlsruhe
gunnar.Loh@mri.bund.de

Prof. Jochen Mattner
FAU Erlangen
Klinische Mikrobiologie
Wasserturmstr. 3-5
91054 Erlangen
jochen.mattner@uk-erlangen.de

Dr. Olivia Kober
Technical University Munich
Nutrition and Immunology
Gregor-Mendel Strasse 2
85354 Freising
olivia.kober@tum.de

Mr. Giray Korkmaz
UKT Mikrobiologie
AG Frick
Elfriede-Aulhorn Str. 6
72072 Tübingen
giray.korkmaz@med.uni-tuebingen.de

Mr. Orest Kuzyk
University fo Vienna
Department of Microbial Ecology
Althanstr. 12
A-1090 Vienna
orest.kuzyk@univie.ac.at

Dr. Antje Legatzki
Klinikum der Universität München (LMU)
Dr. von Haunersche Kinderklinik
Lindwurmstrasse 4
80337 München
antje.legatzki@med.uni-muenchen.de

Ms. Elena Lobner
TUM
Nutrition & Immunology
Gregor-Mendel-Str. 2
85354 Freising
elena.lobner@tum.de

Dr. Alexander Loy
University of Vienna
Department of Microbiology and Ecosystem
Research
Althanstrasse 14
A-1090 Wien
loy@microbial-ecology.net

Ms. Sarah Menz
University of Tuebingen
Medical Microbiology and Hygiene
Elfriede-Aulhorn-Straße 6
72076 Tuebingen
sarah.menz@med.uni-tuebingen.de

Dr. Georg Munz
Deutsche Forschungsgemeinschaft (DFG)
-Lebenswissenschaften 1-
53170 Bonn
Georg.Munz@dfg.de

Dr. Maureen Ostaff
Robert Bosch Gesellschaft für medizinische
Forschung GmbH
Dr. Margarete Fischer Bosch Institute of
Clinical Pharmacology
Auerbachstr.112
70376 Stuttgart
maureen.ostaff@ikp-stuttgart.de

Dr. Fatima Pereira
University of Vienna
Department of Microbiology and Ecosystem
Science
Althanstr. 14
A-1090 Vienna
pereira@microbial-ecology.net

Prof. Hans-Christian Reinecker
Harvard Medical School
Massachusetts General Hospital
Gastrointestinal Unit; Jackson bldg. R711;
Fruit Street
Boston MA 02114
USA
hans-christian_reinecker@hms.harvard.edu

Ms. Urmi Roy
Helmholtz Center for Infection Research
Microbial Immune regulation
Inhoffenstrasse,7
38124 Braunschweig
Urmi.Roy@helmholtz-hzi.de

Prof. Peter Schierack
Brandenburgische Technische Univerisität
Cottbus-Senftenberg
Fakultät für Naturwissenschaften
Großenhainer Str. 57
1968 Senftenberg
peter.schierack@b-tu.de

Dr. Anne Schöler
Helmholtz Zentrum München
Environmental Genomics
Ingolstädter Landstraße 1
85764 Oberschleißheim
anne.schoeler@helmholtz-muenchen.de

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

Prof. Eric G. Pamer
Memorial Sloan Kettering Cancer Center
Infectious Diseases Service
1275 York Avenue
New York, New York 10065
USA
pamere@MSKCC.ORG

Prof. Manuela Raffatellu
University of California Irvine
Department of Microbiology and Molecular
Genetics; Institute for Immunology
B251 Med Sci I
Irvine, CA 92697-4025
USA
manuelar@uci.edu

Mr. Felix Rost
University Hospital Zurich / University of
Zurich
Gastroenterology and Hepatology
Raemistrasse 100
CH-8091 Zurich
felix.rost@usz.ch

Mrs. Monika Schaubeck
TU Munich
Nutrition and Immunology
Gregor-Medel-str.2
85350 Freising
monika.schaubeck@tum.de

Ms. Franziska Schmidt
Charité Universitätsmedizin Berlin
Medizinische Klinik für Gastroenterologie,
Infektiologie und Rheumatologie
Hindenburgdamm 30
12203 Berlin
f.schmidt@charite.de

Dr. Jutta Schröder-Braunstein
University Hospital Heidelberg
Immunology
Im Neuenheimer Feld 305
69120 Heidelberg
jutta.schroeder-braunstein@immu.uni-
heidelberg.de

Ms. Janice Seidel
UKSH Kiel
IEM
Arnold-Heller-Straße 3
24105 Kiel
seidel.janice@gmail.com

Prof. Christian Sina
Molekulare Gastroenterologie
Medizinische Klinik 1
Ratzeburger Allee 160
23538 Lübeck
christian.sina@uksh.de

Dr. Felix Sommer
University of Gothenburg
Department of Molecular and Clinical
Medicine
Bruna Stråket 16
S-41345 Gothenburg
felix.sommer@wlab.gu.se

Mr. Frank Stämmler
University of Regensburg
Statistical Bioinfomatics
Josef-Engert-Str. 9
93053 Regensburg
frank.staemmler@klinik.uni-regensburg.de

Prof. Bärbel Stecher
LMU München
Medicine
Pettenkoferstrasse 9a
80336 München
stecher@mvp.uni-muenchen.de

Prof. Sebastian Suerbaum
Medizinische Hochschule Hannover
Institut für Medizinische Mikrobiologie und
Krankenhaushygiene
Carl-Neuberg-Str. 1
30625 Hannover
suerbaum.sebastian@mh-hannover.de

Dr. Abdulhadi Suwandi
Hannover Medical School
AG Grassl / Medical Microbiome Research,
Institute for Medical Microbiology and
Hospital Epidemiology
Carl-Neuberg-Str. 1
30625 Hannover
a.suwandi@iem.uni-kiel.de

Prof. Britta Siegmund
Charité
Medicine
Hindenburgdamm 30
12200 Berlin
britta.siegmund@charite.de

Mr. Kirill Smirnov
Helmholtz Zentrum München GmbH
Research Unit Analytical BioGeoChemistry
Ingolstädter Landstraße 1
85764 Neuherberg
kirill.smirnov@helmholtz-muenchen.de

Dr. Ulrich Sonnenborn
Ardeypharm GmbH
Biological Research
Loerfeldstr. 20
58313 Herdecke
sonnenborn@ardeypharm.de

Ms. Sabrina Stebe
University of Tübingen
University Hospital Tübingen
Otfried-Müller-Str. 10
72076 Tübingen
sabrina.stebe@ikp-stuttgart.de

Dr. Till Strowig
Helmholtz Institute fpr Infection Research
Microbial Immune Regulation
Inhoffenstr. 7
38124 Braunschweig
tst13@helmholtz-hzi.de

Ms. Annika Sünderhauf
University of Lübeck
Moleculare Gastroenterologie
Ratzeburger Allee 160
23562 Lübeck
annika.suenderhauf@web.de

Mrs. Sophie Thiemann
Helmholtz Centre for Infection Research
MIKI
Inhoffenstraße 7
38124 Braunschweig
sophie.thiemann@helmholtz-hzi.de

Prof. Herbert Tilg
Universitätsklinik für Innere Medizin I
Innsbruck
Endokrinologie, Gastroenterologie und
Stoffwechselerkrankungen
Anichstrasse 35
A-6020 Innsbruck
Herbert.Tilg@i-med.ac.at

Dr. Valentina Volynets
University of Hohenheim
Institute of Nutritional Medicine
Fruwirtstr. 12
70599 Stuttgart
volynets@uni-hohenheim.de

Dr. Rudolf von Buenau
Ardeypharm GmbH
Biological Research
Loerfeldstr. 20
58313 Herdecke
buenau@pharma-zentrale.de

Dr. Alesia Walker
Helmholtz Zentrum München
Research Unit Analytical BioGeoChemistry
Ingolstädter Landstrasse 1
85764 Oberschleissheim
alesia.walker@helmholtz-muenchen.de

Dr. Stefan Wirtz
FAU Erlangen-Nuremberg
Medical Department 1
Hartmannstrasse 14
91052 Erlangen
stefan.wirtz@uk-erlangen.de

Dr. Ines Yang
Medizinische Hochschule Hannover
Institut für Medizinische Mikrobiologie und
Krankenhaushygiene
Carl-Neuberg-Str. 1
30625 Hannover
yang.ines@mh-hannover.de

Dr. Jessica Tuerk
Yakult Deutschland GmbH
Relation Management
Forumstr. 2
41468 Neuss
jtuerk@yakult.de

Prof. Martin von Bergen
Helmholtz Centre for Environmental
Research
Proteomics/Metabolomics
Permoser Strasse 15
4318 Leipzig
Martin.vonbergen@ufz.de

Prof. Alan Walker
University of Aberdeen
Rowett Institute of Nutrition and Health
M15, Boyd Orr Building; Bucksburn
Aberdeen AB21 9SB
UK
alan.walker@abdn.ac.uk

Prof. Jens Walter
University of Alberta
Chair for Nutrition, Microbes, and
Gastrointestinal Health
4-126A Li Ka Shing Centre for Health
Research Innovation and 7-142 Katz Group
Center
Edmonton, Alberta; T6G 2E1
Canada
jwalter1@ualberta.ca

Dr. Anni Woting
DIfE
Gastrointestinal Microbiology
Arthur-Scheunert-Allee 114 - 116
14558 Nuthetal
Anni.Woting@dife.de

Dr. Mario Zaiss
ETH Lausanne / University of Erlangen
Nuremberg
Science de la vie / Medical Clinic III
Glückstrasse 4
91054 Erlangen
mario.zaiss@epfl.ch