



7th Seeon Conference

Microbiota, Probiota and Host Mikrobiota, Probiota und Wirt

04.- 06. JULY 2014

CONFERENCE CENTER

MONASTERY SEEON / CHIEMSEE

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July 4, 2014



Dear Participant,

On behalf of the German Society of Hygiene and Microbiology (DGHM) and the Organizing Committee, welcome to the 7th Seeon Conference “Microbiota, Probiota 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.

Since 2008 our DGHM section “Microbiota, Probiota 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.

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PROGRAM Friday, July 4

15⁰⁰ - 17⁰⁰ Registration
17⁰⁰ - 17¹⁵ Welcoming: J. Frick, Med. Microbiology + Hygiene, University Tübingen

17¹⁵ – 18⁰⁰ Keynote Lecture: **Maria Rescigno**, Department of Experimental Oncology, European Institute of Oncology, Milan
Intestinal immune homeostasis: a multidisciplinary task

18¹⁵ Dinner

GUT MICROBIOME AND HOST, GNOTOBIOTIC MOUSE MODELS

19³⁰– 21⁰⁰ Chair: A. Steimle, Med. Microbiology + Hygiene, University Tübingen

P. Rausch, Max-Planck-Institute for Evolutionary Biology, Plön
Influence of the Fut2 gene on the dynamics of intestinal microbiome development in house mice

O. Kuzyk, Department of Microbiology + Ecosystem Science, University of Vienna
Fine-scale spatial architecture of intestinal microbial communities in mice

J. Calasan, Chair of Nutrition and Immunology, Technische Universität München
The intestinal microbiota is crucial for the development of Crohn´s disease-like ileitis

M. Beutler, Max-von-Pettenkofer Institut, LMU München
Addressing the influence of S. Typhimurium induced gut inflammation on the Oligo-Mouse-Microbiota

E. Galvez, Microbial Immune Regulation, Helmholtz-Institute Braunschweig
Low Complexity Microbiota [LCM] mice: A stable and defined gut microbiota to study host-microbial interactions

I. Lengfelder, Chair of Nutrition and Immunology, Technische Universität München
Pathogenicity mechanisms of Enterococcus faecalis in chronic intestinal inflammation

21⁰⁰ Drink at the Bar?

PROGRAM Saturday, July 5

08³⁰ – 09¹⁵ Keynote Lecture: **Christian Jobin**, Department of Infectious Diseases & Pathology, University of Florida
Intestinal environment and microbial activities: Impact on colorectal cancer

09¹⁵ - 09⁴⁵ Coffee Break / **Poster at the first glance**

MECHANISMS OF INTERACTION OF PATHOGENS AND COMMENSALS AND THE HOST IMMUNE SYSTEM

09⁴⁵ – 11¹⁵ Chair: G. Grassl, Experimental Medicine Universität Kiel/FZ Borstel

C. Günther, Medizinische Klinik I, Universitätsklinikum Erlangen
IL28 influences Paneth cell homeostasis and renders mice highly susceptible to Salmonella typhimurium infection

A. Steimle, Med. Microbiology + Hygiene, University Tübingen
Influence of proteolytic activity of cathepsins on intestinal homeostasis

D. Hofreuter, Medizinische Mikrobiologie, Hannover Medical School
Exploring the metabolic interface between the gastrointestinal pathogen Campylobacter, its host and the microbiota

F. Schmidt, Med. Klinik I - Gastroenterologie, Charité Berlin
The role of microbiota on intestinal macrophage polarization

J. Raschig, Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart
Knockout of E.coli periplasmic reductases DsbA and DsbB conveys resistance against oxidized hBD1

B. Sovran, Animal Sciences, University Wageningen
The effects of probiotics on the intestinal barrier of fast-ageing ERCC1^{-Δ7} mice

11¹⁵ – 12⁰⁰ Keynote Lecture: **Heike Walles**, Tissue Engineering und Regenerative Medizin, Wuerzburg
Complex 3D human tissue models to study infection mechanisms

PROGRAM Saturday, July 5

12⁰⁰ - 13⁴⁵ Lunch

MECHANISMS OF INFLAMMATION AND HOMEOSTASIS

13⁴⁵ – 15¹⁵ Chair: J. Wells, Host-Microbe Interactomics, Wageningen University

M. Mahapatro, Gastroenterology, Medicine Clinic I, Universitätsklinikum Erlangen,
Interleukin-33 promotes intestinal barrier function by direct effects on epithelial proliferation and differentiation

I. Bruesch, Institute for Laboratory Animal Science, Hannover Medical School
Relation of Cdcs1 and the development of a colitogenic T cell population?

A. Blazejewski, Microbial Immune Regulation, Helmholtz-Institute Braunschweig
Inflammatory Caspases in intestinal homeostasis and inflammation

L.T. Birzele, University Children's Hospital Munich, Germany
Microbiome analysis of mattress dust and nasalswab samples of a childhood asthma study

M. Ostaff, Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart
Alcohol abuse is associated with gastric Paneth cell metaplasia

S. Zeissig, Department of Internal Medicine, University Medical Center, Kiel
XIAP variants and impaired NOD1/2-dependent microbial recognition in Crohn's disease

15¹⁵ – 15⁴⁵ Coffee Break

15⁴⁵ – 18¹⁵ **Poster Slam** (2 minutes / 2 slides) **and Poster discussion**
(J. Frick, Med. Microbiology + Hygiene, University Tübingen)

18¹⁵ – 19⁰⁰ Keynote Lecture: **Trevor Lawley**, Bacterial pathogenesis, Wellcome Trust Sanger Institute, Cambridgeshire
Designing a Bacteriotherapy for Clostridium difficile infection

19⁰⁰ Dinner

20³⁰ Bowling at the Bar

PROGRAM Sunday, July 6

08³⁰ – 09¹⁵ Keynote Lecture: **Kathleen McCoy**, Mucosal Immunology Lab,
Department of Clinical Research, University of Bern
Microbial conditioning during early life impacts immune regulation

09¹⁵ – 09⁴⁵ Coffee Break

09⁴⁵ – 10⁰⁰ **Poster Award**

METAGENOMICS AND METAPROTEOMICS

10⁰⁰ – 11³⁰ Chair: T. Clavel, Chair of Nutrition and Immunology, Technische Universität München

G. Korkmaz, Med. Microbiology + Hygiene, University Tübingen
Dendritic Cell Maturation: a Proteomics Approach

T. Clavel, Chair of Nutrition and Immunology, Technische Universität München
Alterations of fecal microbiota and metabolic landscape in response to oral or intravenous iron therapy in patients with inflammatory bowel diseases

B. Hanson, Department of Microbiology and Ecosystem Science, University of Vienna
Revealing physiological host-microbe interactions in the distal gut by using ¹³C-glucose ureide for site-specific substrate delivery and in vivo stable isotope probing

T. Ölschläger, Institut für Molekulare Infektionsbiologie, University of Wuerzburg
*Complete genome sequence of the probiotic *Escherichia coli* strain Nissle 1917*

A. Walker, Analytical BioGeo Chemistry, Helmholtz Zentrum München
Comparative analysis of gut microbial meta-metabolome in two C57BL6 mouse strains using high resolution mass spectrometry techniques

S.B. Haange, UFZ-Helmholtz Centre for Environmental Research, Department of Proteomics, Leipzig
Community and functional composition of the colon mucosal microbiota from a high fat diet rat model and its response to a change in diet

11³⁰ Lunch and Departure

PROGRAM

Friday,

July 04

INTESTINAL IMMUNE HOMEOSTASIS: A MULTIDISCIPLINARY TASK.

Maria Rescigno

*Department of Experimental Oncology, European Institute of Oncology, Via Adamello 16,
Milan, Italy*

In the intestine, dendritic cells (DCs) are found in the lamina propria (LP) of the villi, in the mesenteric lymph nodes (MLN), lymphoid aggregates and Peyer's Patches (PP). Probably the most represented antigen presenting cells in the gut are those found in the LP as they definitely outnumber the number of DCs found in the MLN or PP. In the mouse, these mononuclear phagocytes can be divided into subgroups depending on the expression of CX3CR1 (the receptor of fraktalkine) and CD103 (αE integrin). CD103+ conventional DCs become tolerogenic in the gut, via their interaction with the local microenvironment and in particular with epithelial cells. Indeed, at steady state, ECs condition anti-inflammatory DCs through the constitutive release of TSLP, TGF-β and retinoic acid (RA). EC-conditioned DCs even though phenotypically activated by bacteria polarize T cells towards a mucosal non-inflammatory T helper-2 phenotype or T regulatory cells. CX3CR1+ cells are instead apt at bacteria and food antigen uptake that they then transfer to CD103+ DCs via gap junctions. The interaction allows the establishment of tolerance to luminal antigens and is fundamental for oral tolerance induction. We also studied the role of endothelial cells in the transfer of bacteria from mucosal sites to systemic sites. Together these results suggest that the induction of homeostasis in the gut is regulated by immune and non-immune cells.

GUT MICROBIOME AND HOST, GNOTOBIOTIC MOUSE MODELS

19³⁰– 21⁰⁰ Chair: A. Steimle, Med. Microbiology + Hygiene, University Tübingen

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Low Complexity Microbiota [LCM] mice: A stable and defined gut microbiota to study host-microbial interactions

I. Lengfelder, Chair of Nutrition and Immunology, Technische Universität München

Pathogenicity mechanisms of Enterococcus faecalis in chronic intestinal inflammation

INFLUENCE OF THE *FUT2* GENE ON THE DYNAMICS OF INTESTINAL MICROBIOME DEVELOPMENT IN HOUSE MICE

Philipp Rausch, Sven Künzel and John F. Baines

Evolutionary Genomics, Max-Planck-Institute for Evolutionary Biology, Plön; GERMANY

The *FUT2* gene encodes an α -1,2-fucosyltransferase responsible for the expression of ABO histo-blood group antigens on mucosal surfaces and bodily secretions. Individuals bearing at least one functional allele are known as “secretors”, whereas those homozygous for loss-of-function mutations, which seem to be maintained by strong selective pressures, display a “nonsecretor” phenotype. A large body of evidence suggests this polymorphism is maintained by numerous trade-offs surrounding host-microbe interactions. Further, nonsecretor individuals are more susceptible to Crohn Disease, which may be mediated by alterations in the microbiome. Here, we investigated the dynamics of microbial communities with respect to genotype using a *Fut2*-deficient mouse model, taking community development and initial colonization into account. We found strong differences in community development, diversity, and composition of microbial communities over time depending on the *Fut2* genotype of individual mice and that of their parents/grandparents. We found communities to be differentiated by *Fut2* genotype early in host development, but this difference fades over time. In contrast, the influence of community complexity appears to increase with time, with the highest diversity in *Fut2* *+/+* mice. Thus, during the process of colonization, we identified patterns of community specialization and stabilization that were strongly influenced by host genotype.

FINE-SCALE SPATIAL ARCHITECTURE OF INTESTINAL MICROBIAL COMMUNITIES IN MICE

Orest Kuzyk¹, Thomas Decker², Alexander Loy¹, David Berry¹

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

²*Immunochemistry Max F. Perutz Laboratories, University of Vienna, Austria,*

The intestine is a multi-compartment organ that acts in consort with a complex microbiome to enable digestion and uptake of diet-derived nutrients. Most knowledge about this microbiome comes from stool samples, which represent a composite picture of intestinal microbial diversity. Each intestinal compartment plays a specific role in the sequential degradation of dietary compounds and thereby provides different niches for microorganisms. Accordingly, sampling of different regions of the intestine has identified the presence of distinct microbial communities characteristic for these different niches. Additionally, host tissues can create physical and chemical microenvironments that select for certain microorganisms at the mucosal boundary, partly via gradients of secreted nutrients and antimicrobial compounds. While these observations indicate that functionally important spatial segregation exists, the magnitude and scale of this structuring is still unresolved. In the present study, we establish an approach to study fine-scale spatial structure of the microbiome and apply it to mice on different diets to examine the effect of dietary polysaccharides on microbiome composition. We cryopreserved regions of native intestinal tissue and contents with minimal disturbance, prepared longitudinal and cross-sectional samples and used laser micro-dissection to isolate small areas containing microbiota (~50x200x10 µm). We chose areas proximal and distal to the mucosal boundary from different intestinal compartments, extracted DNA, and applied bacterial 16S rRNA gene-targeted amplicon sequencing using Illumina MiSeq technology. The results of this analysis will be presented. The approach and knowledge gained in this study will be valuable for future studies of the functional significance of intestinal microniches.

THE INTESTINAL MICROBIOTA IS CRUCIAL FOR THE DEVELOPMENT OF CROHN'S DISEASE-LIKE ILEITIS

Monika Weiher¹, Jelena Calasan¹, Thomas Clavel¹, Manolis Roulis², George Kollias² and Dirk Haller¹

¹*Technische Universität München, Chair of Nutrition and Immunology, ZIEL – Research Center for Nutrition and Food Sciences, Biofunctionality Unit, Gregor-Mendel-Straße 2, 85350 Freising-Weihenstephan, Germany*

²*Biomedical Sciences Research Center Alexander Fleming, Institute of Immunology, Vari 16672, Greece*

Background: Dysbiosis of the human gut microbiota is associated with ileal Crohn's disease (CD). Functional evidence for the causative role of intestinal bacteria in the development of chronic inflammation in the small intestine is lacking. We used the genetically-driven TNF Δ ARE/+ mouse-model of CD-like ileitis in different housing conditions to test the disease-conditioning role of intestinal bacteria.

Methods & Results: Heterozygous TNF Δ ARE and TNF+/+ wildtype mice were housed in germfree (GF), specific pathogen free (SPF) or conventional (CONV) conditions until the age of 18 weeks. The intestinal pathology was assessed by microscopic observation of distal ileal and proximal colonic tissue sections at the age of 18 weeks. No sign of intestinal inflammation was observed in GF TNF Δ ARE mice, whereas SPF and CONV mice developed CD-like ileitis. Inflammation of the proximal colon was observed only in CONV housing. 16S rRNA gene sequencing showed separation of caecal microbial composition according to genotype or ileitis severity. TNF levels in plasma (ELISA) but not ileal tissue expression of TNF (qPCR) was elevated in germfree TNF Δ ARE mice. Finally, Paneth cell loss and expression of spliced XBP-1, a marker of endoplasmic reticulum stress, was absent in germfree TNF Δ ARE mice, suggesting a defect in microbial defense under conditions of chronic inflammation.

Conclusion: The development of Crohn's disease-like ileal inflammation in TNF Δ ARE/+ mice is dependent on the presence of intestinal bacteria. Germfree mice are protected from a loss of anti-microbial defense mechanisms in the ileal tissue, mimicking the human pathogenesis of Crohn's disease.

ADDRESSING THE INFLUENCE OF *S. TYPHIMURIUM* INDUCED GUT INFLAMMATION ON THE OLIGO-MOUSE-MICROBIOTA

Markus Beutler¹, Sandrine Brugiroux¹, Simone Herp¹, Saib Hussain¹, Diana Ring¹, Kathy McCoy², Andrew J. Macpherson² and Bärbel Stecher^{1*}

¹Max-von-Pettenkofer Institut, LMU München, GERMANY

²Department for Clinical Research, University of Bern, SWITZERLAND.

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Salmonella Typhimurium (*S. Tm*) is a food-borne pathogen and is among the most clinically important serotypes that cause salmonellosis in humans with tens of millions infections every year worldwide. The infection is usually self-limiting, however in very young, old or immunocompromised patients the *S. Tm* infection can become life-threatening (more than hundred thousand deaths per year, WHO 2013).

S. Tm induced acute gut inflammation is followed by changes in composition and relative abundance of the bacteria that constitute the resident gut microbiota (commonly detected on phylum level) as well as *Salmonella* overgrowth (Stecher Plos Biology 2007). *S. Tm* benefits from inflammatory host responses which decrease colonization resistance mediated by the beneficial anaerobic microbiota. The mechanisms how *S. Tm* takes advantage of inflammation and thereby outcompetes the microbiota during gastroenteritis are just beginning to be explored. So far it could be shown, that anaerobic electron acceptors (nitrate and tetrathionate), iron availability and neutrophils play a role in *S. Tm* dominance over the resident microbiota (Bäumler-review 2013).

In order to further investigate and prioritize those mechanisms as well as to characterize the shifts in microbiota composition during *S. Tm* induced gut inflammation at a single species level we use a gnotobiotic mouse model based on a novel consortium of mouse-adapted strains, the Oligo-Mouse-Microbiota (Oligo-MM). The Oligo-MM comprises 12 isolates from 5 eubacterial phyla (*Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Verrucomicrobia* and *Proteobacteria*) which stably colonize the gut (over 4 generations). In addition, we developed highly strain specific molecular tools (qPCR, FISH and 454-sequencing) to monitor bacterial composition over time. This makes the Oligo-MM a valuable tool for studying the influence of *S. Tm* induced inflammation on microbial ecology in the gut.

LOW COMPLEXITY MICROBIOTA [LCM] MICE: A STABLE AND DEFINED GUT MICROBIOTA TO STUDY HOST-MICROBIAL INTERACTIONS

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¹Helmholtz Centre for Infection Research, Microbial Immune Regulation, D-38124 Braunschweig, Germany

The gut microbiota plays an essential role in the regulation of metabolic and immune functions. The challenge in the study of host-microbial interaction relies in our capacity to comprehend the effect of single bacterial species on the function of the microbial community. In order to achieve this goal high data reproducibility and selection of stable in-vivo models are essential to investigate the interplay between host genetics and specific bacteria as well as the global microbial community.

To identify a stable gut-microbiota system, we compared C57BL/6N mice carrying a “Low Complexity Microbiota” [LCM] originally derived from the Altered Schaedler Flora and bred at our institute with C57BIC57BL/6N mice purchased from commercial vendors. Using Meta-“omics” approaches on Illumina and PacBio sequencing technologies we analyzed stool samples from these mice and were able to confirm that LCM-HZI mice have a reduced gut-microbiota diversity in comparison with 3 different commercial lines. Notably, our analysis of full 16S rRNA genes using PacBio sequencing demonstrates that the LCM maintained at HZI for 10 years in independent ventilated cages, only differs from the original ASF in gaining few species.

Additionally we assessed if the genetic background has a strong influence on the composition of the LCM by evaluating the microbiota profiles of 10 mouse lines with deficiencies and modifications of the immune system. Strikingly, the result revealed that during four sampling time points there were no genotype-dependent changes in the microbial composition associated within LCM mice.

In summary, after collection and analysis of 273 samples at four time points, covering ten different genotypes, two sequencing platforms and millions of DNA reads, we conclude that LCM-HZI mice at the Helmholtz Institute for Infection Research represent a stable and reproducible in-vivo model for study gut microbiota interactions allowing us to investigate the influence of key bacterial members on host physiology as well as specific interactions between additional bacteria and the host genotype.

PATHOGENICITY MECHANISMS OF ENTEROCOCCUS FAECALIS IN CHRONIC INTESTINAL INFLAMMATION

Sava I¹, Lengfelder I¹, Ocvirk S¹, Hansen JJ², Huebner J³, Murray BE⁴, Sartor RB², Haller D¹

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Background: Inflammatory bowel diseases (IBD) are complex inflammatory conditions and it is widely accepted that genetic and environmental factors together with a loss of immune tolerance to gut bacteria are responsible for the rising incidence of these pathologies. Commensal *Enterococcus faecalis* (*E. faecalis*) is a normal member of the human core gut microbiota, but it has also been identified in increased numbers in the intestine of patients with IBD.

Methods & Results: Using a well-established mouse model of intestinal inflammation (IL10^{-/-}) monoassociated with wild-type *E. faecalis* and deletion mutants, we have already identified three bacterial structures, which have a significant impact on the development of chronic colitis. Gelatinase E cleaves the epithelial tight junction protein E-cadherin in IL10^{-/-} mice leading to enhanced intestinal permeability and increased inflammation and we have also recently demonstrated that enterococcal polysaccharide antigen impacts biofilm formation and in vivo mucus penetration partially contributing to disease development, while functional *E. faecalis* lipoproteins involved in TLR2-mediated activation and recruitment of innate immune cells are required as prime bacterial structures responsible for the colitogenic activity of *E. faecalis*. Next-generation RNA sequencing of *E. faecalis* in monoassociated wild-type and IL-10^{-/-} mice with wild-type identified 98 upregulated genes (≥2-fold) and 142 downregulated genes (≥2-fold) under conditions of colonic inflammation. Among the highest upregulated genes, a gene encoding for a major facilitator superfamily (MFS) transporter and several genes of the Eut locus controlling ethanolamine utilization in *E. faecalis* were identified.

Conclusion: Innate immune activation mediated by *E. faecalis*-derived lipoproteins is the crucial step in the development of colitis in IL-10^{-/-} mice. Transcriptional profiling of the colitogenic bacteria identified additional disease-associated bacterial genes relevant for the fitness in an inflammatory milieu

PROGRAM

Saturday,

July 05

INTESTINAL ENVIRONMENT AND MICROBIAL ACTIVITIES: IMPACT ON COLORECTAL CANCER.

Christian Jobin

Department of Infectious Diseases & Pathology, University of Florida

We have come to appreciate the wide impact of the intestinal microbiota on host homeostasis, whose effect range from barrier function, immunity and metabolic outputs. As such, microbial imbalance where specific function are dysregulated is associated with numerous intestinal disorders including inflammatory bowel diseases (IBD) and colorectal cancer (CRC). The events leading to microbial imbalance and deleterious effect on the host are still unclear. *Enterobacteriaceae*, specifically *Escherichia coli*, are abundant in patients with IBD and CRC. I will present evidence that host inflammation contribute to the resilience of *E.coli* in the intestine and activation of microbial carcinogenic potential, biological activities contributing to the development of CRC in experimental models.

MECHANISMS OF INTERACTION OF PATHOGENS AND COMMENSALS AND THE HOST IMMUNE SYSTEM

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B. Sovran, Animal Sciences, University Wageningen

The effects of probiotics on the intestinal barrier of fast-ageing ERCC1^{-Δ7} mice

IL28 INFLUENCES PANETH CELL HOMEOSTASIS AND RENDERS MICE HIGHLY SUSCEPTIBLE TO SALMONELLA TYPHIMURIUM INFECTION

Claudia Günther¹, Barbara Buchen¹, Manuela Hefele¹, Guiwei He¹, Mousumi Mahapatro¹, Markus F. Neurath¹ and Christoph Becker¹

¹*Medical Clinic, Friedrich-Alexander-University, Erlangen, Germany*

Interferons (IFNs) play a critical role in the antimicrobial host defense and are strongly upregulated after viral infection as they represent an important component of the innate immune response. Despite the fact that IFN- λ , a type III IFN, has been shown to predominantly act on mucosal organs, *in vivo* studies have failed to elucidate a specific, nonredundant function. Here we investigated the influence of type III IFNs on intestinal homeostasis, by hydrodynamic delivery (HD) of a vector encoding the IL28 sequence. Four weeks after IL28 injection in wildtype animals (IL28HDmice) we could observe a constant increase in intestinal epithelial cell (IEC) death. Moreover we could observe that these mice lacked Paneth cells and showed a reduced expression of antimicrobial peptides (AMP), whereas other types of intestinal epithelial cells were not affected by IL28 overexpression. As a consequence of impaired AMP expression, IL28HDmice showed a high lethality compared to control animals after infection with *Salmonella typhimurium*. IFNs signal via the Stat1 pathway and indeed we could observe that Paneth cell loss was prevented in mice with an IEC specific deletion of Stat1. Paneth cell death was associated with increased expression of RIP3 and MLKL, molecules that have been shown to mediate necroptosis. Moreover dying epithelial cells did not show activation of caspase, suggesting a non-apoptotic form of cell death.

Taken together, our data demonstrate for the first time that tightly controlled IL28 signalling is important to maintain Paneth cell and intestinal homeostasis.

INFLUENCE OF PROTEOLYTIC ACTIVITY OF CATHEPSINS ON INTESTINAL HOMEOSTASIS

A. Steimle¹, R. Harmening¹, A. Schäfer¹, K. Gronbach¹, J. Müller¹, T. Reinheckel³, M. Jucker⁴, H. Kalbacher², I. B. Autenrieth¹, J.-S. Frick¹

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²University of Tübingen, Interfakultäres Institut für Biochemie, Tübingen, Germany

³University of Freiburg, Institut für Molekulare Medizin und Zellforschung, Freiburg, Germany

⁴Hertie-Institut für klinische Hirnforschung, Tübingen, Germany

Monocolonization of T cell transplanted Rag1^{-/-} mice with *Escherichia coli* mpk results in efficient proliferation and activation of transplanted T cells whereas *Bacteroides vulgatus* mpk does not induce T cell proliferation. In another model, T cell transplanted Rag1^{-/-} mice harbouring a more complex microbiota with enhanced levels of *Enterobacteriaceae* develop symptoms of a T cell mediated chronic colitis. Administration of *B. vulgatus* mpk before T cell transplantation and during inflammation after T cell transfer protects these mice from colitis induction and leads to healing of the symptoms, respectively. The protective effect of *B. vulgatus* mpk is associated with a lower endotoxicity due to a different Lipid A structure as compared to *E. coli*. We could show that the differences in the Lipid A-structure of these gram negative commensals lead to a different regulation of Cathepsin S and Cathepsin B, endosomal proteases and major regulators of the MHC class II transport to the surface, and thus to different phenotypes of dendritic cells. *E. coli*-Lipid A leads to DCs maturation and therefore efficient T-cell activation, while *B. vulgatus* Lipid A-driven semi-maturation may lead to T cell anergy and apoptosis. Since these differences are essentially based on distinct CatS activity, an inhibitor of this protease could be a potential target for the treatment of colitis. Since CatB plays an important role in generating antigenic peptides to be presented via MHC class II, an inhibitor of this protease could boost the protective effect of a CatS inhibitor. Furthermore, inflammation in the gut is associated with enhanced Cathepsin B activity in colonic epithelial tissues and increased Cathepsin S protein levels can be detected in blood serum, indicating that high proteolytic activities of CatB seem to have a more local impact and high CatS activities a more systemic effect during the inflammatory process.

EXPLORING THE METABOLIC INTERFACE BETWEEN THE GASTROINTESTINAL PATHOGEN CAMPYLOBACTER, ITS HOST AND THE MICROBIOTA

Juliane Mohr¹, Hanne Vorwerk¹, Claudia Huber², Petra Grüning³, Karen Methling⁴, Alexandra von Altrock⁵, Olga Wensel¹, Sabin Bhuj⁶, Kerstin Schmidt-Hohagen⁷, Dietmar Schomburg⁷, Michael Lalk⁴, Wolfgang Eisenreich², Peter Valentin-Weigang³ and Dirk Hofreuter¹

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Campylobacteriosis, the most frequent bacterial enteritis worldwide, is primarily caused by *Campylobacter jejuni* and to a lesser extent by *Campylobacter coli*. *C. jejuni* and *C. coli* exhibit a different spatial distribution in the porcine gastrointestinal tract of juvenile animals: *C. jejuni* colonizes primarily the small intestine, the caecum and in lower quantities the colon, whereas *C. coli* was recovered from the colon, caecum and ileum but not from the jejunum of pigs. We hypothesized that distinct metabolic properties of the two pathogens could account for the ability of *C. coli* to persist in the large intestine more efficiently than *C. jejuni*. By combining phenotype microarray analyses and isotopologue profiling studies, we could identify distinct growth substrate utilization patterns for *C. coli* in comparison to *C. jejuni*. Whole genome sequencing allowed us to elucidate the differences in the catabolic capacities between the two *Campylobacter* species. Furthermore, our metabolome analysis from different gut sections of pigs revealed a clear spatial distribution of nutrients along the intestinal anterior-posterior axis. Interestingly, *C. coli* but not *C. jejuni* is able to utilize certain nutrients that are abundant fermentation products of the microflora found in the large intestine of pigs. This observation suggested that *C. coli* might benefit more from the metabolic activity of the resident bacteria in the large intestine than *C. jejuni*. Taken together our study revealed new insights into how different physiological properties could influence the distinct tissue tropism of two closely related *Campylobacter* species.

THE ROLE OF MICROBIOTA ON INTESTINAL MACROPHAGE POLARIZATION

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Background: In inflammatory bowel disease (IBD) a dysbiosis of the intestinal microbiota is common, with its role in disease still not understood. Since macrophages are central in the inflammatory process and reside in close proximity to luminal bacteria, alterations in microbiota composition might affect the phenotype of intestinal macrophages. Thus we aimed to define the effect of bacterial species altered in IBD on macrophage polarization and function and explored the role of microbiota in macrophage maturation in the gut.

Methods: Human CD14⁺ cells were isolated from peripheral blood and polarized to M1- or M2-cells in the presence of bacterial supernatants. Following polarization cells were stimulated with LPS and production of cytokines and surface marker expression was assessed by flow cytometry. Furthermore differences in immune cell polarization as well as barrier function in the gut between germfree (GF) and specific pathogen free (SPF) mice were assessed by flow cytometry, immunohistology and electrophysiological studies.

Results: Supernatants of *Faecalibacterium prausnitzii* (Fp) significantly increased the expression of M2 markers (CD14, CD163) on M1-macrophages. LPS and supernatants of *Bifidobacterium adolescentis* (Ba) or *Escherichia coli* (Ec) induced similar but not significant effects in these cells, but none of these affected expression in M2-macrophages. In both M1- and M2-macrophages, LPS-induced cytokine release was significantly suppressed if cells were polarized in presence of Fp and Ec supernatants. The total amount of F4/80⁺ macrophages in the lamina propria of terminal ileum was similar in GF and SPF mice, but the subset of F4/80⁺CD11b⁺ cells was increased in GF mice while the amount of CD3⁺ cells was significantly decreased and the number of FOXP3⁺ regulatory T-cells altered. Besides this in GF mice the colonic barrier function was altered with increased transepithelial resistance and modulated flux of mannitol, HRP and FITC-Dextran.

Conclusion: The bacterial supernatants tested have divergent effects on polarized macrophages with selected species favoring M2 polarization and the deficiency of microbiota alters the presence of immune cell subsets and barrier function in the intestine. Thus, targeting the intestinal microbiome might well be a tool to modulate local immune cell accumulation and activity in the gut.

KNOCKOUT OF *E. COLI* PERIPLASMIC REDUCTASES DsbA AND DsbB CONVEYS RESISTANCE AGAINST OXIDIZED hBD1

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Background: Antimicrobial peptides (AMPs) are key innate immune players forming a first barrier against microorganisms. One AMP, the human β -defensin 1 (hBD1) is continuously produced by many tissues including intestinal epithelia. Compared to other defensins, it has only minor antibiotic activity in its native state. After reduction of its three disulfide bridges, it however becomes a potent against anaerobic and aerobic bacteria while the oxidized form nonetheless shows activity against a few specific bacteria, such as *E.coli*. We therefore aimed at investigating the activity against *E.coli* by the different hBD1 forms.

Methods: We used a radial diffusion assay to check hBD1 activity against different *E.coli* mutants. A nutrient poor gel was prepared which contains the logarithmic phase bacteria. Reduced or oxidized hBD1 were pipetted into punched wells to allow diffusion into the gel. An overlay-gel was poured onto plates after 3h; after 20h the inhibition zone was checked.

Results: A lack of redox proteins DsbA and DsbB allows *E.coli* to acquire resistance against oxidized hBD1 in contrast to reduced hBD1. However both oxidized and reduced hBD1 exhibit antimicrobial activity against wildtype *E.coli* and other mutants.

Conclusion: Our data support the idea that the bacterial redox system is in part responsible for the antimicrobial mode of action of oxidized hBD1. We further hypothesize that the two redox forms of hBD1 have different antimicrobial killing mechanisms.

This work was funded by the EU (ERC Starting Grant) and the Robert-Bosch-Foundation.

THE EFFECTS OF PROBIOTICS ON THE INTESTINAL BARRIER OF FAST-AGEING ERCC1- Δ 7 MICE

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In humans ageing is associated with heightened basal level of inflammation and the impairment of innate cell migration and pattern-recognition receptor signaling. Microbiota changes during ageing may also affect host physiology and even the rate of aging itself (1). The aim of this study was to investigate, the effects of ageing on intestinal morphology, including mucus properties and microbiota in fast-ageing *Ercc1- Δ 7* mice, and the impact of probiotic supplementation on the aging phenotype. The lifespan of *Ercc1- Δ 7* mice is decreased from 118 weeks to around 20 weeks, compared to wild-type mice (2). Thus it is possible to apply probiotic treatment over their entire adult lifetime. Mice were administered probiotic strains *L. plantarum* WCFS1, *L. casei* BL23, *B. breve* DSM20213, or PBS/glycerol as a control by intra-gastric gavage three times a week. At 16 weeks, the mice were sacrificed and jejunum, ileum, and proximal colon were fixed in Carnoy's fixative and stained for histology (haematoxylin and eosin, PAS/Alcian Blue, Crossmon, FISH, and immunohistochemistry). Like naturally aged mice, 16-week-old *ERCC1- Δ 7* mice show marked changes in intestinal morphology and homeostasis compared to young adult mice. In aged *ERCC1- Δ 7* mice the colonic mucosa had thickened and there was immune cell infiltration. Furthermore, in aged mice, the mucus layer was thinner and more easily penetrable by luminal bacteria compared to wild-type mice of the same age. Administration of *L. plantarum* WCFS1 showed beneficial effects on intestinal barrier, with a decrease in mucosal thickening, a thicker mucus layer, and less bacterial infiltration into the mucus than controls, whereas administration of *L. casei* BL23 appeared to have no effect. In contrast, *B. breve* DSM20213 showed adverse effects on intestinal barrier compared to untreated mice. We conclude that ageing has marked effects on the intestinal barrier, including mucosal thickening, immune cell infiltration, and mucus depletion. The impact of long-term probiotic supplementation may ameliorate or worsen deterioration of the mucosal barrier, depending on the strain.

COMPLEX 3D HUMAN TISSUE MODELS TO STUDY INFECTION MECHANISMS

Maria Steinke, Jan Hansmann, Florian Groeber, Marco Metzger, Matthias Schweinlin and Heike Walles

Department Tissue Engineering and Regenerative Medicine, University Hospital Fraunhofer IGB-Unit Wuerzburg "Regenerative Medicine for Musculoskeletal Diseases and Oncology"

Simple 2D cell culture systems or animals are highly artificial models to study infection and penetration mechanisms of obligate human pathogens. We aim to develop novel human infection models based on engineered human tissue models.

Tissue Engineering is a multidisciplinary field that combines engineering, physical and biological sciences and medicine with the overall goal to restore or to replace damaged tissues or organs. The principle of tissue engineering is based on the isolation of cells from patient biopsies, set up co-culture of different primary cell types that are cultured in vitro together with a scaffold and building a functional piece of human tissue. These tissues can be used as in vitro test systems to test the biocompatibility of materials, drugs and cosmetic products or to study cell-cell, cell material or tissue-microorganism interaction.

Vascularization is a major challenge in creating tissues ex vivo. Complex tissue engineered constructs exceeding a thickness of 100-200 μm need a vascular system in order to supply the cells with oxygen and nutrients and moreover remove waste products. This restricts generation of tissues with an appropriate size for clinical application and complex tissues such as the bone.

We developed 3D vascularized tissues based on decellularized porcine small bowel segments and preserved tubular structures of the capillary network within the collagen matrix which is functional associated with one small vein and artery (biological vascularized scaffold - BioVaSc). This vascularized matrix enables the generation of a functional artificial vascular network and vascularized tissues as trachea, bone, skin, fatty tissue, intestine and liver.

During the presentation the application of the skin, trachea and intestinal model for infection studies will be given.

MECHANISMS OF INFLAMMATION AND HOMEOSTASIS

13⁴⁵ – 15¹⁵ Chair: J. Wells, Host-Microbe Interactomics, Wageningen University

M. Mahapatro, Gastroenterology, Medicine Clinic I, Universitätsklinikum Erlangen, Interleukin-33 promotes intestinal barrier function by direct effects on epithelial proliferation and differentiation

I. Bruesch, Institute for Laboratory Animal Science, Hannover Medical School
Relation of Cdc5 and the development of a colitogenic T cell population?

A. Blazejewski, Microbial Immune Regulation, Helmholtz-Institute Braunschweig
Inflammatory Caspases in intestinal homeostasis and inflammation

L.T. Birzele, University Children's Hospital Munich, Germany
Microbiome analysis of mattress dust and nasal swab samples of a childhood asthma study

M. Ostaff, Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart
Alcohol abuse is associated with gastric Paneth cell metaplasia

S. Zeissig, Department of Internal Medicine, University Medical Center, Kiel
XIAP variants and impaired NOD1/2-dependent microbial recognition in Crohn's disease

INTERLEUKIN-33 PROMOTES INTESTINAL BARRIER FUNCTION BY DIRECT EFFECTS ON EPITHELIAL PROLIFERATION AND DIFFERENTIATION.

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Background and Aims: The small intestine comprises an array of epithelial cells that serves distinct functions during its time course. Many pathways are known to regulate this programming of intestinal stem cell. The balance between various cells of absorptive lineage and cells of secretory lineage (Goblet, Paneth and Enteroendocrine) is highly necessary to maintain homeostasis. Paneth cells serve as immune cells of the gut by secreting various anti-microbial peptides leading to a healthy microflora. However, under pathological conditions the intestinal epithelium is forced to undergo many changes eventually disrupting homeostasis.

Methods: We generated an inducible transgenic mouse expressing IL-33 specifically in gut. For expression of the cytokine mice were injected intraperitoneally with Tamoxifen. Cre-mediated recombination was genotyped by PCR on tail DNA. To analyse the role of IL-33 directly on epithelium we used the in-vitro cultivation of organoids from C5BL/6 mice. Localization of IL-33 was studied in IL-33LacZ/LacZ reporter mice.

Results: We observed high expression of IL-33 in the gut of TLR-ligand challenged mice. IL-33 was specifically expressed by cells which are located around the intestinal crypt bottom, where stem cells are located. Overexpression of IL-33 in transgenic mouse led to increased expression of anti-microbial peptides and goblet cell markers. Signaling of IL-33 into intestinal epithelial cells in vivo and in organoid cultures in vitro was associated with altered intracellular signalling governing proliferation and differentiation of intestinal epithelial cells.

Conclusions: Our data demonstrates a novel mechanism of IL-33 signalling and its significant consequences on intestinal epithelial cell function. Therefore it plays a role in maintaining intestinal homeostasis and tackling foreign challenges.

RELATION OF CDCS1 AND THE DEVELOPMENT OF A COLITOGENIC T CELL POPULATION

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Inflammatory bowel disease (IBD) is a common disease in humans. The pathogenesis is not fully understood yet. Current scientific opinion is that the host microbiota interacts with the environment and the genetic background in a T cell mediated autoimmune response. It is known that in the interleukin-10-deficient mouse model (Il10^{-/-}) the susceptibility to colitis development depends on the background strain. B6.129P2-Il10tm1Cgn/JZtm (B6-Il10^{-/-}) are partially resistant whereas C3Bir.129P2-Il10tm1Cgn/JZtm (C3Bir-Il10^{-/-}) are prone to develop IBD.

The aim of this study was to investigate the relation between a naive T cell subset and Cdc1 (cytokine deficiency induced colitis susceptibility), one quantitative trait locus for IBD susceptibility.

T cell subsets isolated from B6-Il10^{-/-} mice and from two congenic strains B6.C3Bir-Cdc1-2 and 3 (BC-R2 and BC-R3) were transferred to B6.129S7-Rag1tm1Mom (B6-Rag) mice to induce colitis. As an in vivo tool, magnetic resonance imaging was performed and compared to the histological scoring of the colons. Gene expression of a distinct T cell subset was analysed by microarray analyses comparing the susceptible strains C3Bir-Il10^{-/-}, BC-R2, BC-R3 to the resistant B6-Il10^{-/-}.

Microarray analyses revealed 48 genes that were differently expressed between susceptible and resistant strains. In the transfer colitis model development of a colitogenic T cell population depended on the Cdc1 locus. Severity of the inflammation is addicted to the donor strains and to the T cell population.

INFLAMMATORY CASPASES IN INTESTINAL HOMEOSTASIS AND INFLAMMATION

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Caspases are a group of proteases which play an important role in mammalian physiology, including homeostasis and host defense. Inflammatory caspases which have primary functions in innate immunity, include caspase-1, caspase-11 and caspase-12 in mice. Caspase-1, the prototypic inflammatory caspase, is best known for its function in inflammasome complexes that are formed after detection of perturbations of cellular homeostasis. The formation of inflammasome leads to activation of caspase-1 which in turn activates the cytokines interleukin (IL)-1 β and IL-18 and a form of cell death called pyroptosis. Inflammasomes are not only involved in the regulation of inflammation, they also play a crucial role in maintaining the homeostasis. Especially in the intestine, where numerous microorganisms reside, they contribute to balance immune responses to combat invading pathogens, but to spare the microbial communities residing in the gut.

During the last decade several studies have addressed the function of caspases and specific inflammasomes during intestinal homeostasis and inflammation, however, unknowingly they were hampered by two complications, host genetics and the intestinal microbiota. To clarify the functions of Caspase-1 and Caspase-11 we generated Casp-1^{-/-} and Casp-11^{-/-} mice on a pure C57BL/6 background. Moreover, we rederived both lines into a barrier facility to maintain them with a defined microbiota which we confirmed by 16S rRNA sequencing of the intestinal microbiota. Our results indicate, Casp-1^{-/-} mice are strongly protected from DSS colitis compared to wt control mice, which is in contrast to many of the previous studies that had suggested predominantly protective functions. Further studies will be required to clarify substrates and cell types involved in this phenotype. An important tool to answer the latter question are mice conditionally deficient in Caspase-1 which we have started to characterize. In summary our result suggest that Caspase-1 exacerbates disease during DSS-induced colitis, but further experiments are required to investigate whether the specific composition of the microbiota may influence the role of Caspase-1 during intestinal inflammation.

MICROBIOME ANALYSIS OF MATTRESS DUST AND NASALSWAB SAMPLES OF A CHILDHOOD ASTHMA STUDY

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Asthma is a complex disease associated with genetic and environmental determinants like growing up on a farm. Previously, we found an inverse association between diversity of microbial exposure on farm environments and childhood asthma based on SSCP and cultural data. Here we wanted to investigate the microbial exposure in more depth. 86 mattress dust (md) and the corresponding nasal swab (ns) samples from children growing up on a farm or not (asthmatics and non-asthmatics) of the Austrian part of the GABRIEL study were selected. 16S rRNA gene fragment (V3-V5) analysis by GS-FLX Titanium (Roche) was performed and analyzed with QIIME and R. The main detected phyla differed in their abundance between md and ns samples [*Proteobacteria* (17/46%), *Firmicutes* (53/37%), *Actinobacteria* (16/11%) and *Bacteroidetes* (11/4%)], but were similar between farm and non-farm children of the same sample material. Two third of the md OTUs and about one third of the ns showed an abundance of <1%. The top rank most abundant OTUs of ns (genus *Moraxella*, *Streptococcus*, *Staphylococcus*) belong to the human flora. On an individual level, the species richness and diversity (Shannon index) was significantly higher in the md samples than in the nose. Within the md it was higher in the farm samples. A negative association of bacterial richness with asthma could be seen in only the non-farm group of the md samples ($p=0.024$). These results suggest, bacteria detected in the nose are to a less extent environmental origin and the mattress dust samples probably reflect better the environmental exposure.

ALCOHOL ABUSE IS ASSOCIATED WITH GASTRIC PANETH CELL METAPLASIA

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¹*Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, 70376 Stuttgart, Germany*

Background: Excessive alcohol consumption has been associated with gastrointestinal dysbiosis, liver diseases, and cancer. Furthermore alcohol has direct necrotizing effects and can promote oxidative stress in gastric mucosal cells. If and how it might affect innate epithelial defence has however not been studied. Antimicrobial Peptides (AMPs) are crucial in protecting epithelia against pathogens and in maintaining a beneficial homeostasis towards commensal microbiota. In this study we analysed the effects of heavy alcohol abuse on gastric and duodenal AMP expression.

Methods: From 21 patients with chronic alcohol abuse and 15 controls, biopsies from corpus, antrum, and duodenum were examined for mRNA expression of major mucosal AMPs via real-time PCR. HD-5 protein was additionally analysed by immunohistochemistry. Moreover we are performing stimulation experiment using ethanol and reporter gene assays to study the effects of alcohol on gastric cells in vitro.

Results: Analysed AMPs exhibit characteristic mRNA patterns. Not surprisingly, Paneth cell (PC) specific α -defensins HD5 and HD6 were highest in duodenal mucosa. Interestingly however, at gastric locations, alcoholics exhibited high levels of PC products, hinting to the possibility of PC metaplasia, which was confirmed via HD-5 immunohistochemistry. We are currently analysing a potential link between alcohol and Wnt signalling activity.

Conclusion: Heavy alcohol consumption is associated with gastric Paneth cell metaplasia. Whether this might be a protective, a pathogenesis contributing, or simply a side effect will require further mechanistic studies.

This work was funded by the Robert-Bosch-Foundation.

XIAP VARIANTS AND IMPAIRED NOD1/2-DEPENDENT MICROBIAL RECOGNITION IN CROHN'S DISEASE

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Background: Genetic polymorphisms in pattern recognition receptors such as NOD2 are associated with Crohn's disease (CD) as a major form of inflammatory bowel disease (IBD). In addition, functional defects in NOD2-mediated microbial recognition have been observed in CD in the absence of genetic NOD2 variants, the molecular basis of which is unknown.

Methods & Aim: We performed exome and targeted sequencing as well as functional studies to identify genetic variants involved in impaired NOD2 signaling in CD.

Results: Exome and targeted sequencing in CD identified several novel variants in the gene encoding X-linked inhibitor of apoptosis protein (XIAP), a gene previously linked to primary immunodeficiency and fatal Epstein-Barr virus infections. XIAP variants were exclusively found in patients with pediatric-onset CD and were associated with severe and selective defects in NOD1- and NOD2-mediated bacterial recognition as a consequence of impaired association of mutant XIAP with RIPK2 and/or altered XIAP-dependent ubiquitylation of RIPK2. Accordingly, XIAP variants were associated with disruption of NOD1/2-induced, RIPK2-dependent activation of the downstream transcriptional mediator NF- κ B, which abrogated NF- κ B-dependent IL-6 and IL-8 secretion. Lentiviral XIAP reconstitution in monocyte-derived dendritic cells restored NOD1/2 signaling and thus confirmed a causal relationship between XIAP variants and NOD1/2 defects.

Conclusion: These data reveal the presence of XIAP variants in pediatric-onset CD. XIAP variants were associated with severe and selective defects in NOD1/2-dependent microbial sensing and thus contribute to the previously unexplained observation of functional NOD2 defects in the absence of genetic NOD2 variants. Further, the link between XIAP variants and primary immunodeficiency (PID) suggests that primary immune defects, and particularly those associated with PRR-dependent microbial recognition, contribute to the pathogenesis of intestinal inflammation in CD.

DESIGNING A BACTERIOTHERAPY FOR CLOSTRIDIUM DIFFICILE INFECTION

Trevor Lawley

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Faecal microbiota transplantation (FMT) has been used for more than five decades to treat a variety of intestinal diseases associated with pathological imbalances within the resident microbiota, termed dysbiosis. FMT has been particularly effective for treating patients with recurrent *Clostridium difficile* infection who are left with few clinical options other than continued antibiotic therapy. Our increasing knowledge of the structure and function of the human intestinal microbiota and *C. difficile* pathogenesis has led to the understanding that FMT promotes intestinal ecological restoration and highlights the microbiota as a viable therapeutic target. However, the use of undefined faecal samples creates a barrier for widespread clinical use because of safety and aesthetic issues. An emerging concept of bacteriotherapy, the therapeutic use of a defined mixture of harmless, health-associated bacteria, holds promise for the treatment of patients with severe *C. difficile* infection, and possibly represents a paradigm shift for the treatment of diseases linked to intestinal dysbiosis.

PROGRAM

Sunday,

July 06

MICROBIAL CONDITIONING DURING EARLY LIFE IMPACTS IMMUNE REGULATION

Julia Cahenzli, Yasmin Köller, Madeleine Wyss, Markus B. Geuking, [Kathy D. McCoy](#)¹

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Microbial exposure during neonatal and early life profoundly impacts immune system development. The term 'microbial exposure' is not very well defined but includes microbial infections (bacteria or viruses), presence or absence of particular commensal species, the total number of species present (diversity), as well as changes within this microbial composition over time. Epidemiological studies show that alterations within the intestinal microbiota are associated with increased incidence of allergic and autoimmune disorders.

We found that germ-free and mice with a low diversity microbiota early in life develop elevated serum IgE levels. This hygiene-mediated hyper IgE is a hallmark of immune dysregulation. B cells in neonatal germ-free mice undergo isotype switching to IgE within mucosal lymphoid tissues in a CD4 T cell- and IL-4-dependent manner. The clinical relevance of hygiene-induced hyper IgE and immune dysregulation manifested in increased mast cell surface-bound IgE and exaggerated oral-induced systemic anaphylaxis. Colonization with a diverse microbiota was required to protect from hyper IgE although more complex microbiotas were only protective if a critical level of microbial diversity was reached early in life. Thus, appropriate intestinal microbial stimuli during early life are critical to induce an immunoregulatory network that protects from susceptibility to immune-mediated disorders later in life.

METAGENOMICS AND METAPROTEOMICS

10⁰⁰ – 11³⁰ Chair: T. Clavel, Chair of Nutrition and Immunology, Technische Universität München

G. Korkmaz, Med. Microbiology + Hygiene, University Tübingen
Dendritic Cell Maturation: a Proteomics Approach

T. Clavel, Chair of Nutrition and Immunology, Technische Universität München
Alterations of fecal microbiota and metabolic landscape in response to oral or intravenous iron therapy in patients with inflammatory bowel diseases

B. Hanson, Department of Microbiology and Ecosystem Science, University of Vienna
Revealing physiological host-microbe interactions in the distal gut by using ¹³C-glucose ureide for site-specific substrate delivery and in vivo stable isotope probing

T. Ölschläger, Institut für Molekulare Infektionsbiologie, University of Wuerzburg
Complete genome sequence of the probiotic Escherichia coli strain Nissle 1917

A. Walker, Analytical BioGeo Chemistry, Helmholtz Zentrum München
Comparative analysis of gut microbial meta-metabolome in two C57BL6 mouse strains using high resolution mass spectrometry techniques

S.B. Haange, UFZ-Helmholtz Centre for Environmental Research, Department of Proteomics, Leipzig
Community and functional composition of the colon mucosal microbiota from a high fat diet rat model and its response to a change in diet

DENDRITIC CELL MATURATION: A PROTEOMICS APPROACH

G. Korkmaz¹, T. Popov¹, Marius Codrea², Sven Nahnsen², Ana Velic³, Boris Macek³, J-S. Frick¹

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

ALTERATIONS OF FECAL MICROBIOTA AND METABOLIC LANDSCAPE IN RESPONSE TO ORAL OR INTRAVENOUS IRON THERAPY IN PATIENTS WITH INFLAMMATORY BOWEL DISEASES

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Iron deficiency is a common complication in patients with inflammatory bowel diseases (IBD) and oral iron therapy is suggested to exacerbate IBD symptoms. We performed an open-labelled clinical trial including patients with Crohn's disease (CD; N = 29) or ulcerative colitis (UC; N = 19) as well as control patients with iron deficiency (N = 20) to compare the effects of oral (PO; ferrous sulfate) vs. intravenous (IV; iron sucrose) iron therapy over 3 months. PO and IV treatments were comparable regarding amelioration of iron deficiency, with superior but not significant levels of ferritin and iron saturation in the IV group. Worsening or improvement of disease and quality of life were independent of iron treatment types. Fecal samples from IBD patients were characterized by marked inter-individual differences as well as lower phylotype richness and proportions of unknown *Clostridiales*. Major shifts in bacterial diversity occurred in approximately half of all participants after treatment, independently of disease. In those samples where bacterial profiles shifted, changes in diversity were significantly higher in IBD patients. However, no consistent changes in the occurrence of specific OTUs relative to iron treatment could be identified, suggesting individual-specific responses to treatment. Metabolite analysis in feces using OSC-PLC classification showed a clear separation of both UC and CD from control patients before iron treatment. Separation into IV- and PO-specific metabolite profiles appeared in the control and CD group but not in the UC group. In conclusion, shifts in gut bacterial diversity associated with iron treatment are independent of the route of administration and are more pronounced in IBD patients. Efficiency and clinical outcome of both iron therapies are comparable.

REVEALING PHYSIOLOGICAL HOST-MICROBE INTERACTIONS IN THE DISTAL GUT BY USING ¹³C-GLUCOSE UREIDE FOR SITE-SPECIFIC SUBSTRATE DELIVERY AND IN VIVO STABLE ISOTOPE PROBING

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Host diet is a pivotal driver of microbial ecology in the densely populated mammalian distal gut where the microbial degradation of resistant polysaccharides contributes positively to host physiology and energy homeostasis, but identifying the responsible key microorganisms *in vivo* remains a challenge. Glucose is an abundant polysaccharide degradation intermediate, and is a model substrate for analyzing carbohydrate metabolism in the distal gut. Here, we explore ¹³C-glucose ureide, a substrate protected from host degradation, for targeted delivery of ¹³C-labelled glucose to distal gut microbial populations and subsequent *in vivo* stable isotope probing. Initial microcosms containing cecal contents harvested from mice demonstrated the presence of native microbial populations capable of cleaving and degrading glucose ureide. When replicate mice were orally dosed with ¹³C-glucose ureide, ¹³CO₂ was detected in exhaled air with a dose-dependent peak between 6 and 9 hours after dosing, demonstrating that ¹³C-glucose ureide is degraded *in vivo*. The use of ¹³C-glucose ureide, and potentially other ureide-protected substrates, shows great promise as an *in vivo* stable isotope probing method for revealing the metabolic path of glucose (or other substrates) derived carbon, the fate of metabolic products, the interactions between microbial populations, and contributions to host physiology.

COMPLETE GENOME SEQUENCE OF THE PROBIOTIC *ESCHERICHIA COLI* STRAIN NISSLE 1917.

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In spite of *E. coli* Nissle 1917's (EcN) long-term use in medicine (trade name Mutaflor®), its comprehensive investigations by many research groups, and its proven clinical efficacy, the genomic DNA sequence of this probiotic bacterium has only been published as a draft (Cress et al. 2013). Here we report EcN's complete and annotated genome sequence. The determined genome sequence contains 5,441,200 bp, has a GC-content of 50.6 % and encodes 5,324 ORFs, 117 tRNA operons, 53 rRNA operons and 3 complete prophages, as annotated by RAST and Interproscan. The closest related *E. coli* strains are uropathogenic strain CFT073 (genome size 5,231,428 bp; Welch et al. 2002) and asymptomatic bacteriuria causing strain ABU83972 (genome size 5,313,397 bp; Zdziarski et al. 2010). Only 190 ORF's are solely present in EcN, but absent in CFT073 and ABU83972. These genes represent about 0.0035 % of EcN's genome. However, EcN is more similar to CFT073 with respect to genotype and phenotype than ABU83972 (Vejborg et al., 2010). The now available genome sequence of EcN will be helpful in identifying genes and gene products essential for this strains probiotic properties. One such gene cluster is the yersiniabactin determinant which is strongly overexpressed under Mutaflor® production conditions as determined by transcriptome analysis.

COMPARATIVE ANALYSIS OF GUT MICROBIAL META-METABOLOME IN TWO C57BL6 MOUSE STRAINS USING HIGH RESOLUTION MASS SPECTROMETRY TECHNIQUES

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Metabolomics is a powerful tool to investigate different biological questions on a molecular level. We applied a non-targeted metabolomics approach to study metabolite patterns in two different C57BL6 mouse strains and their association to obesity. Here, we observed two factors of diet-induced obesity (DIO): the genetic and the diet background (using three different diets). Moreover, we were interested if DIO is accompanied by altered gut microbial metabolome by using cecal content, which is reflecting the microbial metabolism and liver samples, which are reflecting host metabolism. The comprehensive metabolomics approach reveals that metabolite changes were related to body weight gain, observed in C57BL6 strains, which were on the other hand dependent on fed diets. In detail, metabolite classes that were affected, belonged to unconjugated bile acids, taurine conjugated bile acids but also different bacterial metabolites. Moreover, we were able to discriminate new classes of metabolites that were altered due to diet, genotype or body weight gain.

COMMUNITY AND FUNCTIONAL COMPOSITION OF THE COLON MUCOSAL MICROBIOTA FROM A HIGH FAT DIET RAT MODEL AND ITS RESPONSE TO A CHANGE IN DIET

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The intestinal microbiota is a densely inhabited microbial community that provides many functions for the host including degrading host non-digestible nutrients. The composition of the gut microbiota is unique for each individual and the colonization evolves throughout the entire life and is the result of different environmental influences. The focus of the study was to observe the response of the mucosal microbiota in a high-fat diet model to a change in diet. Therefore, we used a high-fat diet rat model to analyse the response of the gut microbiota to a switch in diet using 16S rRNA gene pyrosequencing, LC-MS metaproteomic analysis hyphenated with protein-based stable isotope probing (protein-SIP, ¹⁵N-fully labelled diet). As a result, we were able to decipher the gut microbiota community structure in regard to taxonomy, enzymatic functionalities and active taxa related to nitrogen utilisation over a three day period. In addition, we observed fast changes in the community composition including decline of *Enterobacteriaceae* and *Streptococcaceae*. Identified proteins were assigned to functional categories of which replication, transcription, signal transduction as well as carbohydrate and amino acid metabolism were overrepresented. Microbial active taxa in regard to nitrogen utilisation belonged to the abundant phyla of *Firmicutes*, *Proteobacteria* and *Bacteroidetes* as well as those from the low abundant phyla of *Spirochaetes*, *Deinococcus-Thermi* and *Planctomycetes*. The integrated data analysis after diet change opens the path to understand the complex gut Microbiota in more detail using protein-SIP to identify the active taxa.

POSTER

1 TAXCO – A TOOL FOR PREDICTION OF BACTERIAL INTERACTIONS FROM 16S rDNA SEQUENCING DATA

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Each microbiome is a complex system developing in constant interdependency to its environment. It is intensively studied how different factors like diet, stress and disease influence the composition of the gastrointestinal microbiome both in humans and mice. This is not the case for colonization resistance (CR), even though it has been known since 1967. We aim to develop methods to predict possible interactions between taxa, which might point at their importance for conferring colonization resistance. Metagenomics and 16S rDNA sequencing provide a snapshot of the entire microbial community independent from cultivation-based approaches facilitating the comprehensive study of bacterial interactions in their natural environment.

We present TaxCo, a tool for the prediction of interactions and dependencies between taxa in multiple related 16S rDNA sequencing experiments. It computes correlation coefficients for changes in the relative abundance of taxa and visualizes them as a network. TaxCo was used to study the microbiome in fecal samples of specific pathogen free (SPF) mice that were orally infected with wildtype and mutant strains of *Yersinia enterocolitica* (Ye).

As a proof of concept we were able to show well-known relations on high taxonomic levels, including dysbiosis of *Firmicutes* and *Bacteroidetes*, but the sequencing depth of the pilot experiment was insufficient to compensate for the presence of high proportions of commensals in comparison to low proportions of Ye. Currently, we revise the experimental setup and develop new strategies to overcome these difficulties. We aim to improve TaxCo by enabling in-depth study of selected taxa and developing it further to analyze shotgun metagenomic data.

2 GENERATION OF AN OLIGO-MOUSE MICROBIOTA TO STUDY COLONIZATION RESISTANCE AGAINST ENTEROPATHOGENS

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Russell W. Schaedler was the first researcher who colonized germfree mice with selected murine bacterial strains, known as the “Schaedler Flora” [1]. A few years later, the consortium was modified and termed the “Altered Schaedler Flora” (ASF) [2]. Composed of 8 murine isolates, it’s been widely used as a low complexity microbiota model but currently the strains are not available in public strain collections [3]. Moreover, the ASF has some limitations with regard to a lack of colonization resistance provided against intestinal pathogens [4] and the representation of only few bacterial phyla found in a conventional microbiota [5]. Therefore, we established a novel defined consortium of bacteria, the Oligo-Mouse Microbiota (Oligo-MM) which can be introduced to germfree mice. It includes 12 cultivable murine isolates representing 5 major intestinal phyla: *Actinobacteria*, *Bacteroidetes*, *Proteobacteria*, *Firmicutes* and *Verrucomicrobia*. In order to characterize this defined consortium, we generated draft genome sequences which were assembled and automatically annotated. To further study gut microbiota dynamics, a strain-specific quantitative multiplex PCR assay was established. We show that the majority of the Oligo-MM strains stably colonized gnotobiotic mice for at least 5 generations and provide colonization resistance against oral infection with the enteric human pathogen *Salmonella enterica* serovar Typhimurium. Recently, fluorescence *in situ* hybridization (FISH) probes for the Oligo-MM strains were designed and validated to quantify and localize single bacteria on intestinal cross-sections. Interestingly, the abundance of some of the Gram positive strains was significantly higher when detected by FISH analysis as compared to high throughput sequencing and qPCR. This result points at a systematic under-estimation of intestinal Gram-positive bacteria by current DNA-based detection methods. In conclusion, the Oligo-MM model and its analytical tools will be very useful for future studies on host-microbiota-pathogens interactions.

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3 CD14 MIGHT MODIFY THE INTESTINAL BARRIER FUNCTION IN IBD DEVELOPMENT

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Inflammatory bowel disease is characterised by relapsing inflammation of the gut. The pathogenesis of disease is yet unknown, however disturbance of the intestinal homeostasis through intestinal barrier disruption could likely play a key role in IBD development. In a mouse model based on Interleukin-10 (*Il10*) deficiency severity of disease depends on the genetic background and microbiota status. Within the *Cdcs6* (cytokine-deficiency induced colitis susceptibility) locus *Cd14* was suggested as a major candidate gene leading to colitis susceptibility in C57BL/6J mice.

Barrier function was analysed in C57BL/6J.129S1-*Cd14^{tm1Smg}* (B6-*Cd14^{-/-}*) mice as well as in B6.129S1P2-*Il10^{tm1Cgn}**Cd14^{tm1Smg}* (B6-*Il10^{-/-}**Cd14^{-/-}*) mice, a model of chronic colitis. Intestinal permeability was analysed by Ussing-chamber experiments. To further determine intestinal barrier function gene expression of tight junctions in distal small intestine and caecum was measured by qRT-PCR.

B6-*Cd14^{-/-}* mice showed no differences in Mannitol flux and gene expression of the sealing proteins Claudin4, Occludin and Zonula-occludens1 compared to wildtype controls. However, the gene expression of Claudin4, Occludin and Zonula-occludens1 was decreased in B6-*Il10^{-/-}**Cd14^{-/-}* mice when compared to *Il10*-deficient mice.

Cd14 deficiency seems to have no influence on epithelial tightness under steady state conditions but it presumably untightens the intestinal barrier under inflammatory conditions. Therefore *Cd14* might play a role in modulation of the intestinal barrier function leading to IBD development.

4 MIBC: THE MOUSE INTESTINAL BACTERIAL COLLECTION

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The study of mammalian gut bacterial communities using culture techniques has been neglected in recent years. Obtaining bacteria in culture is not only of benefit to omics approaches thanks to the amendment of genomic databases, it is also essential for the establishment of simplified models to study bacterial functions of particular relevance. To test the hypothesis that the host species of origin of isolates is important with respect to molecular mechanisms underlying colonization and the development of the immune system, we aimed at isolating aerobic and strictly anaerobic bacteria and create the first exhaustive repository of bacterial strains and associated genomes from the mouse intestine. This collection will be made readily accessible to the scientific community and will be of use for the establishment of model bacterial consortia. It comprises to date 65 species across 24 families, gathering dominant commensals in the mouse gut. Genomes of the most prevalent and abundant species of this collection as determined via analysis of own and SRA-derived 16S datasets from the mouse gut are currently being sequenced. At least 10 novel bacteria within the *Firmicutes* are also being described, including two novel genera of butyrate producers within *Clostridium* cluster IV. Genomic analysis of cluster IV as well as comparative effects of mono-colonization by human- and mouse-derived *Intestinimonas* strains in germfree mice will provide insights into host-specific properties of the strains.

5 INNATE IMMUNE CELLS ENHANCE DEFENSIN PRODUCTION BY PROVIDING EXTERNAL WNT FACTORS

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Background: The α -defensins HD5 and HD6 are produced exclusively by small intestinal Paneth cells. These antimicrobials are important factors for a healthy balance between microbiota and the host gut barrier. In Crohn's disease (CD) the expression of these factors is diminished which leads to an impaired host defense. It was shown that the Wnt pathway partly regulates Paneth-defensin expression and in ileal CD, impairments in the Wnt pathway were observed. Since CD is characterized by reoccurring inflammations, we aimed at analyzing the influence of inflammatory processes on Wnt dependent defensin expression.

Methods: We used the supernatant of stimulated PBMCs to prepare inflammation conditioned media (ICM). Transfected cells or freshly isolated biopsies were treated with ICM to investigate the effect on defensin expression. Different plasmids were used to study the influence of the Wnt pathway in this setting. In addition PBMC subpopulations from patients and controls were analyzed for mRNA expression.

Results: ICM is able to induce the expression of HD5 and HD6. The effect is diminished when Wnt-binding sites in the promoters are mutated. Cytokines by themselves are not able to cause this effect. In monocytes of CD patients a significant diminished expression of several Wnt ligands was observed.

Conclusion: Inflammatory settings can induce α -defensin expression. This effect is mediated by the Wnt pathway. In CD patients, innate immune cells produce less Wnt ligands which might lead to a lack of induction of HD5 and HD6 during inflammation. These results offer further evidence for a primary nature of Paneth cell defensin defects in ileal CD patients.

6 IMPACT OF THE INTESTINAL MICROBIOTA ON HOMEOSTASIS

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The development of inflammatory bowel disease (IBD) is not only influenced by genetic predisposition, but environmental factors such as the commensal bacteria within the intestinal tract also play a role.

In mammals, the gut is colonised by a complex microbiota harbouring 10^{14} bacteria of 500 – 1000 different species. The commensal gut microbiota is an important factor in shaping the host immune system and preventing colonization by pathogens.

It could be shown that by changing the composition of the initial microbiom the outcome of transfer colitis can be modified.

In the current study the composition of the intestinal microbiota of T cell transferred Rag1^{-/-} mice was analysed using 454-sequencing of the 16S gene to compare the composition of the gut microbiom (1) of mice prone to disease versus mice that stay healthy and (2) before and during development and after manifestation of colitis . The initial microbiom is important for development of colitis and differs between mice that stay healthy and mice prone to disease. The sequencing data lead to the assumption that the composition of the microbiom is shifted during colitis development but not if homoeostasis is maintained and treatment with bacteria or only LPS have similar effects on the microbiota composition.

7 ANALYSES OF MICROBIOTA DERIVED COLONIZATION RESISTANCE AND THE ROLE OF VIRULENCE FACTORS IN *YERSINIA ENTEROCOLITICA* INFECTION

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The gastrointestinal tract harbors a dense and complex microbial community that has co-developed with its host. In a state of mutual benefit, the host immune defense tolerates this adapted bacterial population, which in turn contributes to host nutrition, physiology and immunity. Furthermore, the microbiota confers colonization resistance (CR) against many enteric pathogens like *Yersinia enterocolitica* (*Ye*). While many host-pathogen interactions in this interplay are already well characterized, the mutual influence of the gastrointestinal (GI) microbiota on *Ye* infection needs to be further elucidated.

In preliminary experiments we infected specific pathogen free (SPF) and germfree (GF) mice orally with *Ye* wildtype and mutant strains deficient in YadA (Δ YadA), lacking the Yersiniabactin irp1 (Δ irp1) or devoid in Yersinia outer protein (Yop) injection (pYV515) (n = 4 mice per group). All three mutant strains were highly virulent in GF mice but were attenuated in establishing intestinal colonization in the presence of a commensal microbiota. It is not clear whether these effects are due to interactions with intestinal commensals or due to the host inflammatory response.

Feces samples of SPF mice were additionally analyzed by 16S rDNA sequencing. By computing statistical correlations between all present taxa we were able to predict possible relations and interactions between commensal bacteria. However, the relatively high proportion of commensal bacteria in the fecal fraction compared to low proportions of *Ye* made the resolution of the given data not sufficient to determine the relation between pathogen, mutant strains and commensals. We are currently establishing new experimental setups including cohousing strategies and local sampling in the small intestine to overcome these difficulties.

8 FETAL EXPOSURE TO MATERNAL INFLAMMATION DOES NOT AFFECT POSTNATAL DEVELOPMENT OF GENETICALLY-DRIVEN ILEITIS AND COLITIS

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Background. We aimed to study the development of genetically-driven ileitis and colitis in response to maternal inflammation using mouse models.

Methods. Disease susceptible (Tnf Δ ARE/+ and IL10 $^{-/-}$) and disease-free (Tnf+/+ and IL10/+) offspring were raised in inflamed and non-inflamed dams. Ileal, caecal and colonic pathology were evaluated in the offspring at 8 or 12 weeks of age. Ly6G-positive cells in inflamed sections from the distal ileum and distal colon were analysed by confocal microscopy. Tnf and Il12p40 gene expression was measured in whole tissue specimens by quantitative PCR. Microarrays were performed on laser micro-dissected intestinal epithelium. Caecal bacterial communities were assessed by Illumina sequencing of 16S rRNA amplicons.

Results. Offspring's disease severity, numbers of infiltrated neutrophils, Tnf and Il12p40 mRNA expression were independent of maternal inflammation. Maternal inflammation regulated 3,345 (Tnf Δ ARE/+) genes in the fetal epithelium, but prenatal gene expression patterns were overwritten after birth. Co-housing experiments revealed no change in phylogenetic diversity of the offspring's caecal microbiota due to maternal inflammation.

Conclusion. Disease risk and activity in ileitis and colitis mouse models were independent of maternal inflammation. Maternal inflammation did not alter the offspring's caecal microbial diversity, demonstrating that changes of the gene expression program in the fetal gut epithelium were not relevant for the development of chronic inflammatory disorders in the gut.

9 IDENTIFYING SINGLE BACTERIAL SPECIES WHICH CAN INTERFERE WITH *SALMONELLA* TYPHIMURIUM INDUCED COLITIS

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The mammalian gut harbors a highly diverse microbiota with more than 500 different species. They contribute to nutrient supply, immune modulation and prevention of enteric infections (colonization resistance). The interactions between the host and its microbiota leading to protection against infections are highly complex and therefore not fully understood. To investigate those interactions we have made use of a reductionist gnotobiotic mouse model system based on a defined and low complex microbiota. These mice are highly susceptible to enteric infection with the human pathogen *Salmonella enterica* serovar Typhimurium and develop colitis within 12 hours after oral infection. In contrast, mice with a complex microbiota show no or delayed colitis. *Salmonella* Typhimurium is evading the host defense mechanisms by exploiting the inflammatory response of the host for its own growth by using mucus derived carbohydrates as energy source and a variety of anaerobic electron acceptors. Our aim is now to investigate if single bacterial species from the microbiota are sufficient to prevent colitis or if a consortium of several species is required for protection.

10 PRECLINICAL INFECTION C57BL/6 MOUSE MODEL OF PSEUDOMONAS AERUGINOSA INFECTION IN THE IMMUNOCOMPROMISED HOST

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The gastrointestinal tract encloses the largest and most complex bacterial community in the human body, the GI microbiota, which protects the host from colonization and infection by pathogenic and opportunistically pathogenic organisms in concert with the host's immune-system.

Hemato-oncological patients commonly suffer from reduced effectiveness of their immune system, both due to the respective ailments themselves and because of iatrogenic measures. Such patients are constantly threatened by nosocomial infections, including with multi-resistant pathogens such as extensively drug-resistant (XDR) *Pseudomonas aeruginosa*, which can cause particularly severe problems with antibiotic treatment and even lead to fatal outcomes.

Colonization with XDR *Pseudomonas aeruginosa* may be favoured by prophylactic broad spectrum antibiotic therapy, disrupting the microbiota-mediated colonization and infection resistance. It is still unclear whether the immunodeficiency, mucositis, the altered microbial pattern, the specific virulence/fitness factors of the pathogens themselves or all these factors together account for the increased susceptibility towards colonization and even bloodstream infection with germs such as *Pseudomonas aeruginosa*.

To better understand the mechanisms underlying colonization, translocation and infection of immunosuppressed hosts with multi-resistant Gram-negative pathogens, this project aims at clarifying the role of the immune system as well as the microbiota using a C57BL/6 mouse model for preclinical evaluation of interventions targeting the GI microbiota. Conventional and germfree C57BL/6 mice in particular and other mouse lines of interest are treated with *Pseudomonas aeruginosa* strains, including XDR strains isolated from hemato-oncological patients suffering from septicemia.

Immunodeficiency or mucositis may then be induced or an alteration in the microbiota caused through the treatment with antibiotics, the changes in microbial pattern analyzed before and after treatment and/or before and after the colonization attempt and the mice's susceptibility to colonization and infection evaluated. Differences as well as longitudinal changes in the mice's microbiota can be analyzed, germfree mice employed directly or colonized with specific germs or another mouse's microbiota and tested for differences in their colonization and infection resistances to better understand and possibly influence mechanisms shaping and affecting the microbiota.

11 USING PROTEIN-BASED STABLE ISOTOPE PROBING (PROTEIN-SIP) FOR INTESTINAL MICROBIOTA ANALYSIS

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Research into the intestinal microbiota has become an important field of research. The commensal bacteria in our gut interact with the host in a number of ways which include conversion of non-digestible substrates into metabolites which can be utilized by the host, synthesis of important vitamins and the regulation of the immune system. Furthermore, changes in the microbiota have been linked to a number of conditions like obesity or diseases such as colitis.

OMICS technologies are major methods for global analysis of the gut microbiota; however do not allow the investigation of the members of the community actively involved in the utilization of distinct substrates. Thus, protein-based stable isotope probing (Protein-SIP) is a promising method that permits the analysis of the substrate flux and their metabolites in the community. Protein-SIP enables one to determine the degree of heavy isotope incorporation by analysing the mass shifts in the mass spectra after LC-MS/MS measurements after providing the community with heavy isotope labelled substrates.

We were able to establish a method for Protein-SIP analysis of the intestinal microbiota and especially the mucosal microbiota using a fully ¹⁵N heavy isotope labelled feed. We were able to determine the optimal labelling times and could detect labelled peptides from 13 bacterial phyla and from 90 bacterial families, indicating these utilized the nitrogen from the feed. Interestingly, the incorporation of ¹⁵N in the majority of these peptides suggested that the nitrogen from the feed only made up between 20% and 50% of the total nitrogen utilized. Integrated data analysis (metaproteomics + protein-SIP) opens the path to understand the complex gut microbiota in more detail to identify the active taxa.

12 LACTOBACILLI EXTRACELLULAR PROTEINS INHIBIT EPEC ADHERENCE TO INTESTINAL EPITHELIAL CELLS

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Infections with the Gram-negative enteropathogenic *Escherichia coli* (EPEC) are a main cause of diarrhea in infants. The adhesion of the pathogen to intestinal cells as well as the injection of several virulence factors into these cells lead to disruption of the intestinal barrier function.

The aim of our ongoing study is to characterize beneficial probiotic 'factors' that interfere with this pathogenic effect. Therefore we modified a quantitative adhesion assay employing different *Lactobacillus* strains (*L. acidophilus* PZ1041, *L. fermentum* PZ1162, *L. fermentum* W151, *L. gasseri* PZ1160, *L. rhamnosus* PZ1121 and *L. rhamnosus* W65) and analyzed their capability to reduce the binding of EPEC E2348/69 to intestinal epithelial cells. We were able to show that the culture supernatants of all of these *Lactobacillus* strains, except not *L. fermentum* W151, are sufficient to decrease the adhesion of EPEC to T84 monolayers significantly. Moreover, quantitative RT-PCRs (qRT-PCR) showed that the reduction of EPEC binding to T84 cells is due to down regulation of several EPEC virulence factors after Lactobacilli culture supernatant application. Using ammonium sulfate precipitated or Proteinase K digested Lactobacilli culture supernatants, we could identify extracellular proteins, particular resistant to Proteinase K, to be responsible for the decrease in EPEC adherence. These proteins were further characterized by MS peptide mapping. Quantitative adhesion assays with the purified proteins proved the involvement of these proteins in the inhibition of EPEC binding to T84 cells, in a dose-dependent manner.

Our findings revealed new regulatory factors that will foster the development of advanced strategies for treatment of EPEC infections.

13 FRIEND OR FOE: DECIDES THE FLAGELLA OF COMMENSAL OR PATHOGENIC BACTERIA ABOUT INTESTINAL INFLAMMATION OR HOMOEOSTASIS?

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In previous work we show that the microbe-associated molecular pattern (MAMP) flagellin, isolated from the commensal strain *E. coli* Nissle, protected mice against a DSS- induced colitis and isolated flagella from pathogenic *S. typhimurium* SL1344 promote intestinal inflammation. In addition, isolated flagellae from commensal *E. coli* MG1655 and *B. subtilis* showed nor IL-8 response *in vitro* neither a protected effect against DSS- induced colitis. Our aim was to analyze the TLR5 activation capacity of different flagellae in the gut from a variety of commensal or pathogenic bacteria, their activity to induce IL-8 host responses and whether the biologic activity correlates with distinct structure patterns in the flagella D1 domain.

14 METASRA: EXPLORATION OF BACTERIAL DIVERSITY AND ECOLOGY USING ALL PUBLICALLY AVAILABLE AMPLICON SEQUENCES

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Sequence Reads Archive (SRA) serves as the primary depository for the increasing stream of data available from Next Generation Sequencing technologies and currently is hosting raw amplicon data from more than 37 000 samples. One half of these samples derived from human, mice and other hosts with the rest originating from various environmental sources. In order to exploit this accumulating sequence pool of knowledge, we have been developing a pipeline that systematically screens for, retrieves, process, and analyze all prokaryotic 16S rRNA gene amplicon datasets from SRA and use them to build sample-specific microbial profiles and databases. Through a web interface, users of this service can submit their bacterial 16S rRNA gene sequences of interest as queries and get answers about their prevalence in different environments together with their abundances and phylogenetic diversity globally or across selected samples. Hence, using metaSRA, users can get easy access to ecologically relevant knowledge about bacteria of importance in their specific field of research, e.g., microbe-host interactions.

15 BACTERIAL INFLUENCE ON GUT HOMEOSTASIS AND INFLAMMATION

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The interaction and competition among commensal bacteria plays an important role during development of IBD. In healthy hosts, the well-balanced interplay between non-pathogenic symbionts supports maintenance of gut homeostasis and contribute to intestinal immunity. In genetically predisposed hosts, pathobionts – a certain group of commensals – can accumulate and trigger inflammation. However which factors cause and favour such accumulations is unknown.

In our model *E. coli* mpk can cause colitis in germ-free *IL-2*^{-/-} mice due to a yet unknown mechanism while *B. vulgatus* mpk – another commensal – can in turn prevent colitis during *E. coli* mpk and *B. vulgatus* mpk co-colonization.

After whole genome sequencing we identified different genes that might play a role in competition among those bacteria or become important during inflammation.

To study those candidate gene sets *in vivo* we will use an invertebrate animal model, the larvae of the greater wax moth *Galleria mellonella*. It is easy to handle, cost-effective and the generation of data can be much faster with a high number of animals. Further it shares homology with the mammalian innate immune system.

16 REGULATION OF INTESTINAL IL-22 DURING INFLAMMATION AND INFECTION IS DEPENDENT ON THE COMPOSITION OF THE GUT MICROBIOTA

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The gut harbors ten times more bacteria than host cells and is one of the most diverse environments on the planet. The intestinal microbiota is not only involved in digestion of dietary components, but also in shaping mucosal immune responses influencing the course of intestinal infections or inflammation. Interleukin 22 (IL-22) is known to play a central role in mucosal immunity to microbial challenge and its expression is shaped by dietary components, like retinoic acid and tryptophan as well as the composition of the microbiota. The biological activity of IL-22 is further regulated by IL-22 binding protein (IL-22bp), an endogenous antagonist of IL-22. In a mouse model of colitis-associated colon cancer (CAC) levels of *Il22* were found upregulated whereas its soluble antagonist *Il22bp* was downregulated and impaired regulation of these factors resulted in increased incidence of CAC. We are interested in identifying the members of the intestinal microbiota that contribute in regulating these factors during intestinal inflammation. For our studies we utilize mice with a low complexity flora (LCM), containing about 8 different bacterial species, mice with a dysbiotic flora (DysM), and mice with a high complexity flora (HCM). By cohousing these mice, e.g. LCM with DysM, the complexity and composition of the microbiota can be modified. *Salmonella* Typhimurium infection and Dextran sodium sulfate (DSS) colitis models were performed in order to mimic chronic inflammation. Our preliminary results demonstrate that regulation of *Il22* is dependent on the microbiota in our models. Detailed characterization of microbiota composition and targeted manipulation thereof will be employed to define specific classes of bacteria involved in regulation of the IL-22 / IL-22bp axis.

17 OUTER MEMBRANE VESICLES – A NOVEL BACTERIAL CELL WALL STRUCTURE THAT PREVENTS FROM COLITIS?

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Depending on the composition of the intestinal microbiota *Rag1*^{-/-} mice develop a chronic colitis upon transplantation with naive T cells from *wt* mice. Oral administration of *Bacteroides vulgatus* mpk into mice suffering from colitis symptoms leads to healing of the disease. We could already show that *B. vulgatus* mpk lipopolysaccharide (LPS) is responsible for the induction of a novel dendritic cell phenotype (that we call semi-mature) maintaining intestinal homeostasis. However, administration of isolated *B. vulgatus* mpk LPS did not provide similar healing effects in inflamed *Rag1*^{-/-} mice compared to administration with whole viable *B. vulgatus* mpk bacteria.

We suppose that another cell wall derived component of *B. vulgatus* mpk could be at least in parts responsible for the observed healing effects: so-called Outer Membrane vesicles (OMVs). Outer Membrane Vesicles derived from Gram-negative bacteria are a delivery device by which both virulence factors and immunomodulatory molecules are transported to dendritic cells. It is already known that OMVs derived from other gram negative bacteria can provide healing features.

We want to investigate if (1) *B. vulgatus* mpk physiologically forms OMVs and if yes, if (2) they are able to induce or at least to support healing of chronic colitis in T cell transplanted *Rag1*^{-/-} mice. Since the signaling pathway of the compounds that are delivered by OMVs is not completely understood yet, we want to (3) further investigate the recognition mechanism of OMVs by Antigen Presenting Cells like Dendritic Cells and if (4) the OMV-treated DCs become semi-mature contributing to homeostasis manifestation.

18 INSIGHTS INTO THE EVOLUTION AND UNEXPECTED LIFESTYLE OF THE MUCUS-DWELLING BACTERIUM *MUCISPIRILLUM SCHAEDLERI*

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Mucispirillum schaedleri inhabits the intestinal mucus layer of rodents and other animals, where it is thought to degrade mucus. We analyzed the genome of *M. schaedleri* ASF457 to gain insights into its lifestyle and confirmed some predicted traits with physiological experiments. Surprisingly, its genome predicts that *M. schaedleri* has limited capacity for degrading host-derived mucus or other complex compounds, but rather utilizes small compounds such as peptides, amino acids, glycerol, and short chain fatty acids. Additionally, it can reduce nitrate and has systems for scavenging oxygen and reactive oxygen species, which accounts for its survival close to the mucosal tissue blooms during inflammation. Interestingly, *M. schaedleri* harbors a type VI secretion system (T6SS) and several putative effector proteins containing eukaryotic domains, which may be involved in interacting with the host and may play a role in inflammation. An examination of individual phylogenies of all genes in the *M. schaedleri* genome indicated extensive lateral gene transfer, primarily from intestinal Epsilon- and Deltaproteobacteria. Though *M. schaedleri* utilizes non-laterally-transferred pathways (e.g. nitrate reduction), laterally acquired pathways from gut organisms (e.g. T6SS and glycerol-P utilization) are likely also important for its survival in the intestine, indicating that lateral gene transfer facilitated its establishment in the gut ecosystem.

19 E. COLI NISSLE PROTECTS AGAINST DSS-INDUCED COLITIS BY ACTIVATION OF CD11c+ INTESTINAL LAMINA PROPRIA CELLS

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Microbe-associated molecular patterns (MAMPs) induce host innate intestinal immunity resulting in inflammation or homeostasis – we found out that the flagella of *E. coli* Nissle (EcN) protect mice against DSS- induced colitis. To identify the target cell addressed by EcN or especially the EcN flagella we used bone marrow chimeric mice in combination with our DSS model. Therefore WT or TLR5^{-/-} mice were irradiated and transplanted with freshly isolated bone marrow from WT or TLR5^{-/-} mice (TLR5^{-/-} =>WT; WT => TLR5^{-/-}). Administration of EcN or EcN mutant strains (EcNΔtcpC, EcNΔfliC or EcN ΔtcpCΔfliC) beside 3.5% DSS showed that TLR5 on myeloid cells is essential for the EcN mediated protective effect. To identify further which myeloid cell facilitated the protection we irradiated WT mice and transplanted a mixture of TLR5^{-/-} and ΔDC bone marrow (TLR5^{-/-} + ΔDC =>WT). Feeding of EcN and EcNΔtcpC revealed that CD11c⁺ TLR5⁺ cells are crucial for the EcN mediated protective effect against DSS- induced colitis.

20 INTESTINAL MICROBIOTA COMPOSITION INFLUENCES RECURRENCE OF ILEITIS IN A MOUSE MODEL OF CROHN'S DISEASE

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Background: Dysbiosis of the intestinal microbiota has been established in patients with Crohn's disease (CD). Using a mouse model of CD-like ileitis, we aimed at characterizing changes in intestinal microbial communities after antibiotic treatments or caecal microbiota transplants (CMT) to identify disease-relevant bacteria and thereby gain a better understanding of microbial involvement in Crohn's disease.

Methods & Results: Wild-type (WT) and heterozygous TNF^{deltaARE/+} mice as a genetically-driven murine model for CD-like ileitis were treated at 8 weeks of age with vancomycin/metronidazole (V/M), vancomycin/metronidazole/norfloxacin/neomycin (V/M/N/Neo) or ampicillin (Amp) for 4 weeks. Antibiotic treatments significantly reduced tissue pathology in the distal ileum of TNF^{deltaARE/+} mice (Control: 5.6±1.2; V/M/N/Neo: 3.6±0.9; Amp: 1.6 ± 1.0; V/M: 0.9±0.2). All antibiotic treatments substantially decreased bacterial diversity and composition but did not affect total bacterial load. Comparative taxonomic analysis identified a decreased abundance of *Bacteroidales* associated with disease protection in all antibiotic treatments. Relapse of CD-like ileitis was observed 6 weeks after V/M treatment and was clearly associated with preceding regain of a disease-conditioning microbiota. Cecal microbiota transfer from wildtype or antibiotic treated mice into TNF^{deltaARE/+} failed to prevent relapse.

Conclusion: Compositional alterations of the gut microbiota in a mouse model of Crohn's disease are associated with disease pathogenesis and the risk of relapse. Identification of specific disease-conditioning bacteria through monoassociation experiments in germfree mice is currently under investigation.

21 GENETIC ANALYSIS OF THE STEM CELL MARKER LGR5 IN IBD

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Background: Inflammatory Bowel Diseases (IBD), chronic inflammations affecting specific locations within or even the whole intestinal tract, are linked to epithelial barrier defects and microbial dysbiosis. Our working group has discovered multiple impairments within the Wnt pathway that could be linked to small intestinal Crohn's Disease (CD), an IBD subgroup. These defects are in part responsible for diminished antimicrobial activity, as they affect the differentiation and function of the specialized antimicrobial peptide producing Paneth cell. Since Wnt is however also mainly involved in stem cell maintenance and epithelial proliferation, we hypothesize these CD associated aberrations might also be accompanied by stem cell defects. We therefore aimed at investigating a potential role of the stem cell marker LGR5 in IBD.

Methods: We isolated genomic DNA of 91 individuals (healthy controls and IBD patients) and performed full exon sequencing (including intron boundaries and part of the 5' and 3' region) of LGR5 via the Sanger method.

Results: We have finalized our analysis for 18 of 19 exons and so far found various partly previously unknown variants in comparison to the NCBI published sequence. Altogether 9 lead to amino acid exchanges, while 4 represent silent single nucleotide polymorphisms (SNP). In addition we could also identify 8 SNPs within the non-coding 5' and 3' regions within close proximity to the gene's coding region.

Outlook: We plan to finalize this study (including haplotype analysis) by the end of 2014. Interesting variants will be selected for high-throughput analysis in large IBD DNA cohorts.

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22 ROLE OF THE ETHANOLAMINE UTILIZATION PATHWAY IN BACTERIAL COMPETITION

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The microbiota plays a central role in many physiological reactions and disruptions can lead to diseases such as IBD. The underlying molecular mechanisms however are still not understood. The main challenge is often the vast number of microorganisms underlying interbacterial interactions as well as their effects on host immunity.

Caenorhabditis elegans is potentially useful to study the role of certain microbes in IBD given the combined power of bacterial and nematode genetics. However, knowledge on the effects of bacteria on its immune system is still rudimentary e.g. it is debated whether pathogenic bacteria have a metabolic advantage when colonizing the intestine thereby contributing to perturbation.

We therefore want to investigate the immune response of *C. elegans* after exposure to different bacteria strains. In further experiments we want to use this system to examine various metabolic pathways specific to pathogenic bacteria or pathobionts (commensals with a pathogenic tendency) to evaluate their role in the colonization process. In previous studies our lab identified the ethanolamine utilization cluster as promising candidate pathway that could enable bacteria to use ethanolamine as a non-fermentable carbon source accessible during inflammation.

23 PHENOTYPIC CHARACTERIZATION OF HUMAN COLONIC MYELOID CELLS IN A MODEL OF INFLAMMATION

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The human colonic lamina propria harbors a large number of immune cells. The molecular mechanisms of their activation in host defense are still unknown. However, damage of the epithelial layer represents an early hallmark of intestinal inflammation.

Here, we used a human intestinal organ culture model to simulate intestinal inflammation by detachment of the epithelia layer (walk-out model). Lamina propria mononuclear cells obtained by culture (WO-LPMCs) were phenotypically characterized by flow-cytometry using a multicolor panel introduced by the Human Immunphenotyping Consortium (HIPC-Panel).

Further, those cells were compared to lamina propria mononuclear cells of digested colonic mucosa (LPMCs) and peripheral blood mononuclear cells (PBMCs) of the same donors.

24 EFFECTS OF BACTERIAL ISOLATES FROM MURINE FECES IN ACTIVATION AND MATURATION OF BMDC

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The gut microbiota plays an important role in the development of inflammatory bowel disease (IBD). The intestinal immune system is permanently exposed to a high variety of commensal bacteria. It could be shown that bone marrow derived dendritic cells (BMDC) will mature completely when stimulated with *Escherichia coli* mpk. In contrast, BMDC will arrest in a semi-mature state after stimulation with commensal *B. vulgatus*. No further maturation was detected after restimulation with *E. coli* mpk. To analyze whether this effect is specific for *B. vulgatus* we isolated three bacterial isolates (presumable *Bacteroides vulgatus*, *Bacteroides sartorii*, unknown gram-negative bacteria) from murine feces and differentiated them according to activation and maturation of BMDC. The identification of the unknown species via biochemical and MALDI-TOF analysis failed. It could also not be identified by sequencing 16s rDNA.

The unknown bacteria revealed proinflammatory effects compared to *B. vulgatus* or *B. sartorii*. As shown by further experiments we will characterize the unknown bacteria via T-cell proliferation assays, analysis of proinflammatory effects using a T-cell transfer model of chronic colitis and whole genome sequencing.

25 PROTEOMICS ANALYSIS OF DISTINCT DENDRITIC CELL MATURATION STATES

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Both *in vivo* and *in vitro*, dendritic cells differ in their response to pathogenic and commensal stimuli. In our previous work it has been shown that the pathogenic *E.coli* strain (*E.coli* mpk2) induces full maturation of the dendritic cells and severe inflammation whereas the commensal strain *Bacteroides vulgatus* (*B.vulgatus* mpk) drives the DCs to a semi-mature state. In semi-mature state it has been shown that DCs suppress inflammation, which is thought to be a contributing factor in prevention of colitis by *B.vulgatus* in mouse models of disease. Several published data underlined the importance of DCs in inflammation process, and we argue that DC maturation state plays an important role in disease manifestation and progress.

The aim of our project is to perform a proteomic analysis of proteins which are either involved in the signaling pathways leading to the change of DC maturation or which characterize the dendritic cell maturation state. To be able to achieve this we use murine bone marrow derived dendritic cells (BMDCs), cultured in the presence of GM-CSF. The samples are stimulated with *B.vulgatus* for semi-maturation and *E. coli* for complete maturation. The resulting cell population is harvested as a whole and sent for shotgun phosphoproteomics and bioinformatical analysis. In our preliminary proteomics analysis we identified several proteins of interest that are differentially regulated on protein level in semi-mature and fully mature dendritic cells. Superoxide dismutase, CD180, cell cycle protein Cdk1 and important proteins in the apoptotic pathway i.e. Caspase-3 are several examples of identified proteins so far. On the other hand in the *B.vulgatus* samples there are proteins which induce T-cell differentiation or which are involved in Ag- presentation with MHC II, such as Cathepsin S, Ras-related proteins, Aminopeptidases. Further analysis will be done in cooperation with the bioinformatics center in Tübingen to have a systemic overview of differential regulation. A group of selected candidates which are predicted to have a significant effect on the maturation state or maturation process will be validated by *in-vitro* experiments using qPCR, Western Blotting, FACS and ELISAs as well as with *in vivo* validation from isolated mouse dendritic cells. The results of this study will hopefully provide us a better understanding of dendritic cell maturation process and the regulation of it in response to pathogenic and commensal bacteria.

26 CD101 IS CRITICAL FOR THE MAINTENANCE OF INTESTINAL TOLERANCE

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CD101 is a negative costimulatory molecule abundantly expressed on intraepithelial lymphocytes (IELs) and intestinal myeloid cells. We have recently shown that the expression of CD101 on donor and recipient cells significantly delays the induction of colitis in RAG1^{-/-} mice after an adoptive T cell transfer. Although these data suggest a critical role of CD101 in the maintenance of intestinal tolerance, the contribution of each individual immune cell subset to the CD101-mediated protection has not been elucidated.

Thus, in order to study the role of CD101 in greater detail, we extended our studies to the dextran sodium sulfate- (DSS-) and *Salmonella*-infection- induced colitis models utilizing different Cre strains in combination with recently generated floxed CD101 knockout (CD101^{fl/fl}) mice. Both acute and chronic DSS- and *Salmonella*-induced colitis in conventional CD101 KO mice, CD11c Cre x CD101^{fl/fl} and CD8 Cre x CD101^{fl/fl} mice were significantly enhanced compared to littermate control mice. As all three different knockout strains exhibited also distinct changes in the intestinal microbial composition, we are currently investigating the interaction of these bacterial and fungal species with the respective CD101-expressing immune subsets.

27 INCREASED FREQUENCIES OF ACTIVATED EFFECTOR CD4+ T CELLS IN THE PERIPHERAL BLOOD AND HEPATIC TISSUE OF PATIENTS WITH NAFL AND NASH

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Background: The prevalence of non-alcoholic fatty liver disease (NAFLD) ranges between 20-35% in Europe and reaches up to 50% in the US. In 5 to 20% of cases there is a progression from NAFL to a non-alcoholic steatohepatitis (NASH) but it is still not well understood why some people develop NASH and other remain with NAFL. The current hypothesis about the pathogenesis is a two hit theory and for both of the two hits the intestinal microbiome seems to be relevant. The “first hit” causes a steatosis which is often observed in patients with metabolic syndrome and the “second hit” in form of bacterial translocation leads to an inflammation in the fatty liver and the development of NASH. Hepatic Th17 cell infiltration was observed in a NASH mouse model and higher IL-17 and IL-21 gene expression in human liver of NASH patients. Intestinal microbiome also interacts with the host immune system and colonization studies in mice have shown that Treg and effector T cells can be induced in the intestinal lamina propria of specific bacteria. We hypothesized that the phenotype of peripheral CD4+ T cells might be predictive for the degree and quality of hepatic T cell infiltration and histopathology in association to specific changes in intestinal microbiome.

Aims: Characterization of CD4+ regulatory T cells (Tregs) and conventional CD4+ T cells in peripheral blood and hepatic tissue in patients with NAFL and NASH. Pyrosequencing analysis of stool samples.

Methods: 50 patients with histology-proven NAFL or NASH and 44 healthy controls were included in this study. PBMCs of peripheral blood and hepatic tissue were characterized by multi-colour FACS analysis. CD4+ T cells were stimulated with PMA and ionomycin for intracellular detection of cytokine production (IL-17, IL-4, INF-g, IL 21). Stools samples were collected for pyrosequencing analysis.

Results: Patients were older and had a higher BMI in comparison to healthy controls. In patients with NAFL and NASH a lower frequency of resting Tregs (CD4+CD45RA+CD25++) was observed in peripheral blood, which was somewhat mirrored by a trend towards more resting Tregs and effector Tregs (CD45RA-CD25+++ cells among hepatic CD4+ T cells. We found changes in effector CD4+ T cells with higher frequencies of IFN-g+, IL-21+ and IL4+ cells among CD4+ T cells of the peripheral blood of patients. In hepatic tissue, higher frequency of IL17+, IFN-g+, IL21+ and IL4+ cells were measured among CD4+ T cells than in the peripheral blood. CD4+ T cells in peripheral blood, and much more so, hepatic tissue contained higher frequencies of HLA-DR+, i.e. activated cells. Pyrosequencing of stool samples is still ongoing.

Conclusions: The peripheral blood and hepatic tissue of patients with all stages of NAFLD contained higher frequencies of activated effector cells among CD4+ T cells. NAFL patients show a “prehepatic” immune cell profile very similar to that seen in NASH. Our data suggest that interfering with the effector CD4+ T cell response might prevent progression from NAFL to NASH. Outstanding results of pyrosequencing analysis of stool samples will give further insight into our hypothesis of enterohepatic immunopathogenesis of NAFLD.

28 EVIDENCE THAT THE GASTROINTESTINAL MUCUS LAYER INTERFERES WITH COLIBACTIN ACTIVITY OF ESCHERICHIA COLI NISSLE 1917

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Recent publications reported the probiotic *E. coli* strain Nissle 1917 (EcN) to harbor the pks island which encodes the genotoxin colibactin and induces fatal DNA double strand breaks (DSB) in eukaryotic cells. In order to investigate the effect of mucus non-mucus producing HeLa, moderate amounts of mucus producing LS174T and the heavy mucus-producing HT29 MTX-E12 cell line was employed. In a first approach LS174T cells were cultivated in DMEM with 10% FCS or with 50% FCS or with 10% FCS plus 1mM propionate and infected with EcN. The latter two culture conditions induced enhanced mucus production and subsequently a smaller number of cells which suffered from DSB as demonstrated by CometAssay®. In a second approach cell viability of EcN infected HeLa and HT29 MTX-E12 cells as the consequence of DSB was analyzed by flow cytometry and the use of propidium iodide and Hoechst 33342. After 24, 48 and 72 hours post EcN infection cell viability was highest for HT29 MTX-E12 cells which had produced a thicker and denser mucus layer than LS174T cells. In contrast, the number of dead cells was highest for the non-mucus producing HeLa cells. These results reveal a protective effect of the mucus (layer) most likely due to the inability of pks-positive *E. coli* to get into direct contact with the epithelial cells because of the mucus barrier. This is in line with earlier studies in which a direct contact between the colibactin producer and the epithelial cells was proven to be essential for the induction of DSB.

29 IDENTIFY THE IMMUNOREGULATORY CIRCUITS RESPONSIBLE FOR INCREASED COLITIS RISK IN RESPONSE TO THE COLITOGENIC MICROBIOTA OF INFLAMMASOME-DEFICIENT MICE

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The gut microbiota of higher mammals consists of trillions of microorganisms representing hundreds of species. In the healthy state this complex ecosystem is maintained by a mutual homeostasis where the microbiota provides increased digestive capacity and the host ensures a nutrient-rich niche. Alteration in the homeostasis due to genetic or environmental factors can initiate various disease conditions for the host. Previous reports showed that deficiencies in the Nlrp6 inflammasome can result in altered or dysbiotic microbiota with a transmissible phenotype of exacerbation of colitis severity. To characterize the function of adaptive immune cells and Il12 family members during dysbiosis, mice deficient in those cell types (B cells / T cells) and cytokines (Il12a / Il12b) will be cohoused with dysbiotic mice and disease severity in comparison to wildtype mice after colitis induction will be characterized. Our preliminary results suggest a role for B cells and the cytokine IL12 in dysbiosis-exacerbated DSS colitis. Detailed elucidation of changes in the immunoregulatory network driven by colitogenic microbiota including isolation of lamina propria lymphocytes, measuring cytokine expression and defining microbiota composition using 16S ribosomal sequencing will add a new insight towards the contribution of dysbiotic flora in disease development.

30 SHIGA TOXIN BACTERIOPHAGE RESISTANCE OF E. COLI NISSLE 1917 (EcN)

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Objective: In previous work EcN was shown to have antagonistic activity against EHEC during co-incubation. In this study it was tested if probiotic EcN can be transformed into a Stx-producer by Stx-phage infection. This question addresses an important safety aspect of EcN to be used as a potential therapeutic of EHEC infections.

Methods: Phage lysates were generated by induction of bacteria with mitomycin C. This was followed by purification with a CsCl density gradient. Phage isolates from EHEC serotypes O157:H7, O26:H11, O103:H2, O111:H⁻, O104:H4 and O145:H25 were used in the experiments to infect EcN and the *E. coli* K12 strains MG1655, HB101 and DH5 α . Infection was assessed by *stx*-PCR, phage plaque assay, Stx-ELISA and K⁺-efflux assay. Furthermore, transmission electron microscope pictures of the phage isolates were taken.

Results: In our experiments EcN was not infected by the tested Shiga toxin phage isolates. Infection of MG1655, HB101 and DH5 α by phage isolates from EHEC O157:H7, O26:H11, O104:H4 and O145:H25 could be confirmed via phage plaque assay, *stx*-PCR and Stx-ELISA. The K⁺-efflux assay with MG1655 / EcN and the phage isolate from EHEC O157:H7 showed inhibition of phage DNA uptake with EcN. These results indicate EcN to be safely used for treatment of EHEC infections due to the inability of Stx-phages to infect this probiotic *E. coli* strain.

Outlook: We are currently investigating the mechanisms responsible for the observed bacteriophage resistance of EcN.

31 GALLERIA MELLONELLA AS ALTERNATIVE MODEL TO STUDY INFLUENCE OF BACTERIAL FACTORS ON INNATE IMMUNITY

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Several factors are thought to be involved in the development of IBD, genetic susceptibility of the host and environmental factors as well as intestinal microbiota and the host immune system. Focus on bacteria-host as well as bacteria-bacteria interaction during colonization will help to better understand composition of intestinal microbiota and their role in induction or prevention of IBD and other autoimmune diseases.

In previous work our group assessed the influence of different lipopolysaccharide (LPS) endotoxicity on maintenance of gut homeostasis and inflammation in different mouse models.

We want to generate a high throughput model apart from the common mouse models to assess the impact of those bacterial strains and intend to use the lepidopteran insect model *Galleria mellonella*, which is widely used to study host-microbe interactions. It is especially interesting as its innate immune system shows high similarity with the mammalian system and if it produces comparable results.

32 COLONIC CROHN'S DISEASE AND DISTURBED BETA-DEFENSIN EXPRESSION – A ROLE FOR HDACs?

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Background: Epithelial antimicrobial peptides (AMPs), such as defensins, play a key role as a first line of intestinal defense and in shaping the gut microbiota. Inflammatory bowel diseases (IBD) such as Crohn's disease (CD) have a multifactorial pathogenesis characterized by disturbed host microbe interaction and defects in the network of mucosal AMPs. Colonic CD is for instance associated with a diminished upregulation of the inducible beta-defensin HBD2. Epigenetic mechanisms, e.g. the possible role of histone modifying enzymes, could help to better understand the interplay between environment, genome, and microbiome in IBD. We therefore aimed at studying class I histone deacetylases (HDAC)1 and 2 in patients and to analyze their potential role in HBD2 expression.

Methods: HDAC1 and 2 expression was analyzed in colonic biopsies from CD patients and healthy controls by real-time PCR. CaCo2 cells were stimulated by inflammatory mediators while HDACs were inhibited by SAHA or MS-275 and checked for HBD2 mRNA.

Results: We found significantly lower expression levels for HDAC 1 and 2 in the colon of CD patients as compared to controls. In addition, we found a significantly enhanced inflammation-dependent induction of HBD2 when simultaneously inhibiting HDAC function. Conclusion: Aberration in HDAC mediated epigenetic control might be involved in colonic CD. HDACs seem to have an important role in controlling on demand AMP production and therefore in epithelial homeostasis and antimicrobial defense.

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33 THE PROBIOTIC *E. COLI* NISSLE 1917 DOES NOT ENGAGE IN CONJUGATION WITH EHEC

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The probiotic *Escherichia coli* strain Nissle 1917 (EcN) has been successfully used for almost a century in the treatment of several intestinal diseases, e.g. diarrhea, ulcerative colitis and constipation. An important feature for its application as a drug is its biological safety. During co-cultivation experiments we observed that EcN does not act as recipient for a conjugative, virulence-associated plasmid of EHEC O104:H4 strains in contrast to other *E. coli* strains. To prove EcN's genetic stability liquid mating assays were performed. Spontaneous rifampin-resistant mutants of EcN and *E. coli* K-12 strains (MG 1655, DH5 α and HB101) were selected for differentiation. The EHEC O104:H4 strain TY3730 which harbors a conjugative plasmid conferring resistance against extended-spectrum beta-lactamases (pESBL) was used as donor. Co-cultivation experiments were performed for 24 h and transconjugants were selected on LB-agar plates containing rifampin and ampicillin. No transconjugants were detected for EcN. In contrast, the conjugation rate of *E. coli* MG 1655, DH5 α and HB101 was 1.4 %, 2.3 % and 0.01 %, respectively. Furthermore, an involvement of EcN's cryptic plasmids and capsule could be ruled out by testing corresponding isogenic mutants of EcN for their recipient ability. Other factors that might be involved in this effect are currently under investigation. Additionally, the observed conjugation resistance of EcN will be reconfirmed by using other conjugative plasmids as well as by performing surface mating experiments.

34 PROBIOTIC INTERVENTION IN FAST AGEING ERCC1- Δ 7 MICE AFFECTS MUCOSAL AND SYSTEMIC IMMUNITY

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Ageing profoundly affects the immune system. Evidence is increasing that the gut microbiota, which composition alters significantly upon ageing, has an important role in changing immune functions. Thus, we hypothesized that modulation of gut microbiota composition via intervention with probiotics might modulate the ageing process. To study this, we used a fast ageing Ercc1- Δ 7 mouse model, resembling the features of normal ageing at an accelerated rate. The median lifespan of Ercc1- Δ 7 mice is 20 weeks, compared to 118 weeks in wild-type controls. This enabled the study of long-term (10 weeks) intervention with probiotics on systemic and mucosal immunity. Three probiotic strains were tested: *L. plantarum* WCFS1, *L. casei* BL23 and *B. breve* DSM20213, or PBS/glycerol as a control. At 6 or 16 weeks, the mice were sacrificed and all major immune organs were harvested. We used multicolour flow cytometry for assessing immune cell frequencies in several tissues (spleen, mesenteric lymph nodes (MLN), Peyer's Patches (PP), bone marrow (BM), blood and thymus). Immune competence was evaluated by culturing spleen and BM cells and assessing their proliferation capacity, cytokine response and antibody production. *L. casei* BL23 strongly increases the frequency of neutrophils in spleen and BM, which is a sign of inflammatory conditions. This is confirmed by splenomegaly and worse survival. *L. casei* BL23 treatment also induces strong Th17 (spleen) and Treg (spleen and MLN) polarisation. *B. breve* DSM20213 induces only minor changes, mainly in mature B cells in the BM. *L. plantarum* WCFS1 shows also minor changes in the immune system. We observed a decrease in B cells in the BM in selective differentiation stages and a trend towards more Tregs in MLN but not in spleen. Increased IL-17 production by spleen cultures, supplemented with ConA for 4 days, support the findings of increased Th17 frequency by *L. casei* BL23 in the spleen. IgA production by B cells from mice treated with *B. breve* DSM2013 was significantly decreased. IgA is important in maintaining mucosal barrier integrity. BM-derived macrophages and dendritic cells from *L. casei* BL23 treated mice showed also differences compared to the control. Distribution of cell populations in central and peripheral immune organs (bone marrow, thymus, spleen, mesenteric lymph nodes, Peyer's Patches) change with age, and this change is differentially affected by probiotic treatment. It is remarkable that *L. casei* BL23 which induces worse survival and most inflammatory conditions, also shows a significant increase in Tregs. Probably, accumulation of Tregs is not always positive in the context of ageing

35 **INTESTINAL SERINE PROTEASE PATTERN CORRELATES WITH THE COMMUNITY STRUCTURE OF THE INTESTINAL MICROBIOTA**

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Background: Fecal proteolytic activity is increased in UC patients and TNBS colitis. The underlying reasons and responsible proteases as well as the physiological consequences of the increased proteolytic activity are unclear. The aim of this study is to understand the correlation between origin of intestinal proteases, intestinal microbiota and intestinal inflammation.

Methods: We measured total proteolytic activity and serine protease pattern in cecal contents of wildtype (WT) and TNF^{deltaARE/+} mice. Serine proteases were identified by LC/MS-MS analysis.

Results: TNF^{deltaARE/+} mice did not show changes in cecal serine protease pattern and total proteolytic activity compared to WT mice. Antibiotics (AB) treatment significantly increased total protease activity and low molecular weight (LMW) serine proteases, e.g. trypsin and chymotrypsin-like elastase, in WT and TNF^{deltaARE/+} mice. Interestingly, the increase in total proteolytic activity following AB-treatment was accompanied by a drop in bacterial diversity and reduction of ileal inflammation. Recurrence of inflammation after therapy was associated with a reappearance of the disease-related microbial community and serine protease pattern.

Conclusion: The present findings indicate that the observed shifts in serine protease pattern and total proteolytic activity are mainly due to changes in the intestinal microbial composition, resulting in reduced inactivation of pancreatic host proteases. Inflamed-tissue derived proteases and microbe-derived proteases seem to play a minor role for the total proteolytic activity.

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