9th Seeon Conference

Microbiota, Probiotics and Host

Mikrobiota, Probiotics und Wirt

24.- 26. June 2016

Conference Center

Monastery Seeon / Chiemsee

For more information:

www.seeon-conference.de

June 24th, 2016

Dear Participant,

On behalf of the German Society of Hygiene and Microbiology (DGHM) and the Organizing Committee, welcome to the 9th Seeon Conference “Microbiota, Probiotics and Host”!

The dramatic increase of chronic inflammatory and degenerative diseases particularly in the industrialized world implies a dynamic interaction of disease susceptible genomes with an enormously complex environment. Nutrition-related factors together with components of mucosa-associated microbial ecosystems especially in the gastrointestinal system emerged as prime environmental triggers for the development and modification of metabolically-driven and inflammation-mediated pathologies.
Our DGHM section “Microbiota, Probiotics and Host” has established a visible community of talented young and senior scientists across various disciplines including basic science, genetics, and clinical disciplines such as gastroenterology, medical microbiology and immunology, as well as nutritional medicine. During last years, the activities of our DGHM section have made an important contribution to the formation of the DFG Priority Programme “MICROBIOTA – a Microbial Ecosystem at the Edge between Immune Homeostasis and Inflammation” (SPP 1656). The “Seeon Conference” has become a known platform to critically discuss the role of microbe-host interactions in health and disease sharing cutting-edge science and technologies. Basis mechanisms of the host’s microbiome are discussed at the interface of metabolic and immune functions aiming to be implemented in therapy and prevention of chronic inflammatory, atopic and metabolic diseases. Thank you in advance for your contribution to this meeting. Your willingness to participate and share your expertise is greatly appreciated.

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. Barbara Stecher
Max von Pettenkofer-Institut
Microbiota + Infection
Pettenkoferstr. 9a
80336 München
Tel.: +49-(0)89-5160-5448
stecher@mvp.uni-muenchen.de

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PROGRAM Friday, June 24

15^{00} - 17^{00} Registration
17^{00} - 17^{15} Welcoming: J. Frick, Med. Microbiology + Hygiene, University Tübingen

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17^{15} – 18^{00} Keynote Lecture - **Claudia Mauri** (Immunology, University College London, UK): ***Induction of Regulatory B cells by Intestinal Microbiota***
Chair: J. Frick, Tübingen

18^{15} Dinner

MECHANISMS OF INFLAMMATION AND HOMEOSTASIS

19^{30}– 21^{00} Chair: G. Grassl, Hannover

Buchen B, Medical Clinic 1, Friedrich-Alexander-University, Erlangen, Germany
A single viral protein is able to disrupt intestinal immune homeostasis in vivo

Kitowski V, Medical Clinic 1, Friedrich-Alexander-University, Erlangen, Germany
Batf3 is a critical regulator of T-cell driven colitis

Kufer T.A, Institute for Medical Microbiology, Immunology and Hygiene, Cologne, Germany; University of Hohenheim, Institute of Nutritional Medicine, Stuttgart, Germany
Nod1 signalling is linked to F-actin remodelling

Lobner E, Chair of Nutrition and Immunology, Technische Universität München, Freising-Weihenstephan, Germany
Microbiota-dependent signals link ATF6-driven erUPR to colonic tumorigenesis

Roy U, Helmholtz Centre for Infection Research, Braunschweig, Germany
Distinct requirements for the microbiota for induction of anti-bacterial Th17, Th17/22 and Th22 CD4+ T cell population

Steimle A, Institute of Medical Microbiology and Hygiene, University of Tübingen, Germany
Symbiotic gut commensal bacteria act as cathepsin S activity regulators – a novel approach to treat autoimmune diseases

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21^{00} Drink at the Bar?

PROGRAM Saturday, June 25

08^{30} – 09^{15} Keynote Lecture - **Dana Philpott** (Immunology, University of Toronto, Canada): ***Autophagy and Control of Intracellular Bacteria***
Chair: M. Hornef, Aachen

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

IMPACT OF THE GUT MICROBIOTA ON INFECTION AND METABOLISM

09^{45} – 11^{15} Chair: A. Steimle, Tübingen

Clavel T, TU Munich, ZIEL Institute for Food and Health, Freising, Germany
Impact of early life intervention with Bifidobacteria on infant fecal microbiota and metabolite profile

Hefele M, Medical Clinic 1, Friedrich Alexander University, Erlangen, Germany
Caspase-8 maintains the intestinal barrier against pathogen challenge

Herp S, Max-von-Pettenkofer Institut, LMU München, GERMANY
Exploring the role of the commensal Mucispirillum schaedleri in enteric Salmonella enterica serovar Typhimurium infection

Just S, ZIEL Institute for Food and Health, Technische Universität München, Freising, Germany
Gut-derived Coriobacteriaceae increase white adipose tissue deposition in mice

Rausch P, Institute for Experimental Medicine, Christian-Albrechts-University of Kiel, Kiel, Germany; Max Planck Institute for Evolutionary Biology, Plön, Germany
The guts of blood-group antigens: B4galnt2 alters pathogen susceptibility through the intestinal microbiome

Wells J, Top Institute Food and Nutrition, Wageningen, The Netherlands; Host-Microbe Interactomics Group, Wageningen University and Research Center, Wageningen, The Netherlands
Age-associated impairment of the mucus barrier function is associated with profound changes in microbiota and immunity

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11^{15} – 12^{00} Keynote Lecture - **Harry J. Flint** (The Rowett Institute of Nutrition and Health, University of Aberdeen, UK): ***Dietary modulation of gut microbiota and metabolites***
Chair: T. Clavel, Freising

PROGRAM Saturday, June 25

12^{00} - 13^{45} Lunch
12^{30} – 13^{30} Guided Geological Tour

GUT MICROBIOTA FUNCTIONS AND ECOLOGY

13^{45} – 15^{15} Chair: G. Loh, Karlsruhe

Hanson B, Dep. of Microbiology and Ecosystem Science, University of Vienna,
Exploring distal-gut microbial ecology and host-microbe metabolic interactions by in vivo stable isotope probing with ^{13}C-glucose ureide

Iljazovic A, Helmholtz Centre for Infection Research, Braunschweig, Germany
Microbial Interactions of Prevotella Spp. within the Intestinal Ecosystem

Kabbert J, RWTH Aachen, Institute of Molecular Medicine, Aachen, Germany
The inner coat counts

Neville A, Host-Microbiota Interactions Lab., Wellcome Trust Sanger Institute, UK
Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation

Pereira F, Dep. of Microbiology and Ecosystem Science, University of Vienna
Identifying and sorting host compound foragers from the gut microbiota by heavy water-based activity labelling and Raman microspectroscopy

Van Best N, Inst. of Med.Microbiology, RWTH University Hospital Aachen, Germany; Dep. of Med. Microbiology, NUTRIM, Maastricht University, The Netherlands
Postnatal establishment of the enteric microbiota

15^{15} – 15^{45} Coffee Break

15^{45} – 18^{15} **Poster Slam** (2 minutes / 2 slides) **and Poster discussion**
(J. Frick, Med. Microbiology + Hygiene, University Tübingen)

18^{15} – 19^{00} Keynote Lecture - **Philipp Engel** (Fundamental Microbiology, University of Lausanne, Schweiz): ***The honey bee gut microbiota - a versatile model for microbial symbiosis***
Chair: B. Stecher, München

19^{00} Dinner

PROGRAM Sunday, June 26

08^{30} – 09^{15} Keynote Lecture - **Pieter de Groot** (Internal Medicine, University of Amsterdam, Netherlands): ***Fecal transplantation to dissect causal role of gut microbiota in human disease***
Chair: J. Baines, Kiel

09^{15} – 09^{45} Coffee Break

09^{45} – 10^{00} **Poster Award**

METAGENOMICS AND EVOLUTION

10^{00} – 11^{30} Chair: D. Berry, Vienna

Haange SB, Department of Molecular Systems Biology, Helmholtz Centre for Environmental Research-UFZ, Leipzig
Gastric bypass surgery markedly perturbs the community structure and the functional composition of the intestinal microbiota

Lange A, Interfacultary Institute for Microbiology and Infection Medicine, Department for Medical Microbiology and Hygiene, University of Tübingen, Tübingen, Germany
Extensive mobilome-driven genome diversification in mouse gut-associated Bacteroides vulgatus mpk

Loh G, Max Rubner-Institut, Karlsruhe, Germany
Effect of dietary zinc on the horizontal transfer of antibiotic resistance genes in the intestine

Sommer F, Institute of Clinical Molecular Biology, Kiel University, Germany
The role of DUOX2 in shaping the intestinal microbiota and its effect on host physiology

Schierack P, Institute of Biotechnology, Brandenburg Technical University Cottbus - Senftenberg, Senftenberg, Germany
Intestinal Escherichia coli colonization in a Mallard duck population over four consecutive winter seasons

Zioutis C, Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, University of Vienna
Rapid genetic diversification of Bacteroides thetaiotaomicron in the murine gut

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11^{30} Lunch
12^{30} Departure

PROGRAM
Friday,
June 24

INDUCTION OF REGULATORY B CELLS BY INTESTINAL MICROBIOTA

Claudia Mauri

Centre for Rheumatology Research, Division of Medicine, University College London

Growing empirical evidence suggests that targeting the intestinal microbiota may offer new possibilities for the prevention and/or treatment of autoimmune disease. However, recent work by this laboratory has demonstrated that perturbation of the microbiota using antibiotic-treatment reduces not only the severity of autoimmune disease, but also the number and functional capacity of regulatory B cells (Bregs), a subset of B cells with suppressive capacity. We are currently investigating how the microbiota can be targeted for therapy without disturbing immune homeostasis. …

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19^{30}– 21^{00} Chair: G. Grassl, Hannover

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Nod1 signalling is linked to F-actin remodelling

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Microbiota-dependent signals link ATF6-driven erUPR to colonic tumorigenesis

Roy U, Helmholtz Centre for Infection Research, Braunschweig, Germany
Distinct requirements for the microbiota for induction of anti-bacterial Th17, Th17/22 and Th22 CD4+ T cell population

Steimle A, Institute of Medical Microbiology and Hygiene, University of Tübingen, Germany
Symbiotic gut commensal bacteria act as cathepsin S activity regulators – a novel approach to treat autoimmune diseases

A SINGLE VIRAL PROTEIN IS ABLE TO DISRUPT INTESTINAL IMMUNE HOMEOSTASIS IN VIVO

Buchen B. [1], Günther C. [1], Murtadak V. [2], Stürzl M. [2], Cesarman E. [3], Ballon G. [3], Neurath M. F. [1] and Becker C. [1]

[1]Medical Clinic 1, Friedrich-Alexander-University, Erlangen, Germany
[2]Department of Surgery, University Hospital, Erlangen, Germany
[3]Deparment of Pathology and Laboratory Medicine, Cornell University, New York, USA

Recently it has been demonstrated that mice which lack caspase-8 expression in intestinal epithelial cells (IECs, Casp8ΔIEC-mice) spontaneously developed inflammatory lesions in the terminal ileum and showed a high amount of necroptotic Paneth cell death, indicating dysregulated antimicrobial immune cell functions in IECs. On a cellular level, the caspase-8 activity is regulated by cellular FLIPs (cFLIPs), which are expressed in two different isoforms, cFLIPlong and cFLIPshort. Interestingly certain viruses, like herpesviruses and poxviruses, express a viral FLIP (vFLIP) which shares structural similarities with the short isoform of cFLIP, suggesting that viruses express this protein to influence the host cell death machinery during infection.

To elucidate the consequence of vFLIP expression on cell death regulation and gut homeostasis, we analysed mice, which expressed vFLIP from Kaposi's sarcoma associated herpesvirus (KSHV) in IECs (vFLIPVillinCre-tg). These mice spontaneously developed a severe inflammation accompanied by a high amount of immune cell infiltration into the gut tissue, underlined by an increased expression of proinflammatory markers. According to our hypothesis that vFLIP is able to influence the caspase-8 activity, vFLIPVillinCre-tg mice showed Paneth cell depletion and pronounced cell death in the small intestine, resembling the phenotype of Casp8ΔIEC mice. Furthermore we could discover a dysregulation of the NFκB pathway in the intestinal epithelium of vFLIPVillinCre-tg mice, which might further contribute to the inflammatory phenotype. Taken together, KSHV-vFLIP expression in IECs promotes the disruption of the intestinal immune homeostasis and might play a role in the induction of inflammation and cell death during enteric infection.

BATF3 IS A CRITICAL REGULATOR OF T CELL DRIVEN COLITIS

V. Kitowski[1], M. F. Neurath[1], K. Hildner[1]

[1]*Medical Department 1, University Hospital Erlangen, Germany*

Inflammatory bowel diseases (IBD) as Crohn's disease (CD) and ulcerative colitis (UC) represent a group of chronic immune-mediated disorders that are linked to a genetically defined susceptibility (e.g. Nod2, IL-23R, Stat3 etc.) and additional, poorly defined environmental triggers. Interestingly, alterations of the composition of intestinal microbiota are frequently detected in IBD. However, whether this observation is cause or effect, i.e. triggers the colitogenic immune response or is a result of the immune-mediated colitis remains or reflects both possibilities is still highly controversial. The precise impact of distinct immune cell subsets on the composition and functionality of intestinal microbiota in the steady state and during intestinal inflammation has not been studied in detail yet.

The AP-1 transcription factor Basic leucine zipper transcription factor, ATF-like 3 (Batf3) controls related CD8α+ and CD103+CD11b- dendritic cell (DC) differentiation and therefore represents a suitable model to study the role of distinct DC subsets in various in vivo scenarios. Previous studies have already elucidated that Batf3-deficiency confers protection in several bacterial infection models while certain viral and parasitic infections are not controlled in the absence of Batf3.

In this study we therefore sought to investigate whether Batf3 deficiency affects the initiation, promotion and outcome of colitis employing a series of murine intestinal inflammation models. Our data indicate that Batf3 specifically controls T cell driven but not largely innate immune system mediated colitis formation. Most importantly, increased colitis susceptibility turned out to be transferable to co-housed wildtype mice suggesting the presence of altered microbial communities in the absence of Batf3. Consequently, further studies revealed that Batf3-deficiency allow the formation of a transmissible dysbiotic state of the intestinal microbiota that predisposes to and promotes the exacerbation of T cell-driven intestinal inflammation in a however Batf3-deficiency- independent manner.

In summary, Batf3 and most likely Batf3-dependent DCs control the intestinal microbial homeostasis hereby preventing fatal T cell driven colitis formation.

NOD1 SIGNALLING IS LINKED TO F-ACTIN REMODELLING

Bielig H.[1], Lautz K.[1], Braun P.R.[2,3], Menning M.[1], Machuy N.[2], Brügmann C.[1], Barisic S.[4], Eisler S.A.[4], Birte Zurek B.[1], Sansonetti P.J.[5,6,7], Hausser A.[4], Meyer T.F.[2] and Kufer T.A.[1,8]

[1]*Institute for Medical Microbiology, Immunology and Hygiene, Cologne, Germany*
[2]*Department of Molecular Biology, Max Planck Institute for Infection Biology, Berlin, Germany*
[3]*Steinbeis-Innovationszentrum Center for Systems Biomedicine, Falkensee, Germany*
[4]*Institute of Cell Biology and Immunology, University of Stuttgart, Stuttgart, Germany*
[5]*Unité de Pathogénie Microbienne Moléculaire, Institut Pasteur, Paris, France*
[6]*INSERM U786, Institut Pasteur, Paris, France*
[7]*Microbiologie et Maladies Infectieuses, Collège de France, Paris, France*
[8]*University of Hohenheim, Institute of Nutritional Medicine, Stuttgart, Germany*

NOD1 is an intracellular pathogen recognition receptor that responds towards bacterial mDAP-type peptidoglycan and contributes to anti-bacterial innate immune responses, adaptive immunity and tissue homeostasis. We have shown earlier that in human cells, NOD1 localizes to F-actin rich structures and that NOD1 is recruited to the entry sites of the cytoinvasive bacterial pathogen Shigella. This and work by others suggested that NOD1-induced signaling relies on actin remodeling, however, the details of the connection of NOD1 and the actin cytoskeleton remained elusive.

Using a druggable-genome wide siRNA screen we recently identified the cofilin phosphatase slingshot homolog 1 (SSH1) as a novel component of the NOD1 pathway. SSH1 is a phosphatase that regulates the activity of the key actin severing protein cofilin and thereby controls the dynamic of the actin cytoskeleton.

We show that NOD1 directly interacted with SSH1 at F-actin rich sites. Depletion of SSH1 impaired NOD1-mediated NF-kappaB activation and pro-inflammatory cytokine release. By contrast, chemical inhibition of actin polymerization using cytochalasin D enhanced Nod1-mediated NF-kB responses in myeloid and epithelial cells and compensated loss of SSH1. Finally, we show that cofilin activity is linked to NOD1 signaling. Our data thus revealed that NOD1 requires SSH1/cofilin network-induced changes in the actin dynamic for signalling. This suggests that bacterial induced changes in the F-actin dynamics converge into the NOD1 signalling pathway to induce or enhance innate immune responses, a hypothesis we are currently testing.

MICROBIOTA-DEPENDENT SIGNALS LINK ATF6-DRIVEN ERUPR TO COLONIC TUMORIGENESIS

Lobner, Elena[1]; Kober, Olivia[1]; Berger, Emanuel[1]; Clavel, Thomas[2]; Lagkouvardos, Ilias[2]; Weber, Achim[3]; Janssen Klaus-Peter[4]; Haller, Dirk[1, 2]

[1] Chair of Nutrition and Immunology, Technische Universität München, Freising - Weihenstephan, Germany; [2] ZIEL – Institute for Food & Health, Technische Universität München, Munich, Germany;[3] Institute of Surgical Pathology, University Hospital Zurich, Zürich, Switzerland; [4] Department of Surgery, Technische Universität München, Munich, Germany.

Background and aim: Activation of the endoplasmic reticulum unfolded protein response (erUPR) contributes to the pathogenesis of inflammatory bowel diseases (IBD) and might increase the risk for the development of colorectal cancer. However, mechanistic evidence for a causative role of erUPR in the oncogenic tissue transformation is lacking. The activating transcription factor 6 (ATF6) mediates one of three branches involved in sensing and signaling of erUPR. To address the role of ATF6-mediated erUPR signaling in intestinal epithelial cells (IEC), we generated Villin-Cre-driven IEC-specific transgenic mice overexpressing the activated form of ATF6 (nATF6IEC).

Results and methods: Homozygous nATF6IEC tg/tg mice spontaneously developed colonic adenomas independent of inflammatory processes, with an incidence of 100% at 12 weeks of age. In contrast, heterozygous nATF6IEC wt/tg mice reveal fully activated erUPR but fail to spontaneously develop tumors.

High-throughput 16S-rRNA gene sequencing of caecal microbiota showed a clear separation of bacterial communities according to the tumor-promoting genotype and reduced bacterial diversity was already developed at a pre-tumor stage in homozygous nATF6IEC tg/tg mice. Loss of mucin-filled goblet cells was associated with increased microbial penetration of the mucus barrier in homozygous nATF6IEC tg/tg mice.

Germ-free housing of nATF6IEC tg/tg mice was shown to prevent tumor formation and epithelial hyperproliferation, even in the presence of activated erUPR. Antibiotic treatment induced a shift in microbial composition, but not microbial load, and antagonized hyperproliferation and tumor incidence. Most importantly, the transfer of pre-conditioned microbiota into germ-free recipients reestablished the tumorigenic phenotype in nATF6IEC tg/tg mice, clearly demonstrating the causative role of bacterial communities in colonic adenoma formation.

The relevance of this newly generated mouse model is evident through the observed correlation between high ATF6 activation and unfavorable colorectal cancer progression in a cohort of 104 patients.

Conclusion: Microbiota-derived signals are integrated into activated erUPR of the epithelium to cause colonic tumor formation. Loss of goblet cell functions allowed dysbiotic bacterial communities to penetrate the mucus barrier and to induce epithelial hyperproliferation in the absence of tissue inflammation. Thus, the presence of erUPR in IBD patients might represent an inflammation-independent risk factor for the development of colorectal cancer.

DISTINCT REQUIREMENTS FOR THE MICROBIOTA FOR INDUCTION OF ANTI-BACTERIAL TH17, TH17/22 AND TH22 CD4+ T CELL POPULATION

U. Roy[1], E. Galvez[1], M. Basic[2], A. Bleich[2], R. Flavell[3], S. Huber[4], T. Strowig[1]

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

[2] *Medical University Hannover, Hannover, Germany.*

[3] *Yale University, New Haven, USA.*

[4] *University Hospital Hamburg-Eppendorf, Hamburg, Germany.*

The intestinal microbiota is a complex microbial ecosystem that influences numerous physiological processes in the host including the resistance to microbial infections. Numerous studies have highlighted that germfree compared to conventional mice are characterized by enhanced susceptibility to many pathogens. Which members of this diverse community are responsible for these effects is less well understood.

We are specifically interested in the bacteria that are responsible for inducing the development of “innate-like” CD4+ T cell subsets producing the immunoregulatory cytokines IL-17 and IL-22 rapidly after infection with enteropathogens. To look into the in vivo regulation of these cytokine-producing cells we are using IL-17(GFP), Foxp3(RFP) and IL-22(BFP) reporter mice. In our gnotobiotic mouse model, we observed IL-22+IL-17+ and IL-22+IL-17- CD4+ T cells are only found after Salmonella Typhimurium infection in the cecum of mice harbouring segmented filamentous bacteria (SFB), but were absent in mice with a microbial community lacking SFB. IL17+IL22- CD4+ T cells are found in small intestine induced by SFB as reported previously. Moreover, SFB are sufficient to induce these subsets as germfree mice monocolonized with SFB had similar frequencies of these subsets. Using markers like CCR6, CD44, CD62L we could demonstrate that these subsets have mostly an activated memory cell-like phenotype. Gene expression profile by RNAseq revealed distinct properties of these SFB modulated T cell subsets including differential expression of IL-17F and IFN-□.

Our findings demonstrate that SFB are not only essential to induce steady-state Th17 in the small intestine, but also provide signals resulting in the development of CD4 T cell subsets that are distinct of Th17 and are characterized by the ability to produce IL-22 and IL-17 cells rapidly after infection in the intestine.

PROGRAM
Saturday, June 25

ROLE OF ATG16L1 IN BACTERIAL AUTOPHAGY – LINK WITH CROHN'S DISEASE..

Matthew Sorbara, Elisabeth Foerster, Stephen Girardin and Dana Philpott[1]

[1]*Department of Immunology, University of Toronto, Toronto, Canada*

Our group studies a family of innate immune receptors called the Nod-like receptors (NLRs). These proteins represent an intracellular surveillance system for detecting microbial and host-derived danger signals. In particular, we study Nod1 and Nod2, which detect peptidoglycan (PG) from the bacterial cell wall and trigger inflammation. Detection of these PG by Nod1 and Nod2 triggers a signal transduction cascade that culminates in the activation of NF□B and the production of pro-inflammatory mediators. Moreover, Nod triggering can regulate autophagy. Importantly, Nod1 and Nod2 have been implicated in inflammatory bowel disease (IBD), in particular Nod2 has been associated with Crohn's disease, yet a clear understanding of how dysfunctional Nod activation leads to aberrant inflammation is still lacking. ATG16L1 is a protein involved in autophagy and has also been linked to Crohn's disease. We showed previously that Nod1 and Nod2 interact with ATG16L1 and this association promotes autophagy of intracellular bacteria. Since Nod1 and Nod2 are important for triggering bacterial-induced autophagy, and autophagy can influence the immune response, our current work aims to examine how autophagy might regulate the cytokine response to infection and Nod1 or Nod2 stimulation.

IMPACT OF THE GUT MICROBIOTA ON INFECTION AND METABOLISM

09^{45} – 11^{15} Chair: A. Steimle, Tübingen

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Impact of early life intervention with Bifidobacteria on infant fecal microbiota and metabolite profile

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The guts of blood-group antigens: B4galnt2 alters pathogen susceptibility through the intestinal microbiome

Wells J, Top Institute Food and Nutrition, Wageningen, The Netherlands; Host-Microbe Interactomics Group, Wageningen University and Research Center, Wageningen, The Netherlands
Age-associated impairment of the mucus barrier function is associated with profound changes in microbiota and immunity

IMPACT OF EARLY LIFE INTERVENTION WITH BIFIDOBACTERIA ON INFANT FECAL MICROBIOTA AND METABOLITE PROFILE

Monika Bazanella[1], Tanja V. Maier[2], Thomas Clavel[3], Ilias Lagkouvardos[3], Marianna Lucio[2], Maria X. Maldano-Gòmez[3], Chloe Autran[5], Thomas Skurk[3], Jens Walter[4], Lars Bode[5], Philippe Schmitt-Kopplin[2,3], Dirk Haller[1,3]

[1] TU Munich, Chair of Nutrition and Immunology, Freising, Germany; [2] Helmholtz Center Munich, Oberschleißheim, Germany; [3] TU Munich, ZIEL Institute for Food and Health, Freising, Germany; [4] University of Alberta, Edmonton, Canada; [5] University of San Diego, San Diego, California, …

Development of the gut microbiota in infants is a dynamic process and the impact of early-life intervention with bifidobacteria-supplemented formula on intestinal bacterial communities is unknown.We designed a randomized, double-blinded, placebo-controlled intervention trial with 106 healthy neonates receiving infant formula with or without a mixture of four bifidobacteria (B. bifidum, B. breve, B. infantis, B. longum). High-throughput 16S rRNA amplicon sequencing and high-resolution mass spectrometry (UPLC-MS) were used to analyze fecal samples collected over a period of two years. Distinct clusters of bacterial communities and metabolite profiles were observed between formula- and breast-fed infants at early age but then converged over time. A core microbiota, i.e. taxa detected across all infants at least once in the first year of life, was identified, including molecular species classified as two Bifidobacterium spp., and one each Escherichia-Shigella, Streptococcus, and Enterococcus species. Effects of bifidobacteria-supplemented formula were marginal and occurred primarily at early age, including lower relative abundance of Bacteroidaceae, specifically Bacteroides fragilis, and the presence of two specific OTUs within bifidobacteria and lactococci. These shifts were accompanied by the presence of lipid-related and unknown metabolites. One main hallmark of formula-fed infants was increased species richness and Shannon effective diversity, which was not significantly affected by the intervention. Interestingly, none of the formula-derived bifidobacteria were detected in feces after two years. Independent of bifidobacteria supplementation, levels of pyruvate and lactate were high in breast-fed infants, while propionate and butyrate were abundant in both formula-fed groups.In conclusion, infant formula compared to breast milk influences the assembly and metabolite profile of the early life microbiome, particularly associated with increased bacterial diversity. Effects of bifidobacteria-supplemented formula disappeared shortly after the neonatal stage.

CASPASE-8 MAINTAINS THE INTESTINAL BARRIER AGAINST PATHOGEN CHALLENGE

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 [2] Medical University

Caspase-8 is a central regulator of cell death. The activation status of this protein decides which type cell death is initiated: the caspase cascade mediated apoptosis or the caspase-8 independent necroptosis, which is regulated by the RIP-kinases. Blocking apoptotic cell death in the intestinal epithelium of mice by conditional deletion of caspase-8 (Caspase-8ΔIEC mice), leads to spontaneous development of terminal ileitis, caused by necroptotic cell death of intestinal epithelial cells. This pathology is also driven by the depletion of Paneth cells, leading to a diminished expression of antimicrobial peptides in the small intestine and additionally to an altered intestinal microbiota.

Due to the high sensitivity of Caspase-8ΔIEC mice to the application of bacterial and viral products, mimicked by LPS and Poly(I:C) respectively, we wanted to investigate the role of caspase-8 for intestinal homeostasis during infectious colitis. Therefore we infected control and Caspase-8ΔIEC mice with Salmonella Typhimurium. Interestingly, Caspase-8ΔIEC mice showed high susceptibility to the infection. This resulted in severe weight loss, high lethality and dramatic tissue damage. Excessive cell death caused a breakdown of the intestinal barrier and enabled the pathogenic Salmonella Typhimurium and commensal bacteria to invade into subepithelial areas and reach distant organs, which finally results in a septic shock. Following the pathogen challenge, infiltration of immune cells into the tissue and expression of pro-inflammatory markers was enormously increased. Blocking the IL-1 or Tnfα pathways by injection of an antagonist or genetic deletion respectively, reduced the susceptibility towards Salmonella infection and improved the survival rate, but could not completely protect the animals from tissue damage.

Since commensal bacteria play an important role in defense against pathogens, we further investigated the role of an altered microbiota in Caspase-8ΔIEC mice. Therefore we depleted the microflora of C57BL/6 animals by antibiotic treatment. Following this, the animals were reconstituted by fecal microbiota transplantation with microflora from control and Caspase-8ΔIEC animals and consequently infected with Salmonella Typhimurium. Interestingly, mice from the control group were less susceptible to the infection, while the group reconstituted with Caspase-8ΔIEC microflora showed higher weight loss and inflammation.

In conclusion, our results demonstrate and important role for caspase-8 in maintaining the gut barrier in response to pathogen challenge. Additionally, we identified an important role for the microbial composition in bacterial defense and a partial dependence on several cytokine signaling pathways.

EXPLORING THE ROLE OF THE COMMENSAL MUCISPIRILLUM SCHAEDLERI IN ENTERIC SALMONELLA ENTERICA SEROVAR TYPHIMURIUM INFECTION

Simone Herp[1], Markus Beutler[1], Diana Ring[1], Sandrine Brugiroux[1], Buck Hanson[2], Saib Hussain[1], Michaela Steinberger[2], Alesia Walker[3], Philippe Schmitt-Kopplin[3], David Berry[2] and Bärbel Stecher[1*]

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

[2]*Department of Microbial Ecology, University of Vienna, AUSTRIA.*

[3] *Research Unit Analytical BioGeoChemistry, Helmholtz Zentrum München, GERMANY*

*Correspondence: stecher@mvp.uni-muenchen.de

The mammalian gut harbors a complex consortium of bacteria which contributes to our health in several ways. First of all it plays a role in food digestion and nutrition, furthermore it is important for the maturation of a healthy immune system and last it can protect us from enteric infections. The interactions between single bacterial species and the host in preventing infections are highly complex mechanisms which are not yet fully understood. By using gnotobiotic mouse models we evaluated the contribution of one single bacterial species to colonization resistance, prevention of dysbiosis and inflammation during enteric Salmonella Typhimurium (S. Tm) infection. We found that Mucispirillum schaedleri, a mouse commensal bacterium which is closely associated with the mucus layer, protects efficiently against S. Tm induced intestinal inflammation in different gnotobiotic mouse models. To get a deeper understanding of the underlying mechanism we analyzed M. schaedleri associated mice with respect to differences in mucosal gene expression and metabolic state in. By understanding the interaction between M. schaedleri, S. Tm, the gut microbiota and the host we expect to identify new approaches for preventing pathogen-induced intestinal inflammation.

GUT-DERIVED CORIOBACTERIACEAE INCREASE WHITE ADIPOSE TISSUE DEPOSITION IN MICE

Sarah Just[1], Katrin Wegner[2], Stanislas Mondot[3], Catherine Philippe[3], Geneviève Héry-Arnaud[3], Philippe Gerard[3], Sascha Rohn[2], Patricia Lepage[3], and Thomas Clavel[1]

[1] *ZIEL Institute for Food and Health, Technische Universität München, Freising, Germany,*
[2] *Hamburg School of Food Science, Universität Hamburg, Germany,*
[3] *INRA, MICALIS UMR 1319, Jouy-en-Josas, France*

Introduction: Coriobacteriaceae are dominant members of the human gut microbiota and can metabolize cholesterol-derived host substrates such as bile acids. However, consequences for the host are unknown. The aim of the present study was to characterize the effects of Coriobacteriaceae on metabolic health in vivo. Methods: Male germ-free (GF) C57BL/6N mice were associated at week 5 of age with a consortium of four Coriobacteriaceae (CORIO). At week 10, mice were randomly divided into 3 groups (n = 12 each) fed a control (CD), high-fat (HFD), or bile acid-supplemented (BA) diet for 16 weeks. GF and specific-pathogen free (SPF) mice were used as controls. Results: HFD resulted in increased body weight, and all three colonization groups developed steatosis. All mice fed BA stayed lean. However, those colonized with CORIO showed a significant increase in white adipose tissue (WAT) depots (GF, 37 ± 15; CORIO, 65 ± 15, SPF, 26 ± 16 mg/g body weight; $p < 0.001$), most likely due to hyperplasia. This was accompanied by increased gene expression of leptin, but decreased expression of the bile acid receptor TGR5 and the transcription factor FXR in CORIO vs. SPF mice. CORIO mice fed BA were also characterized by increased levels of cholesterol in systemic plasma and subcutaneous WAT, and by increased hepatic triglyceride content. Measurement of fatty acids in WAT revealed a reduction in stearic, oleic, and eicosenoic acid, which correlated negatively with fat mass. Bile acid composition in caecum and WAT showed increased levels of taurine-conjugates in GF and CORIO mice, whereas SPF mice had higher levels of free and oxo-bile acids. WAT proteomics and lipid profiling analysis will deliver further mechanistic understanding of the model.

Conclusion: Interactions between Coriobacteriaceae and bile acids influence fat tissue deposition in mice, providing specific evidence that gut bacteria can regulate host metabolism.

The authors received financial support from the German Research Foundation (DFG) and the French National Research Agency (ANR).

THE GUTS OF BLOOD-GROUP ANTIGENS: B4GALNT2 ALTERS PATHOGEN SUSCEPTIBILITY THROUGH THE INTESTINAL MICROBIOME

Philipp Rausch[1,3*], Natalie Steck[1,2*], Abdulhadi Suwandi[1], Janice A. Seidel[1], Sven Künzel[3], Kirandeep Bhullar[4], Marijana Basic[5], Andre Bleich[5], Jill M. Johnsen[6,7], Bruce A. Vallance[4], John F. Baines[1,3*], Guntram A. Grassl[1,2*]

[1]Institute for Experimental Medicine, Christian-Albrechts-University of Kiel, Kiel, Germany [2]Models of Inflammation, Research Center Borstel, Borstel, Germany; [3]Max Planck Institute for Evolutionary Biology, Plön, Germany; [4]Department of Pediatrics, Division of Gastroenterology, Child and Family Research Institute, University of British Columbia, Vancouver, British Columbia, Canada; [5]Institute for Laboratory Animal Science, Hannover Medical School, Hannover, Germany; [6]Research Institute, Puget Sound Blood Center, Seattle, WA, USA; [7]Department of Medicine, University of Washington, Seattle, WA, USA

Glycans play important roles in host-microbe interactions and genes facilitating their synthesis are known to be prominent targets of selection. The blood-group-related glycosyltransferase B4galnt2 shows a cis-regulatory mutation that leads to a switch in expression from the gut epithelium to the endothelium of blood vessels. This genetic variation has been maintained in wild mouse populations for a long time despite a naturally occurring bleeding disorder associated to the alleles driving endothelial expression. We hypothesize that variation in B4galnt2 alters the intestinal microbiota and susceptibility to intestinal pathogens, which may explain the maintenance of this variation in mouse populations.

To test to the hypothesis that modulation of intestinal microbial communities through B4galnt2 contributes to resistance against gastrointestinal pathogens we challenged mice expressing B4galnt2 in a tissue-specific manner with a Salmonella infection model and analyzed the taxonomic- and functional genomic differences in the intestinal microbiota with respect to disease susceptibility and genotype. Differences in B4galnt2 expression cause strong alterations in bacterial community composition and the pathological response to Salmonella infection. We demonstrate a significant role of the B4galnt2-dependent microbiota on the susceptibility to intestinal inflammation, an effect transmissible by fecal-transfer. We further identify specific compositional- and functional adaptations of the B4galnt2-dependent microbial communities, which may explain the differences in microbial community resistance against disturbance and their differences in susceptibility to infection and inflammation. This data supports the critical role of B4galnt2 in infections and the role of bacterial communities as a selectable phenotype.

AGE-ASSOCIATED IMPAIRMENT OF THE MUCUS BARRIER FUNCTION IS ASSOCIATED WITH PROFOUND CHANGES IN MICROBIOTA AND IMMUNITY

Bruno Sovran[1,3], Floor Hugenholtz[5], Marlies Elderman[3,4], Adriaan A. Van Beek[1,3], Katrine Graversen[2], Myrte Huijskes[2], Mark V. Boekschoten[1,6], Huub F.J. Savelkoul[3], Paul De Vos[1,4], Jan Dekker[2] and Jerry M. Wells[1,2*]

[1]Top Institute Food and Nutrition, Wageningen, The Netherlands; [2]Host-Microbe Interactomics Group, Wageningen University and Research Center, Wageningen, The Netherlands; [3]Cell Biology and Immunology Group, Wageningen University and Research Center, Wageningen, The Netherlands; [4]University Medical Center of Groningen, Groningen, The Netherlands; [5]Laboratory of Microbiology, Wageningen University and Research Center, the Netherlands; [6]Division of Human Nutrition, Wageningen University and Research Center, Wageningen, The Netherlands

Aging significantly increases the vulnerability to gastrointestinal (GI) disorders but there are few studies investigating the key factors in aging that affect the GI tract. To address this knowledge gap, we used 10 week- and 19 month-old litter-mate mice to investigate microbiota and host gene expression changes in association with ageing. In aged mice the thickness of the colonic mucus layer was reduced about 6-fold relative to young mice, and more easily penetrable by luminal bacteria. This was linked to increased apoptosis of goblet cells in the upper part of the crypts. The barrier function of the small intestinal mucus was also compromised and the microbiota were frequently observed in contact with the villus epithelium. Antimicrobial Paneth cell factors Ang4 and lysozyme were expressed in significantly reduced amounts. These barrier defects were accompanied by major changes in the faecal microbiota and significantly decreased abundance of Akkermansia muciniphila which is strongly and negatively affected by old age in humans. Transcriptomics revealed age-associated decreases in the expression of immunity and other genes in intestinal mucosal tissue, including decreased T cell-specific transcripts and T cell signalling pathways. The physiological and immunological changes we observed in the intestine in old age, could have major consequences beyond the gut.

DIETARY MODULATION OF GUT MICROBIOTA AND METABOLITES

Harry J Flint, Alan W Walker, Sylvia H Duncan, Wendy R Russell, Petra Louis

Rowett Institute of Nutrition and Health, University of Aberdeen, Foresterhill, Aberdeen, UK

Carefully-controlled dietary intervention studies with human volunteers have established that both the species composition of our gut microbiota and its metabolic outputs are influenced by diet. Analysis of bacterial genomes can help to define the degradative capabilities of individual species, but cannot as yet predict the outcome of competition for non-digestible carbohydrates in vivo. Important insights into bacterial competition for substrates can however be gained from anaerobic continuous culture experiments in vitro, where pH and substrate supply can be precisely controlled. Such experiments predict a high degree of species specificity in the response of the microbial community to isolated carbohydrates used as prebiotics1. Much of the non-digestible fibre and starch of dietary origin that reaches in the colon, however, exists in insoluble particles. There is increasing evidence that breakdown of such material is initiated by specialist primary degraders that may represent 'keystone' species. For example, Ruminococcus bromii, a species whose representation in the microbiota increases rapidly in individuals consuming high RS diets, has a superior ability to degrade resistant starch particles that coincides with unique organization of its extracellular amylases into 'amylosomes'2. Meanwhile bacteria that become enriched with wheat bran include butyrate-producing species mainly belonging into the Lachnospiraceae3. The health consequences of these diet-driven microbiota changes depend not only on interactions between bacteria and host cells, but also to a large extent on metabolite production and release. Thus, while fermentation of wheat bran yields a high proportion of health-promoting butyrate, it also leads to the release and transformation of bound ferulic acid3, with further consequences for health. Such investigations into gut microbial communities, together new information from the genomes of human bacteria and from the behaviour of cultured representatives, will help us to unravel the complex effects of diet upon human health and should offer new ways to deliver benefits in the future.

Chung WSF et al BMC Biology 2016; 2. Ze X et al MBio 2015; 3. Duncan SH et al Environ Microbiol 2016

GUT MICROBIOTA FUNCTIONS AND ECOLOGY

13^{45} – 15^{15} Chair: G. Loh, Karlsruhe

Hanson B, Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, University of Vienna,
Exploring distal-gut microbial ecology and host-microbe metabolic interactions by in vivo stable isotope probing with 13C-glucose ureide

Iljazovic A, Helmholtz Centre for Infection Research, Braunschweig, Germany
Microbial Interactions of Prevotella Spp. within the Intestinal Ecosystem

Kabbert J, RWTH Aachen, Institute of Molecular Medicine, Aachen, Germany
The inner coat counts

Neville A, Host-Microbiota Interactions Laboratory, Wellcome Trust Sanger Institute, Hinxton CB10 1SA, UK
Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation

Pereira F, Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, Faculty of Life Sciences, University of Vienna, A-1090 Vienna, Austria
Identifying and sorting host compound foragers from the gut microbiota by heavy water-based activity labelling and Raman microspectroscopy

Van Best N, Institute of Medical Microbiology, RWTH University Hospital Aachen, Aachen, Germany; Department of Medical Microbiology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, The Netherlands
Postnatal establishment of the enteric microbiota

EXPLORING DISTAL-GUT MICROBIAL ECOLOGY AND HOST-MICROBE METABOLIC INTERACTIONS BY IN VIVO STABLE ISOTOPE PROBING WITH ^{13}C-GLUCOSE UREIDE

Buck T. Hanson[1], Orest Kuzyk[1], Thomas Decker[2], Andreas Richter[3], Wolfgang Wanek[3], Douglas Morrison[4], David Berry[1], Alexander Loy[1]

[1]Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, University of Vienna, [2]Max F. Perutz Laboratories, University of Vienna, [3] Division of Terrestrial Ecosystem Research, Department of Microbiology and Ecosystem Science, University of Vienna, [4]Scottish Universities Environmental Research Centre, Stable Isotope Biochemistry Laboratory, University of Glasgow, United Kingdom

Microbial metabolites produced from the fermentation of complex polysaccharides in the distal-gut 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 influence of dietary glucose on distal gut microbial ecology, we have implemented 13C-glucose ureide as a model substrate for colon-specific delivery of isotopically-labeled glucose. When wild-type mice were orally dosed with 13C-glucose ureide, we observed respiration of 13CO2, isotopic labeling of cecal-derived biomass and DNA, in addition to the detection of 13C-labeled microbial-derived metabolites (acetate, propionate, and butyrate) in host blood plasma. The peak time of compound metabolism was associated with increased abundance of 16S rRNA gene phylotypes related to glucose-fermenting members of the Lachnospiraceae and Erysipelotrichaceae that are associated with obesity. To examine the role of dietary-induced shifts in gut microbiota and the fate of colonic glucose, we provided mice with two isocaloric diets: a control starch-based diet or starch-free glucose-rich diet. After three weeks with no significant differences in weight gain, each mouse received an oral dose of 13C-lableled glucose ureide. Using a combination of mass spectroscopy, microscopic, and biomarker analyses, we currently aim to connect shifts in microbial community structure and physiology with the fate of glucose-derived metabolites and contributions to host physiology.

MICROBIAL INTERACTIONS OF PREVOTELLA SPP. WITHIN THE INTESTINAL ECOSYSTEM

Aida Iljazovic, Eric JC Galvez, Till-Robin Lesker, Sophie Thiemann and Till Strowig

Helmholtz Centre for Infection Research, Braunschweig, Germany

Prevotella is a genus of anaerobic bacteria prominently found in the human gastrointestinal tract. The presence of one of its members, P. copri, has been associated with beneficial and detrimental impact on the host, i.e. diet-induced improvement of glucose tolerance but also the onset of rheumatoid arthritis (RA), respectively. The distinct modulation of host physiology may be the result of genomic diversity within the species or specific interactions within the intestinal ecosystem that possibly further modulated by the diet. The lack of diverse intestinal Prevotella spp. isolates prevents a detailed study of these possibilities in in vivo models. We isolated three novel Prevotella species from the gut of mice prone to inflammatory diseases and identified large genomic variability, but also the presence of biomarkers previously associated in P. copri with the induction of RA. The isolates individually colonized the intestine of mice lacking Prevotella spp. in high relative abundance (up to 30%), but when transferred together one specific strain outcompeted the other two. We could identify that this domination depends on other members of the microbiome demonstrating that species competition within a genus are dependent on complex interaction within the ecosystem. Studies are now underway to characterize their influence on inflammatory processes in the intestine. Our study highlights the need of not only identifying potential pathobionts, but also determining their interaction with other microbial members that have the potential to modulate their metabolic capabilities and impact on host physiology.

THE INNER COAT COUNTS

Johanna Kabbert[1], Hedda Wardemann[2] and Oliver Pabst[1]

[1]*RWTH Aachen, Institute of Molecular Medicine, Aachen, Germany*
[2]*German Cancer Research Center, Division of B Cell Immunology, Heidelberg, Germany*

Homeostasis of the mucosal immune system requires a fine balancing of immune responses to the microbiota, foreign antigens and potential pathogens. Various microbiota species show different styles of colonization: Whereas many commensals are typically confined to the lumen of the gastrointestinal tract, others are associated with the mucus layer or even the surface of the intestinal epithelium. Such stratified niches and intestinal homeostasis are maintained by the combined activity of physical, biochemical and immunological barriers. In this setup immunoglobulin A takes a key role.

IgA is the most abundant secretory immunoglobulin found at mucosal surfaces. Mucosal plasma cells (PC) prominently secrete dimeric IgA, which is transported across the intestinal epithelium into the gut lumen by the polymeric Ig receptor. Secretory IgA binds to a fraction of the intestinal microbiota as well as enteropathogens and thereby contributes to gut homeostasis and protection against infection. However, important questions remain: How do different pathways of IgA induction contribute to IgA coating of microbiota? Do specific IgA+ PC clones coat distinct members of the microbiota? Does the anatomical location impact the coating of different bacterial taxa?

In this project we characterize the microbiota binding profile of a previously reported set of recombinant monoclonal antibodies. Benckert and colleagues have sorted single IgA+ and IgG+ PC's from terminal ileum from 3 healthy human and generated a panel of about 200 recombinant monoclonal antibodies (J. Benckert et al., 2011 JClinInvest). Screening these antibodies in ELISA for microbiota reactivity, we observed that about one third of all antibodies reacted to murine and human microbiota isolated from feces.

To further characterize the binding of these antibodies, antibodies were purified, directly labeled and used to stain microbiota isolated from human and murine feces in flow cytometry. Consistent with the ELISA data, we observed binding to gut bacteria with some antibodies binding more than 20% of all bacteria isolated. Antibody-positive microbiota were purified by cell sorting and their composition determined by 16S sequencing. Preliminary results suggest that single monoclonal antibodies bind a spectrum of distinct bacteria.

Further experiments will aim to systemically compare the microbiota-binding profile of individual antibodies and to unravel the mechanisms of intestinal IgA coating and IgA responses to commensal bacteria.

CULTURING OF 'UNCULTURABLE' HUMAN MICROBIOTA REVEALS NOVEL TAXA AND EXTENSIVE SPORULATION

Hilary P. Browne[1], Samuel C. Forster[1,2,3], Blessing O. Anonye[1], Nitin Kumar[1], B. Anne Neville[1], Mark D. Stares[1], David Goulding[4], & Trevor D. Lawley[1]

[1]*Host-Microbiota Interactions Laboratory, Wellcome Trust Sanger Institute, Hinxton CB10 1SA, UK*

[2]*Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, Victoria 3168, Australia*

[3]*Department of Molecular and Translational Sciences, Monash University, Clayton, Victoria 3800, Australia*

[4]*Microbial Pathogenesis Laboratory, Wellcome Trust Sanger Institute, Hinxton CB10 1SA, UK*

Our intestinal microbiota harbours a diverse bacterial community required for our health, sustenance and wellbeing. Intestinal colonization begins at birth and climaxes with the acquisition of two dominant groups of strict anaerobic bacteria belonging to the Firmicutes and Bacteroidetes phyla. Culture-independent, genomic approaches have transformed our understanding of the role of the human microbiome in health and many diseases. However, owing to the prevailing perception that our indigenous bacteria are largely recalcitrant to culture, many of their functions and phenotypes remain unknown. Here we describe a novel workflow based on targeted phenotypic culturing linked to large-scale whole-genome sequencing, phylogenetic analysis and computational modelling that demonstrates that a substantial proportion of the intestinal bacteria are culturable. Applying this approach to healthy individuals, we isolated 137 bacterial species from characterized and candidate novel families, genera and species that were archived as pure cultures. Whole-genome and metagenomic sequencing, combined with computational and phenotypic analysis, suggests that at least 50–60% of the bacterial genera from the intestinal microbiota of a healthy individual produce resilient spores, specialized for host-to-host transmission. Our approach unlocks the human intestinal microbiota for phenotypic analysis and reveals how a marked proportion of oxygen-sensitive intestinal bacteria can be transmitted between individuals, affecting microbiota heritability.

IDENTIFYING AND SORTING HOST COMPOUND FORAGERS FROM THE GUT MICROBIOTA BY HEAVY WATER-BASED ACTIVITY LABELLING AND RAMAN MICROSPECTROSCOPY

F. Pereira[1], B. Sziranyi[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*

The secreted mucus layer that separates the mammalian intestinal epithelium from the lumen provides a habitat and serves as a nutrient source for a subset of gut bacteria. Many inhabitants of this layer can secrete glycan-degrading enzymes, such as sialidases, N-acetylglucosaminidases or L-fucosidades, resulting in the release of easily metabolizable monosaccharides and conferring a competitive advantage for these bacteria in vivo. Indeed, the ability to degrade and utilize mucin glycans is part of the strategy employed by several gut pathogens to successfully colonize the gut. Despite the pivotal role that commensal mucin degraders play in resistance to pathogen colonization and in modulating the host immune response, our knowledge about the mucin-degrading activity and consequent monosaccharide uptake by gut commensals is still limited. To test the capacity of the mouse colon community to forage on mucin and to metabolize monosaccharides originating from O-glycans, we used a recently-developed stable isotope probing approach that employs heavy water (D2O)-based activity labelling and Raman microspectroscopy. With this approach we could observe that a significant percentage of the colon microbial community was stimulated by the addition of each of the O-glycan monosaccharide constituents (sialic acid, fucose, N-acetyl-glucosamine, N-acetyl-galactosamine and galactose), or of mucin itself. Stimulation of the gut community in response to the galactose amendment was more prominent in comparison to the other tested sugars. Unlike the other compounds, galactose is abundant in many diet-derived compounds and therefore a broader set of organisms could be expected to use it. By Raman-based cell sorting of active (deuterated) cells with optical tweezers and subsequent multiple displacement amplification and 16S sequencing, novel gut microbes that can forage on mucosal sugars such as sialic acid and N-acetyl-glucosamine were identified. Examination of the genomes of sorted cells will give additional insights into the physiology of these bacteria and help to dissect the process of mucin degradation in the gut.

POSTNATAL ESTABLISHMENT OF THE ENTERIC MICROBIOTA

Niels van Best[1,3], John Penders[3], Paul H.M. Savelkoul[3], Mathias Hornef[1]

[1]Institute of Medical Microbiology, RWTH University Hospital Aachen, Aachen, Germany
[3]Department of Medical Microbiology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, The Netherlands.

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

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

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

HONEY BEE GUT MICROBIOTA: A VERSATILE MODEL FOR MICROBIAL SYMBIOSIS

Philipp Engel

Philipp.Engel@unil.ch

Department of Fundamental Microbiology, University of Lausanne, Switzerland

Gut microbial communities are important determinants of animal and human health. However, their complex composition displays a formidable challenge for studying symbiotic interactions in the gut. Simple model systems assist the discovery of fundamental principles of gut symbioses. The honey bee, Apis mellifera, represents such a model, because its gut microbiota consists of only eight bacterial species1. These bacteria have longstanding evolutionary associations with their host suggesting symbiotic roles that are likely to play key roles for bee health and biology.

To gain functional insights and to understand the ecology and evolution of these bacteria, we have applied various genomic approaches including metagenomics2, single-cell genomics3, and genome sequencing of cultured isolates4. Overall, these analyses revealed that distinct members of the bee gut microbiota encode genes with putative symbiotic roles in nutrient digestion, such as carbohydrate breakdown and host interaction. Furthermore, we found that the eight bacterial species in the bee gut have substantially diversified suggesting adaptation to different ecological niches. These findings parallel observations from mammals, indicating that in situ diversification of a few bacterial lineages is a common pattern in the evolution of gut communities.

Recently, we have established experiments that allow us to colonize microbiota-free bees with cultured bee gut bacteria. We use this system to investigate the impact of gut bacteria on different aspects of honeybee health and ecology and to understand general aspects of symbiosis in the animal gut.

1. Martinson VG, Danforth BN, Minckley RL, Rueppell O, Tingek S, Moran NA. 2011. A simple and distinctive microbiota associated with honey bees and bumble bees. Mol. Ecol. 20(3) pp. 619.

2. Engel P, Martinson VG, Moran NA. 2012. Functional diversity within the simple gut microbiota of the honey bee. 2012. Proc Natl Acad Sci USA, 109(27):11002-11007.

3. Engel P, Stepanauskas R, Moran NA. Hidden diversity in honey bee gut symbionts detected by single-cell genomics. 2014. PLoS Genet, 10(9): e1004596. doi:10.1371/journal.pgen.1004596.

4. Engel P, Vizcaino MI, Crawford JM. Gut symbionts from distinct hosts exhibit genotoxic activity via divergent colibactin biosynthesis pathways. 2015. AEM. Epub ahead of print. doi: 10.1128/AEM.03283-14.

PROGRAM
Sunday,
June 26

FECAL TRANSPLANTATION TO DISSECT CAUSAL ROLE OF GUT MICROBIOTA IN HUMAN DISEASE

Pieter de Groot (Internal Medicine, University of Amsterdam, Netherlands)

Internal Medicine, University of Amsterdam, Netherlands

Obesity and type 2 diabetes incidence are increasing astonishingly worldwide. Obesity and insulin resistance are influenced by complex host-microbiota interactions. Many association studies have been performed on microbiota composition, but these are complicated by confounding factors such as diet and medication use. Also, association is not causation. We aim to elucidate causation in host-microbe interactions following Koch's postulates in our translational research line.

As adipose tissue inflammation is a key characteristic of insulin resistance, we aimed to identify bacterial DNA in the mesenteric fat of patients scheduled for laparoscopic cholecystectomy. This way, we have found that the mesenteric fat of these individuals harboured significant amounts of Ralstonia pickettii DNA, a gram negative rod. Furthermore, Ralstonia DNA amount in mesenteric adipose tissue correlated with markers of insulin resistance and levels of fecal Ralstonia DNA. We confirmed the validity of these findings in the Gothenborg DIWA cohort (from Karlsson et al, Nature, Jun 6;498(7452):99-103) and found fecal levels of Ralstonia DNA to be significantly elevated in type 2 diabetes and impaired glucose tolerance subjects but not in normal glucose tolerant individuals. Finally, we found that gavage with active Ralstonia induced unslin resistance and adipose tissue inflammation in our DIO mouse model and that vaccination with inactivated Ralstonia DNA prior to gavage could prevent these effects.

Fecal transplantation in clinical trials for metabolic disorders can be used as a tool to mine for such harmful protective bacterial species. In a similar way protective species can be identified. In the FATLOSE trial, in which infusion of lean donor feces attenuated insulin resistance in metabolic syndrome subjects, we identified the butyrate producer Eubacterium hallii as potentially beneficial species. Again, after introduction of E. hallii into our mouse model, we reported an increase in insulin sensitivity and energy expenditure. We are currently undertaking a human dose finding trial to see whether these effects are reproducible in men.

On behalf of Prof. Dr. M. Nieuwdorp MD

METAGENOMICS AND EVOLUTION

10^{00} – 11^{30} Chair: D. Berry, Vienna

Haange SB, Department of Molecular Systems Biology, Helmholtz Centre for Environmental Research-UFZ, Leipzig
Gastric bypass surgery markedly perturbs the community structure and the functional composition of the intestinal microbiota

Lange A, Interfacultary Institute for Microbiology and Infection Medicine, Department for Medical Microbiology and Hygiene, University of Tübingen, Tübingen, Germany
Extensive mobilome-driven genome diversification in mouse gut-associated Bacteroides vulgatus mpk

Loh G, Max Rubner-Institut, Karlsruhe, Germany
Effect of dietary zinc on the horizontal transfer of antibiotic resistance genes in the intestine

Sommer F, Institute of Clinical Molecular Biology, Kiel University, Germany
The role of DUOX2 in shaping the intestinal microbiota and its effect on host physiology

Schierack P, Institute of Biotechnology, Brandenburg Technical University Cottbus - Senftenberg, Senftenberg, Germany
Intestinal Escherichia coli colonization in a Mallard duck population over four consecutive winter seasons

Zioutis C, Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, University of Vienna
Rapid genetic diversification of Bacteroides thetaiotaomicron in the murine gut

GASTRIC BYPASS SURGERY MARKEDLY PERTURBS THE COMMUNITY STRUCTURE AND THE FUNCTIONAL COMPOSITION OF THE INTESTINAL MICROBIOTA

Sven-Bastiaan Haange[1], Nico Jehmlich[1], Constantin Hintschich[2], Mohammed Hankir[2], Florian Seyfried[3], Wiebke Fenske[2] , Martin von Bergen[1,4]

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

[2]Neuroendocrine regulation of energy homeostasis group, IFB Adiposity Diseases, Leipzig

[3]Department of General, Visceral, Vascular and Pediatric Surgery, University of Würzburg, Würzburg

[4]Institute of Biochemistry, Faculty of Biosciences, Pharmacy and Psychology, University of Leipzig, Leipzig

In a rat model the effect of Roux-en-Y gastric bypass surgery (RYGB) on the microbiota from the ileum as well as the colon was investigated and compared to body weight matched animals with sham surgery. We analysed the microbiota inhabiting the mucus layer and the intestinal lumen separately. To resolve the community structure in regard to taxonomy and enzymatic functionalities 16S rRNA gene sequencing and metaproteomics was performed.

The results reveal profound changes in the taxa distribution and the enzymatic functional capacity of the microbiome in the ileum as well as the colon after RYGB. For example, for taxa distribution we observed in the ileum and colon greater prevalence of Actinobacteria especially Bifidobacteriales after RYGB with Firmicutes at lower abundances. Enterobacteriales was also more prevalent in the colon of RYGB than in sham. On a functional level in the ileum the relative numbers of Actinobacteria proteins involved in amino acid metabolism or carbohydrate metabolism were higher in RYGB. In the colon proteins from Clostridia belonging to the function of carbohydrate metabolism or the function energy production were seen at lower levels in RYGB whereas proteins from Actinobacteria which are involved in carbohydrate metabolism or cell motility were observed at higher relative numbers.

EXTENSIVE MOBILOME-DRIVEN GENOME DIVERSIFICATION IN MOUSE GUT-ASSOCIATED BACTEROIDES VULGATUS MPK

A. Lange[1], S. Beier[2], A. Steimle[1], I.B. Autenrieth[1], D.H. Huson[2], J.S. Frick[1]

[1]*Interfacultary Institute for Microbiology and Infection Medicine, Department for Medical Microbiology and Hygiene, University of Tübingen, Tübingen, Germany*
[2]*Algorithms in Bioinformatics, ZBIT Center for Bioinformatics, University of Tübingen, Tübingen, Germany*

Like many other Bacteroides species, Bacteroides vulgatus strain mpk, a mouse fecal isolate which was shown to promote intestinal homeostasis, utilizes a variety of mobile elements for genome evolution. Based on sequences collected by Pacific Biosciences SMRT sequencing technology, we discuss the challenges of assembling and studying a bacterial genome of high plasticity. Additionally, we conducted comparative genomics comparing this commensal strain with the B. vulgatus type strain ATCC 8482 as well as multiple other Bacteroides and Parabacteroides strains to reveal the most important differences and identify the unique features of B. vulgatus mpk. The genome of B. vulgatus mpk harbors a large and diverse set of mobile element proteins compared with other sequenced Bacteroides strains. We found evidence of a number of different horizontal gene transfer events and a genome landscape that has been extensively altered by different mobilization events. A CRISPR/Cas system could be identified that provides a possible mechanism for preventing the integration of invading external DNA. We propose that the high genome plasticity and the introduced genome instabilities of B. vulgatus mpk arising from the various mobilization events might play an important role not only in its adaptation to the challenging intestinal environment in general, but also in its ability to interact with the gut microbiota.

EFFECT OF DIETARY ZINC ON THE HORIZONTAL TRANSFER OF ANTIBIOTIC RESISTANCE GENES IN THE INTESTINE

L. Ciesinski[1], S. Guenther[1], M.G. Doherr[2], L.H. Wieler[3], G. Loh[4]

[1]Center for Infection Medicine, Institute of Microbiology and Epizootics and [2]Institute for Veterinary Epidemiology and Biostatistics Freie Universität Berlin, Germany; [3]Robert Koch Institute, Berlin, Germany; 4Max Rubner-Institut, Karlsruhe, Germany

Infections with antibiotic-resistant bacteria belong to the emerging threats to human health. We speculated that not only misuse of antibiotics but also nutrition-derived stress factors may directly trigger horizontal transfer of antibiotic resistance genes between gut bacteria. To test this hypothesis, we fed diets containing zinc oxide (ZnO) at either 100 or 1,900 ppm to germ-free mice. The latter ZnO concentration may cause toxic stress to enterobacteria. After adaptation to diet, animals were inoculated with an Escherichia coli strain with a non-transmissible sodium azide resistance (experimental day 14) and a Klebsiella pneumoniae strain with a plasmid-encoded cefotaxime resistance (experimental day 21). Fecal material was collected each day and plated on agar plates containing either sodium azide, cefoxatime or both antimicrobial agents. Colony forming units (log10 cfu) of sodium azide-resistant E. coli (recipient strain), cefoxatime-resistant K. pneumoniae (donor strain) and of sodium azide-resistant E. coli with acquired cefotaxime resistance (transconjugants) were determined. Conjugation rates were calculated by dividing cfu of the transconjugant by cfu of the recipient strain. The area under the curve (AUC) approach was applied to integrate data obtained for each mouse, strain and diet group over the range of sampling time points.

Both bacterial strains successfully colonized the intestine of previously germ-free mice but fecal bacterial numbers were highly variable between the animals. Median values for the low zinc diet were log10 9.9 (donor strain), log10 8.7 (recipient strain) and log10 6.0 (transconjugant). When the high zinc diet was fed, median values were log10 10.0 (donor strain), log10 8.5 (recipient strain) and log10 5.6 (transconjugant). No effect of diet was observed when the AUC of the donor and recipient values were compared. In contrast transconjugant AUC was significantly higher for the mice fed with 100 ppm of zinc ($P = 0.025$) indicating that high dietary zinc concentrations may rather inhibit horizontal gene transfer. However, this notion was not supported by conjugation rate data and, thus, our study does not support the hypothesis that ZnO at high levels influences horizontal transfer of antibiotic resistance genes in the intestine.

THE ROLE OF DUOX2 IN SHAPING THE INTESTINAL MICROBIOTA AND ITS EFFECT ON HOST PHYSIOLOGY

F. Sommer[1], E. Jami[1,2], S. Lipinski[1], P. Rosenstiel[1]

[1]*Institute of Clinical Molecular Biology, Kiel University, Schittenhelmstr. 12, 24105 Kiel, Germany*
[2]*Department of Ruminant Science, Institute of Animal Sciences, Agricultural Research Organization, Volcani Center, Bet Dagan 50250, Israel*

The intestinal microbiota contributes to host physiology but also poses a potential infection danger. Reactive oxygen species (ROS) have antibiotic properties and therefore eukaryotes employ ROS as protective component of innate immunity. The main ROS producing enzymes belong to the NADPH oxidase family and within the intestine Duox2 is its most highly expressed member. Intestinal epithelial cells express DUOX2 and pathogenic infection but also the normal microbiota elevate Duox2 expression. We generated DUOX2-□IEC mice, which lack DUOX2 specifically in intestinal epithelial cells. Under basal unchallenged conditions DUOX2□□IEC mice did not display any inflammatory or metabolic phenotype. However, loss of DUOX2 in the intestinal epithelium altered the composition of the mucosal microbiota, for example enriching for the anti-inflammatory commensal Akkermansia muciniphila or depleting several Bacteroidetes taxa associated with energy extraction. We currently extend our analyses by testing whether the altered microbiota in DUOX2□□IEC mice confers differential disease susceptibility under inflammatory or dietary challenge using the DSS colitis model and a high-fat-diet feeding regime. Our data highlight the importance of mucosal ROS in host-microbiota interactions and the selection of a beneficial microbiota during normal homeostasis.

Funded by Deutsche Forschungsgemeinschaft CRC877, CRC1182 and Excellence Cluster Inflammation at Interfaces.

Keywords: ROS / DUOX2 / microbiota / inflammation / metabolism

INTESTINAL ESCHERICHIA COLI COLONIZATION IN A MALLARD DUCK POPULATION OVER FOUR CONSECUTIVE WINTER SEASONS

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

[1]*Institute of Biotechnology, Brandenburg Technical University Cottbus - Senftenberg, Senftenberg, Germany*

[2]*Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany*

E. coli populations are individual, dynamic, and very complex, but are poorly understood. Most studies focusing on aspects of competitive colonization have been performed using mouse models and cell-culture models, bioreactors or just liquid culture media, and were generally done with low numbers of bacterial strains. In the present study we describe the E. coli population dynamics of the wild animal species mallard duck (Anas platyrhynchos) - the most abundant duck species in Germany. Bacterial microflora in mallard ducks are only slightly affected by humans, as they are not continuously fed with defined nutrients and medication. Since it is impossible to study the intestinal microflora of individual wild animals over years, we chose to study a large mallard duck population which guaranteed both a continuous sampling of high numbers of bacteria and a random collection of fecal samples.

We sampled and characterized 100 E. coli isolates each consecutive season. Macrorestriction analysis was used to define isolates variously as multi- or one-year PFGE types. Isolates were characterized genotypically based on virulence-associated genes (VAGs), phylogenetic markers, and phenotypically based on hemolytic activity, antimicrobial resistance, adhesion to epithelial cells, microcin production, motility and carbohydrate metabolism.

Only 12 out of 220 PFGE types were detectable over more than one winter, and classified as multi-year PFGE types. There was a dramatic change of PFGE types within two winter seasons. Nevertheless, the genetic pool (VAGs) and antimicrobial resistance pattern remained remarkably stable. The high diversity and dynamics of this E. coli population were also demonstrated by the occurrence of PFGE subtypes and differences between isolates of one PFGE type (based on VAGs, antimicrobial resistance, and adhesion rates). Multi- and one-year PFGE types differed in antimicrobial resistance, VAGs and adhesion. Other parameters were not prominent colonization factors.

In conclusion, the high diversity, dynamics and stable genetic pool of an E. coli population seems to enable their successful colonization of host animal population overtime.

RAPID GENETIC DIVERSIFICATION OF BACTEROIDES THETAIOTAOMICRON IN THE MURINE GUT

Christos Zioutis[1], Nika Ivanovova[1], Fatima Pereira[1], Madeleine Wyss[2], Andrew J. Macpherson[2], Kathy D. McCoy[2], David Berry[1]

[1]*Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, University of Vienna*

[2]*Department of Clinical Research, University of Bern, Switzerland*

The human genome encodes the ability to degrade a limited diversity of the many polysaccharides present in a normal diet. Members of the gut microbiota therefore play a critical role in breaking down these recalcitrant compounds to provide additional energy for the body. Members of the genus Bacteroides, one of the most abundant bacterial taxa in the gut, encode a large repertoire of polysaccharide utilization genes and therefore are key to the digestion of complex dietary compounds. It is still unclear, however, how members of the microbiota adapt to successfully colonize and occupy niches in the gut. In this study, we investigated the role of rapid genetic diversification in Bacteroides thetaiotaomicron VPI-5482 colonizing the germ-free murine intestine over a four week period. Shotgun genomic re-sequencing was performed at regular intervals on fecal pellets to evaluate the extent of genomic diversification during this period. Analysis of genetic polymorphisms revealed extensive accumulation of single nucleotide polymorphisms as well as structural variations. Several polymorphisms appeared independently in multiple mice, indicating that there is a reproducible process of rapid genetic diversification in the murine gut that may be due to fitness selection. Our findings indicate in the initial assembly of the gut microbiota there are strong selection pressures and that rapid diversification may be an important process in determining the success of colonization. Experimental evolution is therefore a powerful approach to identify novel colonization factors and to unravel the interplay of ecology and evolution in the assembly and function of the gut microbiota.

POSTER

1 AGING IS ASSOCIATED WITH CHANGES OF INTESTINAL MICROBIOTA COMPOSITION AND BARRIER FUNCTION: STUDIES IN C57BL/6J MICE

A. Baumann[1], A. Brandt[1], C.J. Jin[1], A.J. Engstler[1], C. Sellmann[1], C. Schmeer[2], O.W. Witte[2], A. Camarinha-Silva[3], I. Bergheim[1]

[1]Institute of Nutrition, SD Model Systems of Molecular Nutrition, Friedrich Schiller University Jena, Jena, Germany
[2]Hans-Berger Department of Neurology, University Hospital Jena, Jena, Germany
[3]Institute of Animal Science, University of Hohenheim, Stuttgart, Germany

Intestinal microbiota and barrier function has been suggested to be involved in maintaining healthy life-span and longevity. Impairments of intestinal barrier function and changes of intestinal microbiota composition as well as alterations of immune cell composition in the gut are discussed to be critical in the development of low-grade inflammation and decline in elderly. However, the interaction between intestinal microbiota, barrier function, immune system and aging-associated degeneration and decline is only partially understood. Starting from the background, we assessed changes in intestinal microbiota composition, permeability and immune response in the small intestine of old and young mice. Markers of intestinal barrier function, e.g. portal endotoxin levels and protein levels of tight junctions, were determined in portal plasma and intestinal tissue obtained from small intestine of 24 and 3 months old standard chow-fed C57BL/6J mice. Illumina amplicon sequencing of intestinal microbiota was used to characterize the diversity and composition of the microbial communities of the upper parts of the small intestine. Bacterial endotoxin levels in portal plasma were significantly higher in 24 months old compared to 3 months old mice. Protein levels of the tight junction proteins occludin and ZO-1 were significantly lower in the upper parts of the small intestine of 24 months old than of 3 months old mice. While expression of markers of iNKT cells were higher in old mice compared to young mice, expression of F4/80 mRNA was significantly lower in old than in young mice. Furthermore, expression of iNOS and concentration of 3-nitrotyrosine protein adducts was also significantly lower in old than in young mice. These alterations were associated with a significantly loss of bacterial diversity in old-aged mice when compared to young animals. Taken together, our data suggest that increased bacterial endotoxin levels in old-aged mice are associated with marked changes in intestinal microbiota composition, a loss of tight junction proteins and marked changes of intestinal immune system in the upper parts of small intestine.

2 MURINE NOROVIRUS TRIGGERS INTESTINAL INFLAMMATION IN THE DEFINED FLORA COLONIZED SUSCEPTIBLE HOST

S. Bolsega[1], M. Basic[1], A. Smoczek[1]and A. Bleich[1]

[1]*Institute for Laboratory Animal Science and Central Animal Facility, Hannover Medical School, Germany*

Inflammatory bowel disease (IBD), with the two main forms Crohn's disease and ulcerative colitis, is a chronic relapsing inflammatory disorder of the digestive tract. The detailed mechanism driving IBD development is not yet known, however, the interaction of enteric microbiota, environmental and genetic factors contributes to the disease onset. Interleukin-10 deficient (*Il-10*$^{-/-}$) mice, lacking anti-inflammatory IL-10 cytokine, are a model for studying experimental IBD. In this model the inflammation development depends on strain background and microbial environment. *Il-10*$^{-/-}$ mice with C3H/HeJBir background (C3H-*Il-10*$^{-/-}$) are more susceptible to develop colitis than *Il-10*$^{-/-}$ mice on B6 background. Intestinal microbiota contributes to the severity of colitis in this model as well. If *Il-10*$^{-/-}$ mice are housed under germ-free conditions they will stay healthy, whereas in conventional housing conditions they will develop severe IBD. In our recent study we demonstrated that the murine norovirus (MNV) provides a colitogenic trigger in this model, which depends on the presence of microbiota. The aim of this study was to analyze the MNV ability to trigger colitis in a susceptible host colonized with defined minimal flora, the Altered Schaedler Flora (ASF). This bacterial community consists of two Lactobacilli, one Bacteroides, one Flexistipes, and four extremely oxygen sensitive (EOS) Fusobacterium species.

In mice colonized only with ASF no inflammatory lesions were observed, whereas ASF colonized mice, which were subsequently infected with MNV, developed intestinal inflammation. The inflammation was located in proximal colon and characterized by hyperplasia of crypt epithelium and infiltration of inflammatory cells like neutrophilic granulocytes, macrophages, and plasma cells in the intestinal wall. Additionally, the histopathologic findings were consistent with increased expression of proinflammatory cytokines. Furthermore, in the lamina propria increase of cell proliferation and cytotoxic cells producing granzyme B were observed. The intestinal barrier tightness by analyzing the tight junction expression was also determined.

Altogether these date indicate that the MNV triggers intestinal inflammation in C3H-*Il-10*$^{-/-}$ mice colonized with defined Altered Schaedler Flora.

3 BOOSTING MECHANISMS IN THE PROBIOTIC STRAIN LACTOBACILLUS PARACASEI F19

D. Brignone[1], P. Radmann[1], J. Behr[1], R.F. Vogel[1]

[1] Technische Universität München, Department of Technische Mikrobiologie, Gregor-Mendel-Str. 4, 85354 Freising, Germany

Lactobacillus (*L.*) *paracasei* subsp. *paracasei* F19 is a well known probiotic strain commonly used in dairy products. A mixture of probiotics and starter cultures is usually added to the milk as freeze-dried powder to start dairy fermentations; the drying stress leads to a conspicuous loss of viable cells and it can negatively influence the viability of the probiotics in the final product. The use of so-called booster substances can improve the performance of lactic acid bacteria. With a high-throughput proteomic screening, we aim at identifying fitness biomarkers of *L. paracasei* F19.

Common food additives, antioxidants and redox-active compounds were selected as candidate boosters; a wide screening was carried out and a statistical analysis of the growth parameters was performed. Remarkable boosting effects were obtained at laboratory scale. An improvement on the maximum growth rate up to 108% was registered and in some cases the lag phase was reduced up to 17% compared to the control. In the same experimental conditions, freeze dried cells were also boosted by the most effective substances, although to a lesser extent.

With a series of *in vitro* tests the boosters` influence on the strain`s fitness and stress tolerance, which may be part of probiotic characteristics, was monitored. The best boosters were able to improve the tolerance of F19 to the adverse growth condition in the GI tract, such as low pH and presence of reactive oxygen species. The boosters had no effect on the antimicrobial properties of *L. paracasei* against selected food-borne pathogens.

MALDI-TOF MS was used as a fast high-throughput screening method for protein spectra collection. A discriminant analysis of principal components allowed the separation of spectra into groups according to the type of substance added or control conditions; in addition cluster analysis revealed that the most prominent differences in the proteome resulted from booster application in the early exponential growth phase.

In conclusion, the results of the MALDI-TOF MS analysis enable a knowledge base for the identification of biomarkers associated with enhanced metabolic activity and fitness. The data collected could allow the transfer of the knowledge to other probiotics and starter cultures.

4 THE PROBIOTIC E. COLI STRAIN NISSLE 1917 INHIBITIS SHIGA TOXIN PRODUCTION IN EHEC AND PROTECTS E. COLI K-12 STRAINS AGAINST STX-PHAGE INFECTION

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 humans as 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 the 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 its B subunits to the globotriaosylceramide receptors (Gb3) of e.g. enterocytes 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 protein synthesis is blocked and the cells die 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 EHEC strains and Stx production, which can only be traced back in part to the production of antibacterial operating microcins [2]. In the course of our experiments with a transwell permeable system we could elucidate that no direct cell to cell contact is necessary for EcN to downregulate the expression of Stx by EHEC strains. Furthermore, we could reveal during in vitro studies that EHEC strains can convert *E. coli* K-12 strains to become Stx producers themselves which however, can be blocked by the presence of EcN but not by other commensal *E. coli* strains. This rescuing effect could be explained by a reduction of *stx* phage expression of EHEC provoked by the probiotic EcN. Our in vitro results might reflect the in vivo situation where *stx* phages can infect commensal bacteria in the human gut and turn them into Stx producers themselves. These findings encourage us to elucidate the mechanism of the downregulation of the Stx production in EHEC strains by EcN and support the idea of applying EcN as a medication in the treatment of EHEC infections as supplementary probiotic treatment during a human EHEC infection.

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5 RHEA: A MODULAR R PIPELINE FOR MICROBIAL PROFILING BASED ON HIGH-THROUGHPUT 16S RRNA GENE AMPLICONS

Ilias Lagkouvardos[1], Sandra Fischer[1], Neeraj Kumar[1], Thomas Clavel[1]

[1] *ZIEL Institute for Food and Health, Technische Universität München, Freising, Germany*

The importance of 16S rRNA amplicon profiles in understanding the influence of microbes in a variety of environments, including mammalian-associated microbiomes, coupled with the steep reduction in sequencing costs led to a surge of microbial sequencing projects. The rapid popularization of next generation technologies for microbiome sequencing is in stark contrast to the slow transfer of knowledge between the laboratories driving further developments in the field and a high number of laboratories simply interested in using these technologies. Among available pipeline options for high-throughput 16S rRNA gene analysis, the R programming environment for statistical computing stands out for its power and increased flexibility, and the possibility to adhere to most recent best practices and to adjust to individual project needs. Here, we present the Rhea pipeline, a set of R scripts that encode a series of well-documented choices for the downstream analysis of Operational Taxonomic Unit (OTU) tables, including normalization steps, alpha- and beta-diversity analysis, statistical comparison of composition data, and calculation of correlations. Rhea is both a straightforward starting point for beginners and a framework for advanced users who can modify and expand the tool. As the community standards evolve, Rhea will adapt to always represent the current state-of-the-art in microbial profile analysis in the clear and comprehensive way allowed by the R language. Rhea scripts and detailed documentation will be made freely available to the community upon publication.

6 XIAP-DEPENDENT REGULATION OF THE INTESTINAL MICROBIOTA IN THE PATHOGENESIS OF CROHN'S DISEASE

Ms Shreya Gopalakrishnan

Inflammatory bowel disease (IBD) is a group of diseases characterized by chronic intestinal inflammation. It is believed to be caused by interplay of genetic and environmental factors, but the precise etiology of the disease remains unknown. Recent genome-wide association studies (GWAS) have identified over 200 genetic loci that are associated with IBD, studies of which have given us great insights into the pathways involved in intestinal inflammation and possible therapeutic targets. We and others have recently identified a monogenic form of IBD caused by mutations in X-linked inhibitor of apoptosis protein (XIAP) and have described that XIAP mutations are found in about 4% of male children with early onset Crohn's disease (CD), a form of IBD. XIAP has been known to act as a central component of the NOD2 complex, a pattern recognition receptor (PRR) that detects bacterial muramyl dipeptide (MDP). As such, loss-of-function in XIAP is associated with impaired NOD2-dependent recognition of microbes and intestinal inflammation. However, the penetrance of CD in patients with XIAP mutations is about 20%, while patients with NOD2 mutations exhibit CD penetrance of about 1.5 % suggesting the existence of NOD2-independent effects, which may contribute to the intestinal inflammation in patients with XIAP mutations. To this end, Xiap-/- mice were investigated and revealed defects in Paneth cells (PC) - antimicrobial peptide-producing cells located at the intestinal crypt bottom, which are critical for innate immune responses in the gut. Xiap-/- mice, when compared to wildtype (WT) littermates, showed a decrease in PC-dependent antimicrobial peptide production, which arose from a mildly increased PC death that was mediated by tumor necrosis factor alpha (TNF-α) in a manner dependent on microbial recognition. As a consequence of PC death and decreased antimicrobial peptide secretion in the intestine, both the stratification and composition of the intestinal microbiota were altered. In addition to this, Xiap-/- mice also showed increased susceptibility to dextran sulphate sodium (DSS)-induced colitis and Helicobacter hepaticus infection. Together, an increased susceptibility to intestinal inflammation in the absence of XIAP may arise from a combined defect in NOD2-mediated bacterial recognition and PC survival resulting in an altered stratification and composition of the intestinal microbiota.

7 INTESTINAL INFLAMMATION IN A MURINE MODEL OF CROHN'S DISEASE LIKE INFLAMMATION DEPENDS ON THE MICROBIAL ENVIRONMENT

C. Günther[1], M. Hefele[1], B. Buchen[1], H. Dorner[1], H. Neumann[1], S. Bischoff[3], V. Volynets[3], Basic[2], A. Bleich[2], 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]*Institute of Nutritional Medicine, Stuttgart, Germany*

A better understanding of the host-microbial interaction in the context of establishing and maintaining intestinal barrier function is essential for the development of novel strategies for the management of intestinal inflammatory disorders. Although there is now clear evidence that necroptosis strongly contributes to intestinal inflammation and barrier dysfunction, little is known about the contribution of the intestinal microbiota on this particular form of cell death. Whereas Casp8$^{\Delta IEC}$ mice, which represent a novel mouse model of Crohn´s disease like inflammation spontaneously developed an ileitis, they did not display inflammation in other parts of the gastrointestinal tract under steady state conditions (Günther et al., Nature 2011).

Interestingly, our preliminary data now suggest that depending on the microbial environment, Casp8$^{\Delta IEC}$ mice also developed an inflammation in other parts of the intestine under steady state conditions. Accordingly, we discovered that Casp8$^{\Delta IEC}$ mice with the same genetic background housed under different conditions developed inflammatory lesions in diverse parts of the gastrointestinal tract, including the proximal small intestine, the caecum and a severe colitis accompanied by sub-epithelial cysts. Histological analysis demonstrated a marked destruction of the architecture and signs of inflammation including bowel wall thickening, loss of crypt architecture and increased cellularity within the lamina propria. This finding of spontaneous inflammation was further supported by increased expression of the inflammation marker S100A9 and by elevated infiltration of the lamina propria with macrophages and granulocytes. Moreover, colitis in these mice was characterized by massive epithelial cell death, suggesting an impaired barrier function in Casp8$^{\Delta IEC}$ mice housed under different microbial condiotions. In line with these observations, we also identified that some of these mice, developed a spontaneous inflammation in the liver, suggesting an increased translocation of bacteria and their products to the extraintestinal space, which might promote inflammation at distant sides. We further provide evidence that germfree Casp8$^{\Delta IEC}$ mice are protected from spontaneous inflammation in all parts of the intestine, supporting the hypothesis that microbial factors play an essential role in the pathogenesis of the Crohn´s disease like inflammation in these mice.

In summary these data demonstrate that Casp8$^{\Delta IEC}$ mice represent an important novel tool to study the contribution of a disease-relevant microbiota in a murine model for Crohn's disease-like ileitis and colitis, in order to elucidate disease mechanisms underlying chronic inflammation in human patients.

8 CHARACTERISATION OF FLIC ON COMMENSAL ESCHERICHIA COLI ON HEALTHY PATIENTS

T. Hagemann[1], S. Menz [1], J. Frick[1]

[1]*IMIT Dept. of Medical Microbiology and Hygiene, Universität Tübingen, Germany*

The flagellum is a key organelle. Its function goes beyond to propel the bacterial cell and participate in adherence and immune modulation and can stimulate the immune system in a pro- or anti-inflammatory manner

As a whole, the flagellum can be considered as a multi protein complex whose assembly relies on more than 40 genes. The flagellar filament is the resulting structure from the polymerization of the protein FliC. Analysis of the structure of FliC shows that it is organized in five domains: Nterminal-D0-D1-D2-D3-D2-D1-D0-Cterminal. Being the three central domains part of the hypervariable region of the protein.

Previous work performed in our laboratory showed that an insert present in the Cter-D2 domain of the probiotic bacteria Escherichia coli Nissle 1912 was able to protect from colitis on a DSS mouse model. Therefore we raise the question if insertions like that, found in E. coli Nissle are playing a similar role in other E. coli strains. We hypothesize that there might be a correlation between the FliC amino acidic sequence and the occurrence of homeostasis in the gut. To address this we characterized FliC from E. coli isolates from healthy patients. The fliC gene was amplified and bioinformatically analysed in search for the sequence corresponding to the Cter-D2 domain.

As a future approach would be interesting to explore if in patients with intestinal disease there is a lack or remarkable difference in the Cter-D2 domain insertion compared with the one observed on E. coli Nissle

9 VISUALIZING MICROBIAL ACTIVITY IN SPATIALLY STRUCTURED ENVIRONMENTS USING RAMAN MICROSPECTROSCOPY

Jesse P. Harrison[1], Kriti Sharma[2], Elizabeth A. Shank[2], David Berry[1]

[1]Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, University of Vienna, A-1090 Vienna, Austria
[2]Department of Biology, University of North Carolina, Chapel Hill, NC 27599, USA

Microbial communities are essential to human and ecosystem health, playing a key role in processes including nutrient cycling and biodegradation of recalcitrant compounds. Several environments, such as the gut and soil habitats, are characterized by a high degree of spatial complexity, which can significantly influence the distribution and activities of individual microbial cells. Existing experimental methods rarely capture this physical complexity, however, and our knowledge of microbial activities and interactions within most natural environments is almost entirely based on indirect observations.

To improve our ability to investigate microbial activity in situ, this project aims to develop a Raman microspectroscopy platform that enables the direct and real-time tracking of microbial activities within realistic, spatially complex environments. Raman spectra of single cells can be obtained within seconds and this technique is compatible with labelling methods for the monitoring of activities such as lipid and protein biosynthesis.

Our preliminary work shows that the spectra of metabolically active bacterial cells, detected via the uptake of deuterium-enriched water, can be successfully distinguished from the spectra of a key energy source for bacterial growth in the human large intestine (insoluble starch), as well as biologically inert materials. Our data also demonstrate that Raman-based activity measurement is compatible with a commonly used nutrient medium, with highly reproducible results. We anticipate that, once fully established, this experimental platform will have important applications in diverse research areas, including nutritional science and medical microbiology.

10 EXPERIMENTAL EVOLUTION OF POLYSACCHARIDE-DEGRADING BACTEROIDES THETAIOTAOMICRON, AN ABUNDANT MEMBER OF THE HUMAN GUT

N. Ivanovova[1], C. Zioutis[1], F. Pereira[1], D. Berry[1]

[1]*Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, University of Vienna*

The intestinal microbiota is important for human health and nutrition. The genus *Bacteroides* is an abundant and diverse group that is associated with a healthy digestive tract. *Bacteroides* species are particularly adapted to utilization of complex dietary and host-derived compounds via an extensive repertoire of polysaccharide utilisation loci. These are evidence of genomic adaptation to survive in response to availability of various nutrient sources. However, the relationship between metabolic flexibility and fine-scale diversification and adaptation to different nutrient-based niches by *Bacteroides* species has not been extensively studied. Here, we focus on *in vitro* and *in vivo* evolution of one of the most broadly-studied species of this genus, *Bacteroides thetaiotaomicron* VPI-5482. Using antibiotic resistance markers in isogenic populations, we were able to track soft evolutionary sweeps in the first four weeks of colonization of germ-free mice, suggesting accumulation of advantageous mutations in the populations. To further determine the ability of *B. thetaiotaomicron* to digest different carbohydrates, pure cultures were grown in defined minimal medium of single compounds as well as mixtures of compounds such as amylopectin, pectin, and inulin. Bacterial growth was detected by optical density and pH measurements to monitor specific growth rates and generation times. Ongoing *in vitro* experimental evolution experiments of *B. thetaiotaomicron* in the presence of different polysaccharides will shed light on the importance of dietary polysaccharide complexity and rapid adaptation on microbiota assembly and niche saturation.

11 RAPID PCR-BASED DETECTION OF THE PROBIOTIC E. COLI STRAIN NISSLE 1917 IN HUMAN STOOL SAMPLES

B. Klinkert[1], S. Dubbert[1], U. Sonnenborn[1], R. von Buenau[1]

[1]*Division of Biological Research, Molecular Genetics, Ardeypharm GmbH, Germany*

The non-pathogenic *Escherichia coli* strain Nissle 1917 (EcN) is used as a probiotic drug against intestinal disorders and diseases in the pharmaceutical preparation MUTAFLOR®. Recently, the genomic sequence of EcN was published [1] allowing to search for sequences exclusively present in EcN. Moreover, EcN typically contains the two cryptic plasmids pMUT1 and pMUT2 [2]. Plasmids homolog to pMUT1 also exist in *Citrobacter rodentium* (pCRP3), *Klebsiella pneumoniae* (pB1020) and in enterohemorrhagic *E. coli* (EHEC) O157:H7 (strain 86-24) (p9705). Partial sequences of pMUT2 including the *mobABCD* gene cluster were also found in *Plesiomonas shigelloides*. Both plasmids together are exclusively found in EcN.

A specific TaqMan® PCR assay was developed based on amplification of plasmid specific sequences and the detection of an EcN specific genomic region. Probes and primers were designed with the eurofins® multiplex designer program. This assay enables to distinguish EcN from all bacteria including even close relative *E. coli* strains.

In order to investigate the presence of EcN in humans e.g. to determine the duration of colonization after application of MUTAFLOR®, we tested our EcN specific multiplex TaqMan® PCR on human stool samples. Stool samples are very complex with a total bacterial count of 10^{13} colony forming units (CFU) per g stool. Often those samples contain inhibitory substances disturbing accurate PCR. Total DNA was extracted from the samples with the QIAGEN Fast Stool Mini Kit. An additionally included detection of the enterobacteriaceae specific gene *(tuf* gene) [3] serves as a control for inhibitory substances in the stool samples. The here developed PCR assay reached a limit of detection (LOD) of 10^3 CFU EcN per g stool, which is tenfold better LOD compared to classical PCR and agarose gel techniques.

[1] Reister et al.; J. Biotechnol 2014; 187 107-107.

[2] Blum-Oehler et al.; Research in Microbiology 2003; 154 59-66.

[3] Maheux et al.; Water research 2009; 43 3019-3028.

12 DENDRITIC CELL MATURATION: A PROTEOMICS APPROACH

G. Korkmaz[1], T. Popov[1], L. Peisl[1], Marius Codrea[2], Sven Nahnsen[2], Ana Velic[3], Boris Macek[3] 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*

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 in order to define proteins/processes/signalling pathways that define semi-mature and mature dendritic cells. In our analysis we have identified differentially regulated proteins that constitute the core factors in inflammatory pathways and stress response, including iNOS, CCL5, COX2 and RIG1. Further bioinformatical analysis predicted activation in key anti-inflammatory upstream regulators such as ABCA1, PTGER4, SOCS1 and DUSP1 in *B. vulgatus* stimulated DCs. Thus the general pattern we observe in our proteome analysis is that *B.vulgatus* regulates signaling pathways that control inflammation and reduces the expression of proinflammatory genes or increases the expression of anti-inflammatory proteins, thereby having an overall protective effect against a fulminant inflammatory response. Our findings are to be confirmed with functional assays and 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.

13 LIPIDOMIC METHODS TO UNRAVEL THE IMPACT OF THE MICROBIOME ON HOST LIPID METABOLISM

G. Liebisch[1], S. Krautbauer[1], S. Matysik[1], J. Ecker[2]

[1]Institute of Clinical Chemistry and Laboratory Medicine, Regensburg University Hospital, Regensburg, Germany

[2]Nutritional Physiology, Technische Universität München and ZIEL Institute for Food and Health, Freising, Germany

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

Electrospray tandem mass spectrometry (ESI-MS/MS) offers an excellent platform to quantify lipid species with high sample throughput. Major glycerophospholipid and sphingolipid classes are accessible by direct flow injection of crude lipid extracts. Whereas low abundant or isobaric species require frequently liquid chromatographic separation coupled to tandem mass spectrometry (LC-MS/MS). Lipid species quantitation is applicable for biomarker search in large clinical studies as well as basic research in a variety of sample materials including plasma, lipoprotein fractions, cells, tissues and faeces. Moreover, these methods provide insight into dynamics of the lipid species metabolism by administration of stable isotope labelled precursors or lipid species. For example major pathways of the glycerophospholipid metabolism may be profiled using D_9-choline, D_4-ethanolamine and $^{13}C_3$-serine; labelled acetate and fatty acids may be applied to profile fatty acid synthesis, uptake and metabolism.

Taken together, mass spectrometry offers a powerful tool box to study the influence of gut microbiota on host lipid metabolism including resorption of fatty acids, lipid synthesis and storage. Moreover, lipidomic analyses of faecal samples provide insight into lipid profiles of the microbiome and its impact on intestinal lipid modification.

14 PROBEBASE RELOADED – NEW FEATURES OF THE ONLINE RESOURCE FOR RRNA-TARGETED OLIGONUCLEOTIDE PROBES AND PRIMERS

Daniel Greuter[1], Alexander Loy[2], Matthias Horn[2] and Thomas Rattei[1]

[1]Division of Computational Systems Biology, and [2]Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, Research Network Chemistry meets Microbiology, University of Vienna, A-1090 Wien, Austria

probeBase http://www.probebase.net is a manually maintained and curated database of rRNA-targeted oligonucleotide probes and primers (1, 2, 3). Contextual information and multiple options for evaluating *in silico* hybridization performance against the most recent rRNA sequence databases are provided for each oligonucleotide entry, which makes probeBase an important and frequently used resource for microbiology research and diagnostics. Here we present a major update of probeBase, which describes a complete remodeling of the database architecture and environment to accommodate computationally efficient access. Improved search functions, sequence match tools, and data output now extend the opportunities for finding suitable hierarchical probe sets that target an organism or taxon at different taxonomic levels. To facilitate the identification of complementary probe sets for organisms represented by short rRNA sequence reads generated by amplicon sequencing or metagenomic analysis with next generation sequencing technologies such as Illumina and IonTorrent, we introduce a novel tool that recovers surrogate near full-length rRNA sequences for short query sequences and finds matching oligonucleotides in probeBase.

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Loy A, Maixner F, Wagner M, and Horn M. [2007] probeBase – An online resource for rRNA-targeted oligonucleotide probes: New features 2007. *Nucleic Acids Res*. 35: D800-D804.

Loy A, Horn M, and Wagner M [2003] probeBase: an online resource for rRNA-targeted oligonucleotide probes. *Nucleic Acids Res*. 31: 514-516.

15 IMPACT OF SYMBIOTIC B. VULGATUS INDUCED REGULATORY B CELLS ON PRESERVATION AND RECOVERY OF THE INTESTINAL IMMUNE EQUILIBRIUM

JK Maerz[1], A. Steimle[1], A. Lange[1], A. Bender[1], I. B. Autenrieth[1], J.-S. Frick[1]

[1]*University of Tübingen, Interfakultäres Institut für Mikrobiologie und Infektionsmedizin*

In general, B cells positively regulate adaptive immune responses by the production of antibodies and thus facilitate optimal $CD4^+$ T-cell activation. Additionally, B cells modulate the innate immune system via presentation of antigens and the secretion of immune-modulating cytokines. Furthermore, a specific subset of B cells (Bregs) exhibit immunosuppressive functions and can also negatively regulate the immune response in mouse models of autoimmune diseases. Thereby the intestinal microbiota plays a critical role for the induction of different B cell phenotypes either by direct or indirect interaction. In previous experiments we could demonstrate that the symbiotic gut commensal *Bacteroides vulgatus* mpk induces tolerant and tolerogenic bone marrow derived dendritic cells with anti-inflammatory properties *in vitro* and *in vivo* and is therefore involved in prevention of inflammation in a model for experimental colitis.

In order to clarify the influence of the intestinal microbiota composition on B cell-mediated immune responses and the role of Bregs in supporting immune homoeostasis, we analyse the immune system activating capacities of two completely sequenced gut commensal strains (*B. vulgatus* mpk and *E. coli* mpk). We could already show that the stimulation of isolated naïve B cells with symbiotic *B. vulgatus* leads to a reduced B cell proliferation *in vitro* and a diverse differentiation of B cell subsets, particularly regarding the development of Bregs. Moreover, *in vivo* colonization of mice with *B. vulgatus* increases the quantity of IL-10 producing B cells in the spleen and the colonic lamina propia. This effect is depended on the specific antigen recognition via *pattern recognition receptors*, since it is abolished in Toll-like receptor deficient mice.

Further we want to (1) characterize the different B cell subset *in vitro* (B10 cells and T2-MZP Breg cells) after priming B cells with *B. vulgatus* or *E. coli,* (2) to investigate the antigen presentation functions of primed B cells and the linked potential for T cell activation and proliferation, (3) to identify the abundance of B cells with regulatory features in mono- and co-colonized wild-type and $Rag1^{-/-}$ mice to demonstrate the modulation of B cells through different bacteria and (4) to highlight the influence of symbiotic bacteria on B cell-mediated immune response for maintaining immune tolerance by adoptive transfer of specific bacteria primed B cell subsets in mouse models for IBD and Multiple sclerosis.

16 HUMANIZED MOUSE MODEL OF INFLAMMATORY BOWEL DISEASE: FUNCTIONAL CHARACTERIZATION OF DYSBIOTIC GUT MICROBIOTA

Amira Metwaly[1], Ludovica F.Buttó[1], Ilias Lagkouvardos[2], Anna Corraliza[3], Azucena Salas[3], Julian Panes[3], Matthieu Allez[4] and Dirk Haller[1, 2]

[1]Chair of Nutrition and Immunology, Technische Universität München, Freising-Weihenstephan,Germany; [2]ZIEL-Institute for Food and Health, Technische Universität München, Freising-Weihenstephan,Germany; [3]Department of Experimental Pathology, Instituto de Investigaciones Biomédicas de Barcelona-CSIC,IDIBAPS, CIBERehd Spain. [4]APHP, Hôpital Saint Louis, Department of Gastroenterology, INSERM UMRS 1160, Paris Diderot, Sorbonne Paris-Cité University, Paris, France

Background & Aims: Imbalanced microbial composition (dysbiosis) has been linked to the pathogenesis of inflammatory bowel disease (IBD). Hematopoietic stem cell transplantation (HSCT) has proven to be extremely successful in inducing remission in a subset of severe, highly refractory Crohn's disease (CD) patients, possibly by erasing immune responses against microbes. We established a humanized gnotobiotic mouse model to assess the functional role of gut dysbiosis associated with different disease-state, different clinical outcomes or the risk of relapse in IBD patients treated with HSCT or anti-TNF therapy.

Methods: Germ-free mice (TNF^{dARE}) were colonized (8w-12w of age) with fecal samples of CD patients presenting different disease state and clinical outcomes. Microbiome analysis was performed on the samples of human donors and humanized mice and the inflammatory capacity of disease-associated microbiota was evaluated by histopathology, immunostaining and immune-phenotyping.

Results: Humanization of $TNF^{deltaARE}$ mice with CD patients microbiota (dysbiotic or in remission) did not establish disease as per histopathology and plasma cytokines levels, which showed to be at same levels of those in GF mice. The gut bacterial composition at phylum level was similar in human donors and humanized mice with increased Bacteriodetes/Firmicutes ratio. However, at the species level, the bacterial composition was hugely different, especially within the phylum Firmicutes, where a loss of (around 30%) in the comprising operational taxonomic units (OTUs) was observed. Strikingly, some of the most abundant taxa in human sample were not detected in the humanized mice. Immune-phenotyping showed that $TNF^{deltaARE}$ mice displayed higher CD4+ and CD8+ effector T cell population in splenocytes, higher CD8+ activated T cell population in splenocytes and MLNs, and lower CD8+ naïve T cell population in splenocytes compared to WT mice. Furthermore, we observed an increase in CD3+ CD4+ CD25- FoxP3+ cells in both spleen cells and MLNs.

Conclusion: Together, our data shows that IBD phenotype is not recapitulated in the $TNF^{deltaARE}$ humanized mouse. Human donor and humanized mice have similar relative-abundance levels of the major bacterial phyla in the gut. However, analysis of shared bacterial OTUs between the two groups suggests a drastic loss of species, especially within the phylum Firmicutes. This suggests that some bacterial taxa (mostly Firmicutes) exhibit host specificity, and compromising the colonization efficiency. Different approaches are currently being tested to characterize this selective pressure in the mouse. In addition, humanization of $IL10^{-/-}$ mice as a colitis mouse model is under investigation.

17 REGULATION OF HOST CATHEPSIN B ACTIVITY IS ESSENTIAL FOR MAINTAINANCE OF INTESTINAL HOMEOSTASIS

L. Michaelis[1], A. Steimle[1], B. Beifuss[1], R. Harmening[1], A. Schäfer[1], J.-S. Frick[1]

[1]*University of Tübingen, Interfakultäres Institut für Mikrobiologie und Infektionsmedizin*

Bacteroides vulgatus monocolonization has revealed beneficial effects in germfree $Rag^{-/-}$ mice upon adoptive T cell transfer since T cell proliferation is not induced in this model of experimental colitis. In addition, T cell transplanted $Rag^{-/-}$ mice harbouring a complex microbiota with enhanced levels of *Enterobactericeae* develop symptoms of a T cell mediated chronic colitis. Administration of *B. vulgatus* mpk to these mice before T cell transplantation and during inflammation was shown to protect them from colitis or result in recovery from colitis symptoms, respectively. In contrast to *Escherichia coli* mpk, which induces dendritic cell maturation and subsequent T cell activation upon administration, *B. vulgatus* is thought to contribute to intestinal homeostasis by induction of a tolerant and tolerogenic semi-mature phenotype of dendritic cells. However, an underlying molecular mechanism manipulated by *B. vulgatus* has not been clearly described. We assume that *B. vulgatus* prevents from V-type ATPase subunit assembly in dendritic cells and therefore prohibits acidification of the vesicular compartment being a prerequisition for endosomal and lysosomal protease activity. Thus, an increased intravesicular pH impairs the activity of the endosomal protease Cathepsin B (CTSB) which is involved in antigen and MHC class II processing. This dendritic cell semi-maturation does not lead to T cell maturation and prevents from inflammation. Exploring the molecular pathways beneficially influenced by *B. vulgatus* might help to find a potential treatment strategy in order to restore intestinal homeostasis in individuals with inflammatory bowel disorders (IBD). Our focus lies on the inhibition of V-type ATPase and intracellular CTSB. Furthermore, enhanced extracellular CTSB activity in colonic epithelial tissue was shown to contribute to inflammation in the gut. In fact, we could abolish induction of colonic inflammation in a mouse model for T cell mediated experimental colitis using an intracellular CTSB inhibitor. This finding underlies the role of CTSB as a potential drug target for the treatment of IBD.

18 CANDIDA-BACTERIA COINFECTIONS IN THE GUT AND THEIR IMPACT ON DISEASE DEVELOPMENT

M. J. Niemiec[1], M. Kapitan[1], Ilse D. Jacobsen[1]

[1]*Microbial Immunology Unit, Hans Knöll Institute, Leibniz Institute for Natural Product Research and Infection Biology*

C.albicans is an opportunistic fungal pathogen that colonizes most humans asymptomatically – with the primary reservoir being the gastro-intestinal tract. From there, *Candida* can disseminate leading to blood stream infections (BSIs) or candidemia, abscess formation in varies organs, and eventually septic shock. The ability to switch between planktonic yeasts and filamentous hyphae is known to be crucial during *C. albicans* infections. Risk factors for dissemination, for instance severe trauma or surgery, are mutually characterized by a decreased barrier function of the gut epithelial layer. To date, *C. albicans* is the most frequently isolated fungus in systemic infections. While the gut microbiome is composed of diverse fungi and bacteria, also candidemia is often accompanied by a bacterial BSI. These polymicrobial infections are associated with altered severity and mortality indicating changes in damage potential and immune response compared to the respective single-species infections.

Since the understanding of bacterial-fungal interactions in the human gut and its impact on dissemination and disease development are very limited, we aim to investigate the interplay of *C. albicans* with gut-associated bacteria and the human immune system.

For this, we selected 25 gram-positive and gram-negative bacteria from varies sepsis-relevant species, e.g. *Escherichia coli* and *Pseudomonas aeruginosa*. Culture supernatants retrieved under different growth conditions were collected and tested for their potential to inhibit *Candida* growth and hyphal formation. Simultaneously, the damage potential of *Candida*-bacteria coinfections was assessed using an epithelial layer composed of HT29-MTX and C2BBe1 cells.

Most importantly, our preliminary data indicates that the bacterial inhibitory potential towards *C. albicans* is not only species-, but also highly strain-dependent. Similarly, cell damage during bacterial-fungal coinfections was altered heterogeneously.

In future experiments, we aim to dissect the mechanism underlying synergism or antagonism during mixed infections with *C. albicans* and develop a cocolonization model in mice to investigate the impact of the mammalian immune system to the interplay.

19 PREDICTED TLR9-DEPENDENT IMMUNE MODULATION BY (META-)GENOMIC DNA

D. Podlesny, C. Arze, W.F. Fricke
Dept. of Nutrigenomics, University of Hohenheim, Stuttgart, Germany

Pro- and anti-inflammatory immune modulation via Toll-Like Receptor (TLR) 9 is being extensively studied by using synthetic oligodeoxynucleotides (ODNs) as adjuvants in vaccination and cancer therapy, as well as in the experimental treatment of autoimmune diseases. Although incompletely understood, TLR9 activation has been attributed to specific short DNA sequences, including [CG] or [GC]-containing 8-mers. As these sequence motifs naturally occur in microbial genomes and TLR9 is known to respond to stimulation by genomic DNA, the goal of this project was to compare bacterial genomes and metagenomes from the human microbiota with respect to their predicted TLR9 activation potential.

Individual bacterial genomes were analyzed based on a representative subset of complete or draft genome assemblies from 1,644 bacterial and 149 archaeal species from NCBI's RefSeq database. For metagenomic analyses, we focused on the neonatal gut microbiota, for which an immunological relevance of TLR9 modulation had previously been suggested. Metagenomic shotgun sequence data of more than 160 samples obtained from the Human Microbiome Project and NCBI's Short Read Archive were analyzed based on unassembled raw reads. Relative abundances of K-mers of length 2 and 8 nucleotides were determined using the KAnalyze tool and compared in R. As a broader predictive marker for TLR9 activation, relative abundances of the 2-mers [CG] and [GC] were studied, whereas a more detailed analysis was carried out based on a previously described set of 15 immune stimulatory (ISS) and regulatory (IRS) 8-mers. Relative K-mer abundances were studied in the context of variations in G+C contents and taxonomic distance (genomes) or taxonomic composition (metagenomes) of the input data.

Based on our preliminary results, individual genomes show significant variations in the ratio of [CG] and [GC] that appear to reflect pathogenic lifestyles of their bacterial hosts. Additionally, based on relevant 8-mer concentrations, neonatal compared to adult fecal metagenomes were predicted to induce increased activation of TLR9.

Our preliminary data suggests that genome sequence-based *in silico* prediction of TLR9 activation could identify biomarkers for different bacterial lifestyles and support a role of TLR9 stimulation for neonatal immune homeostasis in the intestinal tract.

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

C. Ring[1], K. Dahlke[1], M. Basic[2], A. Bleich[2], M. Blaut[1]

[1]*Department Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam-Rehbrücke, Nuthetal, Germany*

[2]*Institute for Laboratory Animal Science and Central Animal Facility, Hannover Medical School, Hannover, Germany*

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

A. muciniphila exacerbates inflammation induced by *Salmonella enterica* Typhimurium and concomitantly leads to a dramatic shift in the composition of the gut microbiota in gnotobiotic mice. To identify the underlying mechanisms the time course of the events following the infection with *S. enterica* Typhimurium is being investigated in detail. Microbiota composition, histological parameters and cytokine expression, as well as modification in the mucus layer are assessed in a time dependent manner. Candidate proteins and pathways involved in the *A. muciniphila*-mediated effects will be detected by proteome analysis of bacteria and host tissues.

Effects of substrates released from mucin by *A. muciniphila* on *S. enterica* Typhimurium growth independent of the host were investigated by *in vitro* experiments. The obtained results do not support a growth-promoting effect of *A. muciniphila* on *S. enterica* Typhimurium in minimal medium with mucin.

To clarify whether the inflammation-promoting effect of *A. muciniphila* is a general feature of this organism, another mouse model, namely the colitis-prone IL-10 knockout mouse, is being used. Gnotobiotic mice associated with selected bacterial species are additionally associated with *A. muciniphila* to assess the inflammatory response in dependence of the microbiota and the presence or absence of *A. muciniphila*.

21 PEDIATRIC OBESITY IS ASSOCIATED WITH AN ALTERED GUT MICROBIOTA AND DISCORDANT SHIFTS IN FIRMICUTES POPULATIONS

Alessandra Riva[1,2], Francesca Borgo[2], Carlotta Lassandro[3], Elvira Verduci[3], Giulia Morace[2], Elisa Borghi[2], and David Berry[1]

[1]*Department of Microbiology and Ecosystem Science, Division of Microbial Ecology, Research Network Chemistry Meets Microbiology, University of Vienna, Althanstrasse 14, Vienna, Austria,*
[2]*Department of Health Sciences, Università degli Studi di Milano, via di Rudinì, 8, Milan, Italy,*
[3]*Department of Pediatrics, San Paolo Hospital, via di Rudinì, 8, Milan, Italy*

An altered gut microbiota has recently been linked to obesity. However, knowledge about gut microbiota in paediatric obesity is currently very limited. The primary aim of this study was to characterize the composition of the gut microbiota in obese (n=42) and normal-weight (n=36) Italian children between 6 and 16 years of age. Using 16S rRNA gene-targeted sequencing, we evaluated taxa with differential abundance according to weight status and age- and sex-normalized body mass index (BMI z-score). Obesity was associated with an altered gut microbiota, which was characterized by elevated levels of Firmicutes and depleted levels of Bacteroidetes. Correlation network analysis revealed that the gut microbiota of obese children also had increased correlation density and clustering of operational taxonomic units (OTUs). Members of the Bacteroidetes were generally better predictors of BMI z-score and obesity than Firmicutes, which was likely due to discordant responses of Firmicutes OTUs, with some positively and some negatively correlated with BMI z-score. In accordance with these observations, the main metabolites produced by gut bacteria, short chain fatty acids (SCFAs), were significantly higher in obese children, suggesting elevated substrate utilization by the gut microbiota of obese children. Multiple taxa were correlated with SCFA levels, reinforcing the tight link between the microbiota, SCFAs, and obesity. Our results suggest that gut microbiota dysbiosis and elevated fermentation activity may be involved in the etiology of childhood obesity.

22 INVESTIGATING THE IMPACT OF AN INFLAMMATORY GUT MILIEU ON THE MICROBIOTA USING AN IN VITRO CULTURE ASSAY

Patrick Schiller[1], Markus Beutler[1], Sandrine Brugiroux[1], Simone Herp[1], Debora Garzetti[1], Saib Hussain[1], Diana Ring[1] and Bärbel Stecher[1§]

[1]Max von Pettenkofer-Institut, LMU München, Pettenkoferstr. 9a, 80336 München, GERMANY

[§] Corresponding author

The intestinal microbiota efficiently limits enteric infection by pathogens (colonization resistance). Pathogen infection and gut inflammation can alter the intestinal milieu, which can induce a state of dysbiosis and pathogen "blooming". Inflammation-induced dysbiosis is characterized by reduced iron availability and an increased concentration of substrates for bacterial anaerobic respiration (e.g. nitrate, tetrathionate), which is exploited by enteric pathogens, such as *Salmonella enterica* serovar Typhimurium (S. Tm). So far, little is known as to how the inflammatory milieu in the gut affects members of the microbiota.

We established an *in vitro* culture assay of a defined consortium of mouse-derived commensal bacteria, the Oligo Mouse Microbiota (Oligo-MM). The Oligo-MM consists of twelve bacterial strains representing five abundant bacterial phyla of the mouse gut: *Firmicutes, Bacteroidetes, Actinobacteria, Verrucomicrobia* and *Proteobacteria*. The inflammatory gut milieu was mimicked by depleting iron from the culture medium using iron chelators and by adding sodium nitrate to the culture system. Depletion of ferrous iron (Fe^{2+}) by the iron chelator 2,2'-Dipyridyl in the culture system led to reduced growth of most Oligo-MM strains, yet *Enterococcus faecalis* was unaffected by the reduced iron availability. The relative composition of the bacterial strains was altered considerably. Depletion of ferric iron (Fe^{3+}) by DTPA limited growth of bacterial strains in a similar way to treatment with 2,2'-Dipyridyl but changes were less pronounced. Supplementation of sodium nitrate resulted in a slightly shifted microbial composition and increased relative abundance of *Clostridium clostridioforme*.

In summary, our experiments disclose a prominent role of iron-depletion and increased NO3 concentrations in promoting microbial dysbiosis. Changes observed in vitro partially resemble shifts observed in vivo during S. Tm infection in mice. By further exploring the impact of environmental factors on representative members of the gut microbiota we envision to extend the current knowledge on the mechanisms underlying gut inflammation-inflicted dysbiosis and, thereby, contribute to the development of new therapies to prevent pathogen "blooming" and collateral damage of the gut microbiota.

23 ROLE OF INTESTINAL MICROBIOTA ON GUT BARRIER, METABOLIC FUNCTION AND INFLAMMATION IN A HUMANIZED MOUSE MODEL OF DIET-INDUCED OBESITY

Valentina Schüppel[1], Annick Hartstra[2], Max Nieuwdorp[2], Dirk Haller[1,3]

[1]Chair for Nutrition and Immunology, *Technische Universität, Freising-Weihenstephan, Germany; [2]Department of Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands; [3]ZIEL - Institute for Food & Health, Technische Universität, Freising-Weihenstephan, Germany*

Background & Aim: An altered gut microbiota has been linked to chronic inflammatory disorders and low grade inflammation, but also to metabolic diseases including obesity and diabetes. However cause and consequence are still unknown. Colonization of germfree mice is an appropriate approach to test the role of the gut microbiome in the context of obesity and related disorders. The aim of this study was to establish a gnotobiotic mouse model for obesity and metabolic dysfunction using patient-derived human microbiota.

Methods & Results: Human fecal samples were obtained from an obese and insulin resistant patient who underwent fecal microbiota transplantation (FMT) with autologous stool and butyrate tablets. 4 weeks after FMT body mass index and fasting blood glucose were unchanged, but insulin level and inflammation marker improved. Mice were colonized via single gavage with either “pre-FMT” or “post-FMT” fecal microbiota for 4, 8 or 12 weeks receiving control diet. After colonization mice revealed normal body and fat pad weights associated with unaffected fasting blood glucose levels and intact gut barrier independently of human donor treatment and colonization period. Preliminary sequencing data showed a shift of microbiota composition and a dramatic loss in number of bacterial species after 4 weeks of colonization compared to human donor. Additional challenge of colonized mice with palm oil-based high fat diet induced a significant increase in body and fat weight associated with impaired glucose tolerance and elevated permeability of jejunum in both pre-FMT and post-FMT group.

Conclusion: We demonstrated that obesity and insulin resistance cannot be initialized in mice by transferring patient-derived human fecal microbiota. In addition, the transfer of human microbiota into mice resulted in a substantial change in bacterial community structure and bacterial richness/diversity. Diet-induced impairment of glucose tolerance and gut barrier function was similar in microbiota transfer experiment with pre- and post-FMT microbiota.

24 DEVELOPMENT OF AN IMPROVED PRIMARY IN VITRO MODEL OF THE HUMAN SMALL INTESTINE

Matthias Schweinlin[1], Sabine Wilhelm[1], Heike Walles[1,2], Marco Metzger[1,2]

[1] Department of Tissue Engineering and Regenerative Medicine (TERM), University Hospital Würzburg, Röntgenring 11, 97070 Würzburg, Germany

[2] Translational Center Würzburg "Regenerative Therapies for Oncology and Musculoskeletal Diseases" (TZKME), Würzburg branch of the Fraunhofer Institute Interfacial Engineering and Biotechnology (IGB), Röntgenring 11, 97070 Würzburg, Germany

The small intestine in our body represents the organ with the largest contact surface to the environment. Its main function is the absorption of essential nutrients, water and vitamins, and it is a barrier protecting us from toxic xenobiotics and pathogens. It provides an elegant system for stem cell studies as well as aspects of transport mechanisms and barrier functions.

In our study, we applied epithelial cells in a 3D in vitro culture system in order to mimic the microenvironment of the gut in vivo.

Intestinal crypts including stem cells were isolated from human small intestinal tissue samples and co-cultured on a decellularized porcine gut matrix together with intestinal fibroblasts. In vitro models were maintained under static and dynamic conditions for 7 days. Epithelial integrity was tested by FITC-dextran (4kDa) and TEER-measurement. Models were further characterized by qPCR, immunohistochemistry, electron microscopy and transport assays.

Intestinal cells have formed a monolayer including all the differentiated cell types shown by, Mucin2, Villin, Chromogranin A, and Lysozyme immunohistochemistry. Electron microscopy depicted essential functional units of an intact epithelium such as microvilli and tight junctions. FITC-dextran and TEER-measurement proved tightness of the cell layer. Models showed characteristic transport activity for several reference substances.

The development of an in vitro system based on human primary cells provides a promising tool for more predictive preclinical testing with pharmaceutical substances, probiotic active organisms or human pathogenic germs in infection studies. Further functional validation studies are necessary to show stabile enzyme activity, transport function, and batch to batch consistency.

25 COMPARATIVE TRANSCRIPTOME ANALYSIS OF E.COLI NISSLE 1917 (MUTAFLOR®) FROM THE PRODUCTION FERMENTER

[1]Manonmani Soundararajan, [1]Lukas Page, [1]Tobias A. Oelschlaeger

[1]Institut für Molekulare Infektionsbiologie, Josef-Schneider-Str. 2/D15, D97080 Würzburg, Germany

E. coli Nissle 1917 (EcN) is one of the best characterized probiotics and it is the active component of the probiotic preparation “Mutaflor®”, which is used in the treatment of various gastrointestinal disorders. The non-virulent nature, increased genetic stability, fitness factors and safety aspects make EcN an ideal probiotic. Recently, studies have been reported on antagonistic activity of EcN against various Enterohaemorrhagic *E. coli* strains (EHEC) such as the classical EDL933 and also the isolates from the 2011 outbreak (Rund et al, *IJMM*, 2013) which emphasize EcN’s anti-pathogenic capability. EcN is currently produced and sold by Ardeypharm GmbH, Germany. We believe that the industrial culturing conditions determine the properties of EcN as Mutaflor. These characteristics of the “starter culture” in the gut might be important for EcN’s ability to colonize and exert the beneficial effects on the host. In order to identify genes which are highly expressed in the fermenter culture we compared the transcriptome of LB over-night-cultures in our lab with the transcriptome of fermenter cultures from the Ardeypharm Company. This was achieved by isolating RNA from conventional liquid LB culture and the commercial fermenter culture in their stationary phase by QIAGEN RNAeasy midi kit. The RNA was sequenced by differential RNA sequencing (Sharma CM et al., 2014) and quantified using the DESeq software (Anders et al., 2010). Read numbers were compared between the two different cultivation methods. Preliminary analysis of changes in gene expression of the fermenter culture indicates that there is a strong iron deprivation which is evident from several fold up regulation of genes that code for different siderophores. In addition there are also changes in gene regulation of important metabolic pathways such as glycolysis, citric acid cycle and urea cycle which might indicate stress due to the varying level of key ingredients like phosphate, sulfur and nitrate in the fermenter. Also there is indication of glucose saturation in the fermenter which is evident from reduced glycolysis activity and increased osmotic stress. Results from this study indicate certain shortages EcN encounters at least at the end of the fermenter culture.

26 ADJUSTING MICROBIOME PROFILES FOR DIFFERENCES IN MICROBIAL LOAD BY SPIKE-IN BACTERIA

F. Stämmler[1,2],J. Gläsner[2], A. Hiergeist[2], E. Holler[3], D. Weber[3], P.J. Oefner[4], A. Gessner[2], R. Spang[1]

[1]Chair of Statistical Bioinformatics, University of Regensburg, Am Biopark 9, 93053 Regensburg, Germany; [2]Institute of Clinical Microbiology and Hygiene, University Medical Centre, Franz-Josef-Strauß-Allee 11, 93053 Regensburg, Germany; [3]Department of Haematology and Oncology, Internal Medicine III, University Medical Centre, Franz-Josef-Strauß-Allee 11, 93053 Regensburg, Germany; [4]Chair and Institute of Functional Genomics, University of Regensburg, Am Biopark 9, 93053 Regensburg, Germany

Background: Next-generation 16S ribosomal RNA gene sequencing is widely used to determine the relative composition of the mammalian gut microbiomes. However, in the absence of a reference, this does not reveal alterations in absolute abundance of specific operational taxonomic units if microbial loads vary across specimens.

Results: Here we suggest the spiking of exogenous bacteria into crude specimens to quantify ratios of absolute bacterial abundances. We use the 16S rDNA read counts of the spike-in bacteria to adjust the read counts of endogenous bacteria for changes in total microbial loads. Using a series of dilutions of pooled faecal samples from mice containing defined amounts of the spike-in bacteria *Salinibacter ruber*, *Rhizobium radiobacter* and *Alicyclobacillus acidiphilus*, we demonstrate that spike-in-based calibration to microbial loads allows accurate estimation of ratios of absolute endogenous bacteria abundances. Applied to stool specimens of patients undergoing allogeneic stem cell transplantation, we were able to determine changes in both relative and absolute abundances of various phyla, especially the genus *Enterococcus,* in response to antibiotic treatment and radio-chemotherapeutic conditioning.

Conclusion: Exogenous spike-in bacteria in gut microbiome studies enable estimation of ratios of absolute OTU abundances, providing new insights into the structure and the dynamics of intestinal microbiomes.

27 FOOD PROTEINS AND MICROBIOTA IS REQUIRED FOR NORMAL DEVELOPMENT AND FUNCTION OF THE SMALL INTESTINE

Sabrina Hartmann[1], Alexander Visekruna[1], Hans Mollenkopf[2], Krishna Rajalingam[3] and Ulrich Steinhoff[1]

[1]*Institute for Medical Microbiology and Hygiene, Philipps University of Marburg,*

[2] *Max-Planck Institute for Infection Biology, Berlin*

[3]*Institute for Immunology, JGU, University Medical Center, Mainz*

While it is well known that development of the intestinal immune system is shaped by the microbiota, very little information exists about the impact of dietary antigens on development and homeostasis of the intestine. According to the current concept the immune system has to discriminate between (harmless) food and potentially dangerous microbial antigens, but this mechanism is not well understood yet. We thus investigated the immune reactivity against normal dietary proteins in germfree and normofloric mice.

We could show that continuous exposure to dietary protein antigens leads to generation of highly activated T cells localized mainly in Peyer´s Patches (PPs) and to less extent in the small intestinal lamina propria (siLP) of both, conventional and germ-free (GF) mice. Dietary protein activates $CD4^+$ T cells predominantly in Peyer´s patches (PP) and these cells are distinct from regulatory T cells (Tregs). Dietary protein-reactive T lymphocytes remained innocuous due to an equilibrium between activation and apoptosis. Macrophage mediated uptake of apoptotic T cells from the PP but not from other tissues resulted in strong IL-10 expression. In contrast lack of dietary proteins led to a hypocellular, immature small intestinal immune system with reduced $CD4^+$ T and B cells in PP and siLp and altered intestinal microbiome.

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

28 HIGH-RESOLUTION ANALYSIS OF BACTERIAL GWAS SIGNALS IN MICE AND HUMANS

Annika Stüwer [1], Philipp Rausch [1,2], Malte Rühlemann [3], Andre Franke [3], John Baines [1,2]

[1] *Institut for Experimental Medicine, Christian-Albrechts-University of, Kiel, Germany*
[2] *Max Planck Institute for Evolutionary Biology, Plön. Germany*
[3] *Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, Germany*

Numerous recent studies demonstrated the influence of the intestinal microbiota's composition on host health and susceptibility to disease. Accordingly, understanding the extent to which variation in microbial composition is influenced by host genetic variation is a key question in microbiome research. Recent genetic association studies in our working groups identified host genetic loci influencing the genus *Bacteroides* in mice and humans. This genus constitutes a large proportion of the gut microbiome and plays an important role in physiological and pathological processes. The goal of this study is to evaluate the species composition of the *Bacteroides* community in fecal samples of *Mus musculus domesticus* (WSB/PhJ) and *Mus musculus musculus* (PWD/PhJ) breeding lines and their F2 hybrids, which were previously used in a large-scale quantitative trait locus (QTL) mapping experiment. We identified differences in *Bacteroides* community structure between the pure mouse lines and hybrids, which is indicative of species-level adaptation of *Bacteroides* to a specific host background (PWD-*M.m.musculus*). We further investigated a healthy human cohort for potential genetic associations with single *Bacteroides* species and carried out a comparative genomic analysis of chromosomal regions shared between humans and mice. The characterization of host-microbial genetic signals at the bacterial species level in mice and humans will increase our understanding of host-microbial co-adaptation and its physiological and evolutionary relevance.

29 15-LIPOXYGENASE CONTRIBUTES TO THE RESOLUTION OF EXPERIMENTAL COLITIS IN MICE

A. Sünderhauf[1], K. von Medem[1], F. Bär[1]; R. Pagel[2], A. Künstner[3], C. Sadik[3], S. Derer[1], C. Sina[1]

[1]*Molecular Gastroenterology, Department of Medicine 1, University of Lübeck, Germany*

[2]*Institut of Anatomy, University of Lübeck, Germany*

[3]*Lübeck Institute of Experimental Dermatology, University of Lübeck, Germany*

An impaired intestinal barrier accompanied by imbalanced intestinal immune responses are supposed to be responsible for the etiology of inflammatory bowel disease (IBD), a group of chronic, relapsing-remitting diseases. Intestinal epithelial cells express specialized pro-resolution lipid mediators (SPM) receptors ALX/FPR2 and ChemR23. *In vitro,* activation of the latter with diverse SPMs has been shown to downregulate proinflammatory chemokine and cytokine expression, NF-κB activity as well as colon epithelial cell apoptosis, while bacterial clearance from an SPM-pretreated epithelial cell line was enhanced. Therefore we assumed 15-lipoxygenase (Alox15), the key enzyme in the biosynthesis of many SPMs, to play a pivotal role in the resolution of experimental colitis in mice.

Basal gene expression in colon samples of Alox15$^{-/-}$ and C57Bl/6J mice was determined by microarray analysis. Genes found to be differentially expressed were further confirmed via reverse transcriptase quantitative polymerase chain reaction (rt-qPCR) and immunohistochemistry (IHC). Faecal samples were collected from Alox15$^{-/-}$ and WT animals for microbiome analysis via next generation sequencing. Acute experimental colitis was induced in Alox15$^{-/-}$ and C57Bl/6J mice by administration of 4% (v/v) dextran sodium sulphate (DSS) to the drinking water and evaluated by disease activity index (DAI), and murine endoscopic index of colitis severity (MEICS) scores in four independent experiments.

Microarray analysis, rt-qPCR and IHC revealed proliferation marker Ki-67 to be significantly lower expressed in colonic tissue of Alox15$^{-/-}$ mice, while mucosal mast cell markers were increased. Microbiome analysis revealed β-diversity to be significantly different between knockout and wildtype mice, with a depletion of the genus *Akkermansia* in Alox15$^{-/-}$ mice. Finally, deficiency in Alox15 resulted in an aggravated and prolonged colitis, measured by increased DAI and MEICS scores.

With these data we propose the enzyme Alox15 to contribute to the maintenance of intestinal tissue homeostasis and to the resolution of inflammation by balancing cell proliferation and apoptosis. Yet the exact mechanism of how Alox15 activity modulates cell regeneration, mucosal mast cells and intestinal microbiome composition still remains to be determined

30 INTESTINAL EXPRESSION OF THE HISTO-BLOOD GROUP GENE FUT2 INFLUENCES SUSCEPTIBILITY TO INTESTINAL INFLAMMATION

A. Suwandi[1], P. Rausch[2], F. Pereira[3], D. Berry[3], J. F. Baines[2], G. A. Grassl[1]

[1]*Institute of Medical Microbiology and Hospital Epidemiology, Hannover Medical School, Germany*

[2]*Max Planck Institute for Evolutionary Biology, Evolutionary Genomics, Plön, Germany and Christian-Albrechts-University of Kiel, Germany*

[3]*Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, University of Vienna, Austria*

Glycans play important roles in host-microbe interactions. The *fut2* gene encodes a α-1,2-fucosyltransferase responsible for the expression of ABO histo-blood group antigens on the gastrointestinal mucosa and bodily secretions. In humans, loss of function mutations of *fut2* are known as 'nonsecretors'. These individuals have a loss of glycan structures in the gut epithelium. Furthermore, differences in pathogen susceptibility are known to be associated with this loss and the nonsecretor status was identified as a genetic risk factor for developing Crohn's Disease and primary sclerosing cholangitis. However, it remains unclear which aspects of these differences in susceptibility are due to changes in microbial communities, host glycosylation or both.

Using *Salmonella enterica* serovar Typhimurium infections in mice, we observed significantly lower *Salmonella* colonization in the colon and cecum of *Fut2*-deficient mice at day 7 and 14 post infection. Furthermore, decreased histopathological changes were observed in the colon tissue of Fut2 deficient mice. Stronger infiltration of immune cells in Fut2 wildtype mice compared to Fut2 deficient mice was detected by immunofluorescence staining. In addition, fewer bacteria of the normal flora isolated from Fut2 deficient mice were able to take up fucose ex vivo compared to bacteria from wildtype mice. Thus, our data demonstrate that intestinal *Fut2* expression influences *Salmonella* colonization of the intestine and the susceptibility to *Salmonella* induced inflammation which is most likely due to differences in microbiota composition.

31 INTER-INDIVIDUAL VARIABILITY IN THE RESPONSE OF THE MURINE COLON MICROBIOTA TO GLYCOSAMINOGLYCANS

B. Szirányi[1], F. Pereira[1], D. Berry[1]

[1]*Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, University of Vienna, Austria*

The human intestinal microbiota is essential to health and disease. Metagenomic surveys have revealed that each person hosts a distinct microbiota, and the gene content of the microbiota can vary widely between healthy individuals. There are, however, few demonstrated examples of the functional consequences of inter-individual variability in the complex gut microbiota. In this project we used single cell activity measurements of the gut microbiota to study the degradation of two diet- and host-derived complex compounds present in the extracellular matrix of tissues: hyaluronate and chondroitin sulfate. In each experiment, three C57BL/6 mice were sacrificed and the mixed colon contents were incubated for six hours under anaerobic conditions in the presence of 50% D2O and were amended with 3 different concentrations of the studied compounds. Glucose, which is utilized by the majority of the gut microbiota, was used as a positive control. Activity levels were measured by single cell Raman microspectroscopy based on the deuterium incorporation per cell (CD-peak) compared to the CH peak (%CD). In one of three biological replicates we were able to detect a subpopulation of bacterial cells that were strongly stimulated by both of the complex compounds (%CD~20), but not in the other two replicate experiments. 16S rRNA gene amplicon sequencing was carried out on the Illumina MiSeq platform to characterize the microbiota of the biological replicates in order to unravel the differences between the communities and to identify the high-responding populations. As host-derived compounds can serve as a nutrient source for a variety of microorganisms including pathogens, understanding the phenomenon of inter-individual variability in glycosaminoglycan utilization by the microbiota may be of key importance for health and susceptibility to certain diseases.

32 IMPACT OF GENETICS AND PARENTAL MICROBIOTA ON THE GUT MICROBIOME COMPOSITION OF OFFSPRING

Treichel N. S.[1], Schöler A.[1], Prevoršek Z.[2], Stres B.[2], Schloter M.[1]

[1]Helmholtz Zentrum München, Research Unit Environmental Genomics, Neuherberg, Germany
[2]Department of Animal Science, University of Ljubljana, Ljubljana, Slovenia

The microbiota of the gut has great impact on the physical condition of its host. It is involved in the development of innate and adaptive immunity, defends its host against pathogens and influences the degradation and uptake of nutrients. Therefore, the gut microbiome is linked to the metabolic phenotype of the host. In accordance, the transfer of gut microbiota from obese mice to germ-free mice resulted in a higher increase in total body fat compared to the transfer of a "lean microbiome" (*Turnbaugh 2006*).

The mouse gut microbiome is environmentally acquired after birth and in the beginning of life resembles the maternal communities in vaginas. After 21 days the gut microbiome becomes stable and similar to the one in feces of adult mice (*Pantoja-Feliciano 2013*). Beside each individual having its unique microbiome, the gut microbiome composition within a family is more similar in comparison to unrelated individuals and also more similar within monozygotic twin pairs compared to dizygotic twin pairs (*Goodrich 2014*). This raises the question to which degree the genetics of the host has an impact on its gut microbiome.

To investigate to what extend genetics and the maternal microbiota influences the gut microbiome composition, a cross-fostering experiment using two genetically different mouse lines was conducted. Half of a litter was exchanged between one mouse line, which is prone to be obese and another, which remains lean. Also control groups of litters staying with their mothers were investigated. The mice were dissected at three weeks age, and DNA was extracted from the colon with content and the cecum with content. Investigation of the microbiome was performed by paired-end next generation sequencing. The sequences were analyzed at 97% sequence identity with QIIME using the Greengenes database and revealed a distinct clustering of samples according to microbiome type inherited from the foster mother. The effect of the host's genetics is not as explicit, but seems to be stronger within individuals with a "lean microbiome".

This indicates that the inherited maternal microbiota has a higher impact on the on the gut microbiome composition than the host's genetics.

33 ANTIBIOTICS-MEDIATED INCREASE IN LARGE INTESTINAL PROTEASE ACTIVITY IS ASSOCIATED WITH IMPAIRED INTESTINAL BARRIER FUNCTIONS AND AGGRAVATION OF SPONTANEOUS COLITIS

Hongsup Yoon[1], Monika Schaubeck[2], Dirk Haller[1], Gabriele Hörmannsperger[1]

[1]Chair of Nutrition and Immunology, ZIEL – Institute for Food & Health, Technische Universität München, Gregor-Mendel-Str. 2, 85350 Freising, Germany; [2]Max Planck Institute of Neurobiology, Chair of Neuroimmunology, 82152 Planegg-Martinsried, Germany

Introduction: Early exposure to antibiotics (AB) has been associated with increased risk for later development of inflammatory bowel diseases (IBD) but the causal relevance and pathophysiological mechanisms are unknown. Specific AB treatments induce a major increase of the large intestinal protease activity (liPA) via the elimination of bacteria that mediate the physiological inactivation of pancreatic proteases in the large intestine.

Aims and Methods: We hypothesized that the AB-mediated rapid increase in liPA may impair the large intestinal barrier and promote the development of chronic inflammation. In order to investigate this hypothesis, we treated wildtype (WT) and interleukin 10 deficient (IL10-/-) mice with vancomycin/metronidazole (V/M) and investigated the impact of this AB therapy on the liPA, the intestinal barrier function and the subsequent susceptibility towards dextran sodium sulfate (DSS)-induced and spontaneous colitis.

Results: V/M-mediated elimination of anti-proteolytic bacteria in the large intestinal ecosystem resulted in a rise of large intestinal pancreatic trypsin (~20x) and liPA (~5-10x), being comparable to the levels observed in GF mice. Transwell and Ussing chamber analyses using large intestinal epithelial cells or cecal tissue revealed that the high proteolytic activity in cecal supernatants (CS) from V/M-treated or GF mice significantly impaired the epithelial barrier function in a serine protease dependent way. In WT and IL10-/- mice, the acute V/M-mediated increase in liPA was associated with impaired large intestinal barrier functions (Ussing chamber) and increased translocation of orally applied FITC dextran (4 kDa) to the systemic circulation. Importantly, oral co-administration of a serine protease inhibitor, AEBSF, maintained normal intestinal barrier functions in V/M-treated WT mice. Repeated short term V/M treatments of WT mice (2x7 days, at 4 and 8 weeks of age) resulted in chronically increased liPA but did not affect later susceptibility towards DSS-induced colitis (at 12 weeks). However, analogously V/M-treated IL10-/- mice showed accelerated development of chronic colitis, systemic inflammation and large intestinal tumor formation.

Conclusion: V/M treatment results in a rapid and major increase in liPA which is detrimental to the intestinal barrier. Pulsed V/M treatments mediated a chronic increase in liPA as well as accelerated colitis development in IBD susceptible mice. These findings demonstrate that specific AB therapies can indeed promote the development of IBD in disease susceptible organisms and indicate that the increase in liPA may contribute to this long term adverse effect.

34 PGAM5 DEFICIENCY PROTECTS MICE FROM TLR3 INDUCED VILLUS SHORTING

Yu-Qiang Yu, Gui-Wei He, Veronika Thonn, Markus F. Neurath, Christoph Becker

Department of Medicine 1, University Clinics, Erlangen, Germany

Background & Aims: Phosphoglycerate mutase family member 5 (PGAM5) is a mitochondrial phosphatase that can function in the regulation of cell death. However, the exactly function of PGAM5 in vivo is poor understood. The aim of our study was to identify PGAM5 function in intestinal epithelial homeostasis.

Methods: PGAM5 knockout mice were analyzed by histological techniques. Cell shedding in the small intestine has been induced by intraperitoneal injection of poly (I: C) in PGAM5 knockout mice and WT mice. Small intestine damage has been demonstrated by H&E and TUNEL staining. Histological assessment and western blot have been used for measuring the activation of apoptosis, pSTAT1 and pDrp1.

Results: PGAM5 deficient mice show no overt gut phenotype compared to WT mice, no obvious differences have been found between untreated PGAM5 knockout mice and WT mice by H&E and TUNEL staining. Once challenged by poly (I: C) injection, WT mice developed intestine damage, as indicated by cells death and villus shorting, while PGAM5 deficient mice were protected. Interestingly, no rDrp1 activation was observed in WT mice. Both PGAM5 knockout mice and WT mice showed high pSTAT1 and cleaved caspase 3 activation level after poly (I: C) injection.

Conclusions: PGAM5 is not essential for general gut homeostasis. However PGAM5 does play a role in poly (I: C) villus shorting and epithelial cell death regulation.

Participants

PARTICIPANTS 2016

Prof John Baines
MPI Plön / CAU Kiel
Inst. for Experimental Medicien
August-Thienemann-Str. 2
24306 Plön
baines@evolbio.mpg.de

Dr Anja Baumann
Friedrich-Schiller-University, Jena
Model Systems of Molecular Nutrition
Dornburger Str. 29
7743 Jena
anja.baumann@uni-jena.de

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

Ms Desideria Brignone
Technische Universität München
Lehrstuhl für Technische Mikrobiologie
Gregor-Mendel-Strasse 4
85354 Freising
brignone@wzw.tum.de

Mrs Susanne Bury
Institute for molecular infection biology
Infection biologie
Josef-Schneider-Straße
97080 Würzburg
susibury@hotmail.de

Dr Christoph Cichon
UniKlinik Muenster
Institut fuer Infektiologie
Von-Esmarch-Strasse 56
48149 Muenster
cichon@uni-muenster.de

Dr Pieter de Groot
Academic Medical Centre
Department of Internal Medicine
Meibergdreef 9
0 Amsterdam
p.f.degroot@amc.uva.nl

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

Dr David Berry
University of Vienna
Dept. of Microbiol. + Ecosystem Science
Althanstrasse 14
1090 Vienna
berry@microbial-ecology.net

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

Mrs Barbara Buchen
Universitätsklinikum Erlangen
Medizinische Klinik 1
Hartmannstr. 14
91052 Erlangen
Barbara.Buchen@uk-erlangen.de

Mrs Jelena Calasan
Technische Universität München
Lehrstuhl für Ernährung und Immunologie
Gregor Mendel Str. 2
85354 Freising
jelena.calasan@tum.de

PD Dr Thomas Clavel
Technische Universität München
ZIEL
Weihenstephaner Berg 3
85354 Freising
thomas.clavel@tum.de

Mrs Silke Dubbert
Ardeypharm GmbH
Biological Research
Loerfeldstr. 20
58313 Herdecke
silke.dubbert@ardeypharm.de

Ms Claudia Eberl
LMU München
Max von Pettenkofer-Institute
Pettenkoferstrasse 9 a
80336 München
claudia.eberl@outlook.com

Prof Philipp Engel
University of Lausanne
Department of Fundamental Microbiology
Biophore Building
1015 Lausanne
philipp.engel@unil.ch

Prof Harry J. Flint
The Rowett Institute of Nutrition and Health
Gut Health
Greenburn Road; Bucksburn
0 Aberdeen
h.flint@abdn.ac.uk

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

Prof W. Florian Fricke
University of Hohenheim
Nutrigenomics
Fruwirthstr. 12
70599 Stuttgart
w.florian.fricke@uni-hohenheim.de

Ms Shreya Gopalakrishnan
CRTD

FetscherstraÃŸe 105
1307 Dresden
shreya.gopalakrishnan@crt-dresden.de

Dr Georg Gradl
Eurofins Genomics
Global Sales Manager Next Generation Sequencing
Anzinger Str. 7a
85560 Ebersberg
georggradl@eurofins.com

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

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

Dr Claudia Günther
Friedrich-Alexander-University
Lab. of Mucosal Immune Regulation + Intest. Cell Biology
Hartmannstrasse 14
91052 Erlangen
C.Guenther@uk-erlangen.de

Mr Sven Haange
Helmholtz Centre for Environmental Research
Department of Molecular Systems Biology
Permoserstraße 15
4318 Leipzig
sven.haange@ufz.de

Mr Thomas Hagemann
Universität Tübingen
Dept. of Medical Microbiol. + Hygiene
Elfriede-aulhorn-Straße, 6
72076 Tübingen
thomas.hagemann@med.uni-tuebingen.de

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

Dr Buck Hanson
University of Vienna
Dept. of Microbiol. + Ecosystem Science
14 Althanstrasse
1090 Wien
hanson@microbial-ecology.net

Dr Jesse Harrison
University of Vienna
Dept. of Microbiol. + Ecosystem Science
Althanstrasse 14
1090 Vienna
harrison@microbial-ecology.net

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

Prof Kai Hildner
University Hospital Erlangen
Medical Department 1
Ulmenweg 18
91054 Erlangen
Kai.HIldner@uk-erlangen.de

Ms Aida Iljazovic
Helmholtz Center for Infection Research
Vaccinology and Applied Microbiology
InhoffenstraÃŸe 7
38124 Braunschweig
aida.iljazovic@helmholtz-hzi.de

Mrs Daniela Janosch
Pharma-Zentrale GmbH
Biological Research
Loerfeldstrasse 20
58313 Herdecke
janosch@pharma-zentrale.de

Ms Johanna Kabbert
Uniklinikum RWTH Aachen
Molekulare Medizin, AG Oliver Pabst
Pauwelsstrasse 30
52074 Aachen
jkabbert@ukaachen.de

Dr Birgit Klinkert
Ardeypharm GmbH
R&D
Loerfeldstr. 20
58313 Herdecke
klinkert@ardeypharm.de

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

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

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

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

Ms Nika Ivanovova
University of Vienna
Dept. of Microbiol. + Ecosystem Science
Althanstrasse 14
1090 Wien
ivanovova@microbial-ecology.net

Mrs Sarah Just
TU München
Chair for Nutrition & Immunology
Gregor-Mendel-Str. 2
85354 Freising
sarah.just@tum.de

Ms Vera Kitowski
Universitätsklinikum Erlangen
Medizinische Klinik 1
Hartmannstraße 14
91054 Erlangen
vera.kitowski@uk-erlangen.de

Mr Ali Giray Korkmaz
UKT Medical Microbiology
AG Frick
Elfriede-Aulhorn Str. 6
72076 Tübingen
giray.korkmaz@med.uni-tuebingen.de

Prof Thomas Kufer
University of Hohenheim
Inst. of Nutritional Med., Dep. Immunology
Fruwirth Str. 12
70593 Stuttgart
thomas.kufer@uni-hohenheim.de

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

Dr Gerhard Liebisch
University Hospital of Regensburg
Institute of Clinical Chemistry
Franz-Josef-StrauÃŸ-Allee 11
93053 Regensburg
gerhard.liebisch@ukr.de

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

Mr Jan Maerz
Universitätsklinikum Tübingen
Universitätsklinikum Tübingen
Elfriede-Aulhorn StraÃŸe 6
72076 Tübingen
jan.maerz@med.uni-tuebingen.de

Ms Amira Metwaly
Technical University in Munich (TUM)
Chair of Nutrition and Immunology
Goethestrasse.2
85354 Freising
amira.metwaly@tum.de

Ms Lena Michaelis
Universitätsklinikum Tübingen
Inst. für medizinische Mikrobiol. + Hygiene
Elfriede-Aulhorn StraÃŸe 6
72076 Tübingen
lena.michaelis@med.uni-tuebingen.de

Dr Maria Joanna Niemiec
Hans Knöll Institute
Microbial Immunology
Beutenbergstr. 11a
7745 Jena
joanna.niemiec@leibniz-hki.de

Dr Maria de Fatima Pereira
University of Vienna
Dept. of Microbiol. and Ecosystem Science
Althanstrasse 14
1090 Wien
pereira@microbial-ecology.net

Mrs Katharina Läsker
Universität Würzburg
MED 2
Oberdürrbacher Str. 6
97080 Würzburg
k.laesker@googlemail.com

Ms Elena Lobner
Technische Universität München
Ernährung und Immunologie
Gregor-Mendel-Str. 2
85354 Freising
elena.lobner@tum.de

Prof Alexander Loy
University of Vienna
Dept. of Microbiol. + Ecosystem Science
Althanstrasse 14
1090 Wien
loy@microbial-ecology.net

Prof Claudia Mauri
University College London
Centre for rheumatology research
5 University Street
0 London
c.mauri@ucl.ac.uk

Dr Marco Metzger
Fraunhofer IGB
Translational Centre
Röntgenring 11
97070 Würzburg
marco.metzger@igb.fraunhofer.de

Dr Anne Neville
Wellcome Trust Sanger Institute
Host-Microbiota Interactions Laboratory
Genome Campus Hinxton
0 Cambridge
an7@sanger.ac.uk

Dr Tobias Ölschläger
Uni Würzburg
Inst. f. Molekulare Infektionsbiologie
Josef-Schneider-Str. 2 / D15
97080 Würzburg
t.oelschlaeger@uni-wuerzburg.de

Dr Matthias Pfeiffer
Eurofins Genomics
Key Account Manager Austria / Bavaria
Anzinger Str. 7a
85560 Ebersberg
matthiaspfeiffer@eurofins.com

Prof Dana Philpott
University of Toronto
Dept. of Immunology
1 King's College Circle
0 Toronto, Ontario
dana.philpott@utoronto.ca

Dr Philipp Rausch
Max Planck Institute for Evolutionary Biology
Evolutionary Genomics
August-Thienemann-Str. 2
24306 Plön
rausch@evolbio.mpg.de

Ms Alessandra Riva
University of Milan
Department of Health Science
San Paolo Hospital, via di Rudini' 8
24142 Milan
alessandra.riva@unimi.it

Ms Urmi Roy
Helmholtz Centre for Infection Research
Vaccinology
Inhoffenstrasse, 7
38124 Braunschweig
Urmi.Roy@helmholtz-hzi.de

Mr Patrick Schiller
Max-von-Pettenkofer-Institute
Bacteriology
Pettenkoferstr. 9a
80336 Munich
schiller@mvp.uni-muenchen.de

Ms Valentina Schüppel
Technische Universität München
Chair of Nutrition and Immunology
Gregor-Mendel-Straße 2
85354 Freising
valentina.schueppel@tum.de

Dr Ulla Schwertassek
Fraunhofer Institute for Cell Therapy and Immunology
Therapy Validation – Preclinical Models Unit

Perlickstr. 1
4103 Leipzig
ulla.schwertassek@izi.fraunhofer.de

Mr Daniel Podlesny
University of Hohenheim
Nutrigenomics
Fruwirthstr. 12
70599 Stuttgart
daniel.podlesny@uni-hohenheim.de

Mrs Christiane Ring
DIfE
GAMI
Arthur-Scheunert-Allee 114 - 116
14558 Nuthetal
Christiane.Ring@dife.de

Prof Philip Rosenstiel
Kiel University
Institute of Clinical Molecular Biology
Schittenhelmstr. 12
24105 Kiel
p.rosenstiel@mucosa.de

Prof Peter Schierack
Brandenburg University of Technology
Institute of Biotechnology
Großenhainer Str. 57
1968 Senftenberg
peter.schierack@b-tu.de

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

Mr Matthias Schweinlin
University Hospital Würzburg
Dept. of Tissue Engineering + Regenerative Med.
Röntgenring 11
97070 Würzburg
matthias.schweinlin@uni-wuerzburg.de

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

Dr Felix Sommer
Kiel University
Institute of Clinical Molecular Biology
Schittenhelmstr. 12
24105 Kiel
f.sommer@ikmb.uni-kiel.de

Ms Manonmani Soundararajan
Institute for Molecular Infection Biology (IMIB)
Infection Biology
Josef-Schneider-Str. 2/D15
97080 Würzburg
manonmani.soundararajan@stud-mail.uni-wuerzburg.de

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

Prof Ulrich Steinhoff
Universität Marburg
Medical Microbiology
Hans-Meerweinstr. 2
35043 Marburg
ulrich.steinhoff@staff.uni-marburg.de

Ms Annika Stüwer
UKSH, Kiel
Institut for Experimental Medicine
Michaelisstrasse 5
24105 Kiel
stuewer@evolbio.mpg.de

Dr Abdulhadi Suwandi
Medizinische Hochschule Hannover
Institut für Med. Mikrobiol. + Krankenhaushygiene
Carl-Neuberg-Straße 1
30625 Hannover
suwandi.abdulhadi@mh-hannover.de

Ms Nicole Treichel
Helmholtz Zentrum München
Research Unit Environmental Genomics
Ingolstädter Landstr. 1
85764 Neuherberg
nicole.treichel@helmholtz-muenchen.de

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

Mr Frank Stämmler
University Regensburg
Institute of functional Genomics
Am Biopark 9
93053 Regensburg
frank.staemmler@ukr.de

Mr Alexander Steimle
Universitätsklinikum Tübingen
Inst. für med. Mikrobiol. + Hygiene
Elfriede-Aulhorn StraÃŸe 6
72076 Tübingen
alexander.steimle@med.uni-tuebingen.de

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

Mrs Annika Sünderhauf
Molekulare Gastroenterologie
Medizinische Klinik 1
Ratzeburger Allee 160
23538 Lübeck
annika.suenderhauf@uksh.de

Ms Barbara Szirányi
University of Vienna
Dept. of Microbiol. + Ecosystem Science
Althanstraße 14
1090 Wien
kuszogeb@gmail.com

Mr Niels van Best
Uniklinik RWTH Aachen
Med. Microbiology
PauwelsstraÃŸe 30
52074 Aachen
nvanbest@ukaachen.de

Dr Rudolf von Bünau
Ardeypharm GmbH
Biologische Forschung
Loerfeldstr. 20
58313 Herdecke
vbuenau@ardeypharm.de

Mr Hongsup Yoon
Technische Universität München
Chair of Nutrition and Immunology
Gregor-Mendel-Str. 2
0 Freising-Weihenstephan
ga54req@mytum.de

Mr Christos Zioutis
University of Vienna
Microbiology and Ecosystem Science
Althanstrasse 14
1090 Vienna
zioutis@microbial-ecology.net

Prof Jerry Wells
Wageningen University
Animal Sciences
De Elst 1
0 Wageningen
jerry.wells@wur.nl

Mr Yuqiang Yu
University Clinics, Erlangen
Medicine 1
Hartmannstrasse 14
91052 Erlangen
Yuqiang.Yu@uk-erlangen.de