# 6th Seeon Conference

## Microbiota, Probiota and Host

## Mikrobiota, Probiota und Wirt

28.- 30. June 2013

Conference Center

Monastery Seeon / Chiemsee

**For more information:**

**DGHM@wzw.tum.de**

**http://www.dghm.org/red/fachgruppen/**

June 28, 2013

Dear Participant,

On behalf of the German Society of Hygiene and Microbiology (DGHM) and the Organizing Committee, welcome to the 6th 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 the newly founded 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 clinicial 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 DFGPriority Programme “MICROBIOTA – a Microbial Ecosystem at the Edge between Immune Homeostasis and Inflammation” (SPP 1656). The “Seeon Conference” has become a known platform to critically discuss the role of microbe-host interactions in health and disease sharing cutting-edge science and technologies. Basis mechanisms of the host’s microbiome are discussed at the interface of metabolic and immune functions aiming to be implemented in therapy and prevention of chronic inflammatory, atopic and metabolic diseases.

Thank you in advance for your contribution to this meeting. Your willingness to participate and share your expertise is greatly appreciated.

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

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

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# PROGRAM Friday, June 28

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

| | |
|---|---|
| $17^{15}$ – $18^{00}$ | Keynote Lecture: **Karsten Kristiansen**, Department of Biology, University of Copenhagen<br>***You'll never walk alone – you and your gut microbiota and why it matters*** |

| | |
|---|---|
| $18^{15}$ | Dinner |

## GUT MICROBIOME AND HOST

$19^{30}$– $21^{00}$ Chair: G. Graßl, Experimental Medicine, Universität Kiel/FZ Borstel

S. Bollmann, Max-von-Pettenkofer Institut, LMU München
*Quantitative single-cell analysis of colicin Ib production in Salmonella enterica serovar Typhimurium*

I. Flade, Institute of Medical Microbiology and Hygiene, University of Tübingen
*The dichotomic role of LPS: induction or prevention of intestinal inflammation*

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*Metaproteome analysis and molecular genetics of rat intestinal microbiota reveals section and localization resolved species distribution and enzymatic functionalities*

P. Rausch, Max-Planck-Institute for Evolutionary Biology, Plön
*Alteration of inflammatory responses through the interaction of gut microbiota and blood group related antigens*

A. Walker, Department of Environmental Sciences, Helmholtz Zentrum München
*Discovery of gut microbial metabolome in Type 2 diabetes*

M. Weiher, Chair of Nutrition and Immunology , Technische Universität München
*Antibiotic-induced alteration of the gut microbiota protects TNFdeltaARE mice from Crohn's disease-like ileitis*

| | |
|---|---|
| $21^{00}$ | Drink at the Bar? |

# PROGRAM Saturday, June 29

$08^{30}$ – $09^{15}$ Keynote Lecture: **Alexander Loy,** Department of Microbial Ecology, University of Vienna
***Metabolic individuality in the intestinal wilderness - a novel single-cell approach to study in vivo function of intestinal microbiota members***

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

## PROBIOTIC MECHANISMS / ANTIMICROBIAL COMPOUNDS

$09^{45}$ – $11^{15}$ Chair: C. Becker, Medical Department 1, Universitätsklinikum Erlangen

L. Courth, Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart + University of Tübingen
*Regulation of α-defensin expression by inflammatory processes and bacteria*

M. Meijerink, Host-Microbe Interactomics, Animal Sciences, Wageningen University
*The effect of non-replicating probiotic strains in a Salmonella infection mouse model*

S. Menz, Institute of Medical Microbiology and Hygiene, University of Tübingen
*Flagella and TcpC as probiotic factors of E. coli Nissle 1917*

M.J. Ostaff, Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart
*Paneth cells, Wnt ligands, and intestinal Crohn's Disease*

J.M. Wells, Host-Microbe Interactomics Group, Wageningen University
*Intestinal REG3γ plays a protective role against intestinal infection with both Gram-positive and Gram-negative pathogens*

D. Zhurina, Institute of Microbiology and Biotechnology, University of Ulm
*Exploring Bifidobacterium bifidum S17 for potential players in host-microbe interactions by genomic and proteomic approaches*

$11^{15}$ – $12^{00}$ Keynote Lecture: **Nadine Cerf-Bensussan**, INSERM U989, Université Paris Descartes-Sorbonne Centre and Institut IMAGINE
***Interactions of Segmented Filamentous Bacterium with the host immune system: lessons from gnotobiotic mice***

# PROGRAM Saturday, June 29

$12^{00}$ - $14^{00}$ Lunch

## MECHANISMS OF INFLAMMATION

$14^{00}$ – $15^{30}$ Chair: M. Heimesaat, Microbiology, Charité Berlin

C. Günther, Medical Clinic 1, Friedrich-Alexander-University Erlangen
*Caspase 8 in intestinal epithelial cells regulates immune homeostasis in the gut*

T. Kruis, Campus Benjamin Franklin, Charité Universitätsmedizin Berlin
*The interaction of macrophages and the microbiota –*
*a modulator of the intestinal barrier?*

C. Kunst, Department of Internal Medicine, University of Regensburg
*Physiologic TLR9-CpG-DNA interaction is essential for the homeostasis of the intestinal immune system*

S. Lipinski, Institute of Clinical Molecular Biology, CAU Kiel
*RNAi screening identifies FRMPD2: a scaffolding protein controlling NOD2-mediated immune responses*

B. Sovran, Host-Microbe Interactomics Group, Wageningen University
*Homeostatic mechanisms preventing ileitis in mice with absent or deficient Muc2 production*

A. Wittmann, Institute of Medical Microbiology and Hygiene, University of Tübingen
*TLR2- and TLR4-Mediated Amelioration of DSS-Colitis by Induction of CD103-Expressing Dendritic Cells*

$15^{30}$ – $16^{00}$ Coffee Break

$16^{00}$ – $18^{00}$ **Poster Slam** (2 minutes / 2 slides) **and Poster discussion**
(J. Frick, Med. Microbiology + Hygiene, University Tübingen)

$18^{00}$ – $18^{45}$ Keynote Lecture: **Nicola Harris**, Global Health Institute, Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland
***Regulation of chronic inflammatory diseases by the microbiota***

$18^{45}$ Dinner

$20^{30}$ Bowling at the Bar

# PROGRAM Sunday, June 30

$08^{30}$ – $09^{15}$ Keynote Lecture: **Christine Josenhans**, Institute for Medical Microbiology and Hospital Epidemiology, Hannover Medical School
***H. hepaticus colitis models as an example for interplay of bacterial colitogenic factors, microbiota and the host defence***

$09^{15}$ – $09^{45}$ Coffee Break

$09^{45}$ – $10^{00}$ **Poster Award**

## MECHANISMS OF INFECTIONS

$10^{00}$ – $11^{30}$ Chair: G. Loh, Gastrointestinal Microbiology, DIFE

S. Brugiroux, Max von Pettenkofer Institut, LMU Munich
*Generation of an Oligo-Mouse Microbiota to study colonization resistance against enteropathogens*

M.M. Heimesaat, Inst. for Microbiology + Hygiene, Charité – University Med. Berlin
*Campylobacter jejuni induces acute non-self-limiting enterocolitis in gnotobiotic IL-10-/- mice via Toll-like-receptor-2 and -4 signaling*

R. Lakra, Medicine Clinic I, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU)
*The C.rodentium T6SS (Type 6 Secretion System) is Important for the Effective Gut Colonization*

L. Maier, ETH Zurich, Institute of Microbiology, Switzerland
*Multiple factors influence the occurrence of population bottle neck(s) during Salmonella Typhimurium colitis*

S. Ocvirk, Chair of Nutrition and Immunology, Technische Universität München
*Enterococcus faecalis polysaccharide antigen and lipoproteins mediate virulence in chronic inflammation and in infection models*

N. Steck, Inst. for Experimental Med., University of Kiel / Research Center Borstel
*Complex role of proteases in Salmonella-induced intestinal fibrosis*

$11^{30}$ Lunch and Departure

# PROGRAM
# Friday,
# June 28

# YOU'LL NEVER WALK ALONE – YOU AND YOUR GUT MICROBIOTA AND WHY IT MATTERS

Karsten Kristiansen

*Department of Biology, University of Copenhagen and BGI-Shenzhen*

*Abstract:* The importance of the gut microbiota for regulation of metabolism and immune functions is well established today, but the exact molecular mechanisms by which bacteria in the gut exert their actions still remain elusive. Evidence has been presented that the gut microbiota also affects general behavior adding fascinating novel facets to the studies of the gut microbiome. Our laboratory has during the last couple of years been involved in large-scale metagenomics projects in collaboration with BGI-Shenzhen using high throughput Illumina-based sequencing of total fecal DNA. These studies have primarily been focused on humans and mice, but have now been extended to encompass several other species including pig and fish. In this lecture I will first summarize our data on the mouse and human gut microbiota, pointing to differences and similarities. Using mice as a model of obesity, we have been able to identify specific bacteria associated with leanness or obesity per se irrespective of the low grade of inflammation that generally accompanies the obese state. In large scale studies of human cohorts we have recently described changes in the gut microbiota that characterize obese individuals and individuals with type 2 diabetes, revealing characteristic changes in the diversity and functional competences of the gut microbiota. I will conclude the lecture by discussing possible functional consequences and perspectives of these findings.

*Lecturer:* Professor Karsten Kristiansen is professor of Molecular Biology and Head of Department of Biology at the University of Copenhagen. After graduation from the University of Copenhagen, he held postdoctoral positions at the Max-Planck-Institut für Molekulare Genetik in Berlin and at the Institut de Biologie Physico-Chimique, Fondation Edmond de Rothschild, in Paris. Before moving to his current position in Copenhagen in 2008, he served as Professor and Head of Department of Molecular Biology, later fused to form the Department of Biochemistry and Molecular Biology at the University of Southern Denmark. He is also professor at and senior advisor to BGI-Shenzhen. Karsten Kristiansen is internationally recognized for his work on adipose tissue development and function, and during recent years for work on genomics and metagenomics. Karsten Kristiansen is now heading a large international laboratory comprising 9 postdocs and 18 PhD students. Karsten Kristiansen has published 217 articles in refereed journals, including several publications in Science, Nature, Nature Biotechnology, Nature Structural Biology, Nature Genetics, and Cell. He has supervised 130 graduate students and served as opponent/external examiner in connection with 66 Ph.D. or doctoral dissertations at universities in Denmark, Norway, Sweden and France.

# GUT MICROBIOME AND HOST

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# QUANTITATIVE SINGLE-CELL ANALYSIS OF COLICIN IB PRODUCTION IN SALMONELLA ENTERICA SEROVAR TYPHIMURIUM

Stefanie Bollmann[1] and Bärbel Stecher[1]*

[1]*Max-von-Pettenkofer Institut, LMU München, GERMANY*
**Correspondence: stecher@mvp.uni-muenchen.de*

Colicins are protein toxins (40-80 kDa) produced by and toxic for members of the Enterobacteriaceae family (i.e. E. coli, Salmonella spp.). These toxins are known to be expressed only by a fraction of a (genetically identical) population (phenotypic heterogeneity). Individual bacteria producing colicin are lysed upon colicin release, which is referred to as division of labor and may eventually increase the overall fitness of a strain in competition with a colicin-sensitive enterobacterial community. Thus, colicin production by a subfraction of bacteria serves as a common good for the whole population.

Here we aim to show that colicin Ib (cib), produced by enteropathogenic Salmonella enterica serovar Typhimurium, is heterogeneously expressed, and that heterogeneous expression increases the fitness of the pathogen against competitors. In order to this hypothesis, we generated chromosomal and plasmid-based promotor gfp-reporter strains. We first validated the gfp-reporter strains by a “side by side” comparison with detection of the colicin Ib protein in single cells by immunofluorescence. Using the reporter strains we next aim to investigate environmental cues leading to stochastic expression in vitro and in vivo in the gut. Using a gnotobiotic mouse model we want to determine the benefit of heterogeneous colicin expression for S. Tm upon competition against other Enterobacteriaceae. Using the collective experimental data we will generate a mathematical model explaining the link between heterogeneity of colicin Ib expression in a S. Tm population to provide new fundamental insights into microbial interactions in the gut and their consequences for pathogen competition against the intrinsic microbiota.

# THE DICHOTOMIC ROLE OF LPS: INDUCTION OR PREVENTION OF INTESTINAL INFLAMMATION

Kerstin Gronbach[1, 12#], Isabell Flade[1, 12#], Otto Holst[2], Buko Lindner[3], Hans Joachim Ruscheweyh[4], Richard P. Darveau[5], Sarah Menz[1, 12], Patrick Adam[6], Bärbel Stecher[7, 12], Andreas Kulik[8], Daniel Huson[4], Ingo B. Autenrieth[1, 12] and Julia-Stefanie Frick[1, 12*]

*[1]Institute of Medical Microbiology and Hygiene, University of Tübingen, Germany, and Divisions of [2]Structural Biochemistry and [3]Immunochemistry, Research Center Borstel, Airway Research Center North (ARCN), Member of the German Center for Lung Research (DZL), Germany,and [4]Algorithms in Bioinformatics, ZBIT Center for Bioinformatics, University of Tübingen, Germany, and [5]Department of Periodontics, School of Dentistry, University of Washington, 1959 NE Pacific St., Seattle, WA 98195-7444, USA and [6]Institute of Pathology, University of Tübingen, Germany, and [7]Max von Pettenkofer-Institute of Hygiene and Medical Microbiology, Ludwig-Maximilians-University, Munich, and [8]Institute for Microbiology, University of Tübingen, Germany, and [12] German Centre for Infection Research (DZIF)*
*[#]equal contribution*

*Background:* The intestinal microbiota is crucial for shaping mucosal immunity and in inflammatory bowel diseases (IBD) the mucosal immune system interacts inappropriately with the intestinal microbiota. We showed for the first time that the composition of the intestinal microbiota results in different endotoxicity of the intestinal microbiota and that differential endotoxicity has a crucial impact on IBD development.
*Methods:* We colonized T-cell-transferred *Rag1*$^{-/-}$ mice with two different types of complex intestinal microbiota characterized by 16S rDNA amplicon sequencing.
*Results:* (i) Endo$^{lo}$-microbiota defined by low proportion of *Enterobacteriaceae* but high proportion of *Bacteroidetes* and exhibiting low entotoxicity resulted in mucosal immune homoeostasis, while (ii) the high endotoxic Endo$^{hi}$-microbiota consisting of high proportion of *Enterobacteriaceae* but low proportion of *Bacteroidetes* resulted in $T_H1/T_H17$-driven colitis. We hypothesized that colitogenicity of Endo$^{hi}$-microbiota might be related to the higher endotoxic activity of lipopolysaccharide (LPS) from *Enterobacteriaceae* compared to LPS from *Bacteroidetes*. Administration of *E. coli* JM83 (wildtype LPS) or *E. coli* JM83 *htrBhtrB*$_{PG}$ (mutated LPS; lower endotoxicity like LPS of *Bacteroidetes*) or LPS isolated from these strains to mice resulted in exacerbation or prevention of colitis, respectively.
*Conclusion:* Depending on the endotoxicity of their LPS commensals may favour immune stimulation resulting in immune homoeostasis or colitis. This principle might aid the design of novel probiotics that can be employed in the therapy of human inflammatory bowel disease.

# *METAPROTEOME ANALYSIS AND MOLECULAR GENETICS OF RAT INTESTINAL MICROBIOTA REVEALS SECTION AND LOCALIZATION RESOLVED SPECIES DISTRIBUTION AND ENZYMATIC FUNCTIONALITIES*

N. Jehmlich[1], S.B. Haange[1,2], A. Oberbach[2,3], N. Schlichting[2,3], F. Hugenholtz[4], H. Smidt[4], H. Till[2], J. Seifert[1,7], M. von Bergen[1,5,6]

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

[2] Department of Pediatric Surgery, University Hospital of Leipzig, Leipzig, Germany

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

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

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

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

[7] Institute of Animal Nutrition, University of Hohenheim, Stuttgart, Germany

Intestinal microbiota is a complex microbial community with by far greatest part consisting of bacterial species. The microbiota functionality is crucial for the metabolism of the host and the microbiota is strongly interlinked with the host`s immune system. Since many functions are redundant among different species and even phyla the actual activity is more relevant than the mere phylogenetic assessment. Hence we focus on functional analysis by metaproteomics.

We established a method to isolate and investigate the rat gut microbiota not only from feces and the gut content, but especially from the intestinal mucus. A higher resolution of the microbiota structure was achieved by separating the lower intestine into cecum, proximal colon and distal colon and then separating each segment into mucus and contents for analysis. Differences in phylogeny and enzymatic functionality were observed along the lower gut as well as between mucus and content environments. Using high performance LC-MS/MS in total 2802 non-redundant bacterial proteins were assigned. Furthermore, samples from the rat colon mucus and feces were sequenced by 16S rRNA gene pyrosequencing. The major phyla from which bacterial proteins came from were *Firmicutes*, *Bacteroidetes* and *Proteobacteria*. The most prevalent protein functionalities observed were those involved in translation, energy conversion as well as carbohydrate and amino acid metabolism. The results showed a clear difference between the bacterial communities found in the colon mucus and feces. Further investigation of the microbiota using protein based stable isotope probing (protein-SIP) to elucidate uptake and fluxes of nutrients will still be carried out.

Reference: Haange et al., J Proteome Res. 2012 Nov 2;11(11):5406-17

# *ALTERATION OF INFLAMMATORY RESPONSES THROUGH THE INTERACTION OF GUT MICROBIOTA AND BLOOD GROUP RELATED ANTIGENS*

Philipp Rausch* & Natalie Steck*, Guntram Grassl[#] & John F. Baines[#]

*, [#] equal contributions

Glycans on mucosal surfaces play an important role in host-microbe interactions. We previously demonstrated the influence of intestinal *B4galnt2* expression on the composition of the murine intestinal microbiota. To determine whether the glycans produced by this highly selected glycosyltransferase gene influence the host susceptibility to enteric pathogens, we investigated *Salmonella typhimurium* induced gastroenteritis in mice. Although intestinal *Salmonella* colonization was enhanced in *B4galnt2*-deficient mice, they developed significantly less pathology in the cecum compared to wild type mice one day post-infection. Cecal inflammation in *B4galnt2*-deficient animals was associated with significant differences in the microbial communities and decreased gut inflammation compared to wild type animals. Furthermore, a higher phylogenetic diversity of the intestinal microbiota, as present in *B4galnt2*-deficient mice before the induction of inflammation, was predictive for a reduced inflammatory response. In summary, our results support the hypothesis that variation in intestinal *B4galnt2* expression may alter susceptibility to diseases such as infectious gastroenteritis and might explain the evolutionary patterns which arose in wild mouse populations.

# DISCOVERY OF GUT MICROBIAL METABOLOME IN TYPE 2 DIABETES

A. Walker[1], M. Lucio[1], B. Pfitzner[2], M. Scheerer[3], S. Neschen[3], M. Hrabé de Angelis[3], A. Hartmann[2], P. Schmitt-Kopplin[1,4]

*Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany*

*[1] Department of Environmental Sciences, Research Unit Analytical BioGeoChemistry*

*[2] Department of Environmental Sciences Research, Unit Microbe-Plant Interactions, Research Group Molecular Microbial Ecology*

*[3] Institute of Experimental Genetics*

*[4] Technische Universität München, Chair of Analytical Food Chemistry, 85350 Freising - Weihenstephan, Germany*

Type 2 Diabetes (T2D) is one of major global epidemics that burden our health insurance through the enormous financial costs, emerging in T2DM etiopathology. Thus, it is a complex metabolic disorder whereby genetic predisposition but also environmental influences are discussed to be risk factors for developing T2D.

Previously, metabolomics studies were performed investigating the role of metabolism in T2D, analyzing plasma and urine samples in different species such as mice, rats but also humans. Here, we applied a metabolomics approach using solely fecal samples of leptin receptor deficient mouse model (*db/db*), which represents a good model for T2D. We used different mass spectrometry (MS) techniques to define, reveal and identify the fecal metabolome patterns in diabetic mice, concerning host but also the microbial role in T2DM. Based on this study, we were trying to find biomarkers of T2D by using alone fecal samples, which then could provide a non-invasive biological matrix to perform clinical studies. Finally, the application of MS based metabolomics highlighted different metabolite classes that were involved in T2D, including fatty acids, bile acids, arachidonic and linoleic acid metabolism. Furthermore, we wanted to emphasize the role of sulfur containing metabolites in fecal samples of diabetic mice.

# ANTIBIOTIC-INDUCED ALTERATION OF THE GUT MICROBIOTA PROTECTS TNF$^{DELTAARE}$ MICE FROM CROHN'S DISEASE-LIKE ILEITIS

Monika Weiher[1], Thomas Clavel[2], Marina Schmidt[1], Alesia Walker[3], Ines Martinez[4], Jens Walter[4], Philippe Schmitt-Kopplin[3], George Kollias[5] and Dirk Haller[1,2]

[1]*Chair of Nutrition and Immunology*

[2]*Junior Research Group Intestinal Microbiome, ZIEL – Research Centre for Nutrition and Food Science, Biofunctionality Unit, Technische Universität München, Gregor-Mendel-Str. 2, 85350 Freising, Germany*

[3]*Institute of Ecological Chemistry, Helmholtz Zentrum München, Neuherberg, Germany*

[4]*University of Nebraska, Dep. of Food Science and Technology, Food Industry Complex, Lincoln, USA*

[5]*Institute of Immunology, Biomedical Sciences Research Center Alexander Fleming, Vari 16672, Greece*

*Background:* Crohn's disease is associated with dysbiosis of the gut microbiota. To test the causative role of intestinal microorganisms in the development of genetically-driven chronic inflammation and to identify bacterial taxa associated with disease, we used the TNF$^{deltaARE}$ mouse model of Crohn´s disease-like ileitis.

*Methods & Results:* Antibiotics (vancomycin (0.25 g/l) and metronidazole (1 g/l) for 4 weeks) substantially reduced ileitis in TNF$^{deltaARE}$ mice (histopathology of 1.6 ± 1.2 *vs.* 4.9 ± 0.8 in the antibiotic *vs.* control group; n = 5-6; $p < 0.001$), but had no effect on tissue pathology in the caecum and proximal colon. Four weeks after antibiotic treatment, recurrence of ileitis was observed (score of 4.1 ± 1.5). High-throughput 16S rRNA gene sequencing showed marked changes in bacterial diversity and composition in the caecal lumen and the ileal mucosa. Total bacterial counts were not affected. In control mice, *Firmicutes* and *Bacteroidetes* were the dominant phyla (> 82 % total sequences). Antibiotics increased the occurrence of lactobacilli in the ileal mucosa (19.6 ± 27.6% *vs.* 76.3 ± 19.8%), whereas the proportion of *Bacteroides* was reduced (7.2 ± 10 % vs. 15.0 ± 6.0 %). In the caecal lumen *e.g.* the proportion of *Echerichia/Shigella* was increased (0.0. ± 0.0 *vs.* 12.3 ± 5.0), whereas *Alistipes* were decreased (5.5 ± 3.0 vs. 0.0 ± 0.0). Fluorescensce *in situ* hybridization (EUB-338 probe) showed changes in spatial distribution of ileal bacteria in TNF$^{deltaARE}$ mice, relative to the epithelial layer. Metabolite profiling of caecal content by FT/ICR-MS indicated changes in bile acid and carnitine metabolism. Mono-association and caecal microbiota transplant experiments are currently underway to address the causative role of gut bacteria in experimental ileitis.

*Conclusion:* Our findings indicate an essential role of intestinal bacteria in the development of Crohn's disease-like ileitis.

*Keywords:* Intestinal microbiota, ileitis, caecal microbiota transplant, TNF$^{deltaARE}$ mice.

# PROGRAM
# Saturday, June 29

# METABOLIC INDIVIDUALITY IN THE INTESTINAL WILDERNESS - A NOVEL SINGLE-CELL APPROACH TO STUDY IN VIVO FUNCTION OF INTESTINAL MICROBIOTA MEMBERS

Alexander Loy

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

The animal and human intestinal mucosa secretes an assortment of compounds to establish a physical barrier between the host tissue and intestinal contents, a separation that is vital for health. Some pathogenic microorganisms as well as members of the commensal intestinal microbiota have been shown to be able to break down these secreted compounds. Our understanding of host-compound degradation by the commensal microbiota has been limited to knowledge about simplified model systems because of the difficulty in studying the complex intestinal ecosystem in vivo. Stable isotope labeling combined with high-resolution imaging of single cells overcomes previous technical limitations and allows observing which microbial cells in the intestine utilize host-derived compounds. We added stable isotope-labeled threonine intravenously to mice and combined fluorescence in situ hybridization with high-resolution secondary-ion-mass-spectrometry imaging to characterize utilization of host proteins by individual bacterial cells. We show for the first time that two bacterial species, Bacteroides acidifaciens and Akkermansia muciniphila, are important host-protein foragers in vivo. Using gnotobiotic mice we show that microbiota composition determines the magnitude and pattern of foraging by these organisms, demonstrating that a complex microbiota is necessary in order for this niche to be fully exploited. These results underscore the importance of in vivo studies of intestinal microbiota and the approach presented in this study will be a powerful tool to address many other key questions in animal and human microbiome research.

# PROBIOTIC MECHANISMS / ANTIMICROBIAL COMPOUNDS

$09^{45}$ – $11^{15}$ Chair: C. Becker, Medical Department 1, Universitätsklinikum Erlangen

L. Courth, Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart + University of Tübingen
*Regulation of α-defensin expression by inflammatory processes and bacteria*

M. Meijerink, Host-Microbe Interactomics, Animal Sciences, Wageningen University
*The effect of non-replicating probiotic strains in a Salmonella infection mouse model*

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*Paneth cells, Wnt ligands, and intestinal Crohn's Disease*

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*Intestinal REG3γ plays a protective role against intestinal infection with both Gram-positive and Gram-negative pathogens*

D. Zhurina, Institute of Microbiology and Biotechnology, University of Ulm
*Exploring Bifidobacterium bifidum S17 for potential players in host-microbe interactions by genomic and proteomic approaches*

# *REGULATION OF A-DEFENSIN EXPRESSION BY INFLAMMATORY PROCESSES AND BACTERIA*

L. Courth, M.J. Ostaff, E. F. Stange, J. Wehkamp

*Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology and University of Tübingen, 70376 Stuttgart, Germany*

*Background:* The Wnt-Pathway is important for stem cell proliferation as well as Paneth cell differentiation in the small intestine. Paneth cells are primarily located at the bottom of small intestinal crypts and generate antimicrobial peptides. Their most abundant innate defence molecules, the α-defensins HD5 and HD6, are important players in regulating gut microbiota. Their expression is partly controlled by the Wnt-Pathway and is decreased in ileal Crohn's Disease (CD). In patients with ileal CD we found impairments of Wnt pathway components. Additionally it is well accepted that intestinal microbes can influence CD development. In our studies we want to clarify the role of bacteria in Wnt mediated defensin expression. We also analyze the impact of inflammatory processes in this setting aiming to improve our understanding of microbial and inflammatory contributions in CD pathogenesis.

*Methods:* Modulation of defensin expression is investigated using reporter gene assays in Hek293-cells under different conditions. We treated transfected cells with stimulated peripheral blood mononuclear cell (PBMC) supernatant or heat-killed bacteria. Future experiments will include stimulation of fresh intestinal biopsies followed by mRNA analysis.

*Results:* Stimulated PBMC supernatant can increase Wnt activity (Topflash) and to a smaller extend Paneth cell α-defensin expression. In first experiments bacteria show no influence in cell lines lacking TLR2 and NOD2.

*Conclusions:* Inflammatory processes can impact Wnt activity and HD5/HD6 expression. Additional research on regulatory cytokines and downstream factors could elucidate the mechanism. Different cell lines or biopsies will be used to study the influence of microbes in this context for gaining new insights into the regulation of the gut-microbe homeostasis.

# THE EFFECT OF NON-REPLICATING PROBIOTIC STRAINS IN A SALMONELLA INFECTION MOUSE MODEL

M. Meijerink[1], V. Petit[2], A. Taverne-Thiele[1], N. Winaris[1], J.M. Wells[1], A. Mercenier[1,2]

[1]*Host-Microbe Interactomics, Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, the Netherlands.*
[2]*Nestlé Research Centre, P.O. Box 44, CH1006, Lausanne, Switzerland.*
*marjolein.meijerink@wur.nl*

Diarrheal infections caused by bacterial enteric pathogens including Salmonella, are one of the major causes of childhood morbidity and mortality in developing countries. Probiotics have been shown to influence both innate and adaptive immunity through direct contact with epithelial and immune cells, or by their ability to modify the gut microbiota. Specific probiotic strains have been shown to reduce or prevent Salmonella infection. While probiotics have limited shelf life in liquid products, especially in non-refrigerated conditions, effective non-replicating probiotic strains (NRMs) may offer advantages over their live counterparts especially in terms of processing and storage. In this study we investigated the ability of L. johnsonii La1 NCC533 NRM and B. longum NCC2705 NRM to protect C57Bl/6 mice against infection with Salmonella enteritidis. Both NRMs were previously shown to be superior to their live counterparts in upregulating the synthesis of antimicrobial peptides β-defensin 1 and 2 in T84 cells. Continuous administration of La1 NRM led to increased amounts of innate immunity markers in the ileum, including myeloperoxidase iNOS and Reg3β) as well as increased secretion of IgA. These changes were associated with decreased translocation of Salmonella in the ileum, spleen, liver and mesenteric lymph nodes, as well as less systemic inflammation compared to the Salmonella-infected groups of control mice and B. longum NRM treated mice.

# *FLAGELLA AND TCPC AS PROBIOTIC FACTORS OF E. COLI NISSLE 1917*

S. Menz[1], K. Gronbach[1], A. Bender[1], P. Adam[2], A. Wieser[4], U. Dobrindt[3], S. Schubert[4], T.A. Ölschläger[3], I.B. Autenrieth[1] and J.S. Frick[1]

[1]*Institute of Medical Microbiology and Hygiene, University of Tuebingen, Germany*
[2]*Institute of Pathology University of Tuebingen, Germany*
[3]*Institute of Molecular Infection Biology, University of Wuerzburg, Germany*
[4]*Max von Pettenkofer Institute, University of Munich, Germany*

The probiotic E. coli Nissle strain 1917 (EcN) is as effective as mesalazine in maintenance of remission in ulcerative colitis and shortens the duration of diarrhea in young children. To analyse the probiotic effects of EcN in ulcerative colitis the DSS mouse model of acute colitis was used. In C57BL/6 x TLR5 littermates challenged with 3.5% DSS E. coli Nissle shows protective effects but not in groups which were fed with EcNΔfliC, EcNΔtcpC or EcNΔfliCΔtcpC. So these two factors, the flagella and the protein TcpC seem to be essential in the protective effect of EcN. To get detailed information about the interacting cell type of these two factors bone marrow chimeric mice were used. Therefore irradiated TLR5-/- mice transplanted with wildtyp bone marrow and irradiated WT mice transplanted with TLR5-/- bone marrow were fed with EcN, EcNΔfliC or EcNΔtcpC and 3.5% DSS. The experiments show that dendritic cells play a major role in the protective effect of EcN via the flagella and the protein TcpC.

# *PANETH CELLS, WNT LIGANDS, AND INTESTINAL CROHN'S DISEASE*

M.J. Ostaff, Z. Teltschik, L. Courth, S. Stebe, E. F. Stange, J. Wehkamp

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

*Background:* To control a large amount of microbes intestinal epithelia critically depend on antimicrobial peptides (AMPs). A particularly specialized AMP source is the Paneth cell in the small intestine. The cells most prominent products, the α-defensins HD5 and HD6 are decreased in patients with small intestinal Crohn's Disease (CD). This is linked to different Wnt pathway defects, amongst others a coding variant in LRP6, a Wnt co-receptor, which is associated with an early onset phenotype. We now studied the mucosal expression of different ligands for this receptor, including Wnts, R-Spondins and DKK proteins in patients.
*Methods:* RNA from 114 biopsies (CD patients and controls) was isolated and transcribed into cDNA. Analysed products were quantified by real-time PCR and normalized to β-actin. Patients were grouped according to inflammation and disease location. Expression differences were subject to student t- or Mann-Whitney-tests (depending on distribution) correlation with IL8 or HD6 were tested via Spearman rank analysis
*Results:* mRNA expression of some Wnt pathway influencing ligands is significantly reduced in patients, while others are unchanged or increased in certain subgroups. R-Spondin 2, e.g., exhibits diminished mRNA independent of current inflammation.
*Conclusion and future experiments:* Different Wnt signalling impairments are seen in small intestinal CD. These malfunctions affect the transcription factor and receptor level and, as now reported, also include altered mRNA expression of important ligands. A genetic pathway approach including ligands in addition to classical intracellular signalling compounds might elucidate further causative Wnt variants in small intestinal CD.

*This work was funded by the DFG (SFB685) and the Robert-Bosch-Foundation*

# *INTESTINAL REG3Γ PLAYS A PROTECTIVE ROLE AGAINST INTESTINAL INFECTION WITH BOTH GRAM-POSITIVE AND GRAM-NEGATIVE PATHOGENS*

Linda MP Loonen, Ellen H Stolte, Marcel TJ Jaklofsky, Marjolein Meijerink, Jan Dekker, Peter van Baarlen and Jerry M. Wells

*Host-Microbe Interactomics Group, ASG, Wageningen University, NL*

REG3γ has been proposed to have a protective role against infection due to its bactericidal effect on Gram-positive bacteria, but evidence from *in vivo* studies is lacking. Therefore we generated a REG3γ -/- mouse, to determine its role in intestinal homeostasis and protection against experimental infection. REG3γ -/- mice were phenotyped using histological methods and a range of innate and immune markers. To investigate the antimicrobial role of REG3γ we experimentally infected mice with Gram-positive *Listeria monocytogenes* and Gram negative *Salmonella enteritidis* and measured translocated bacteria, mucosal and systemic markers of infection. REG3γ -/- mice display altered ileal mucus distribution and increased bacterial contact with the epithelium, concomitant with increased inflammatory status of the ileal mucosa and increased expression of IL-22, myeloperoxidase (MPO) and serum chemokines. In response to infection, REG3γ -/- mice showed transcriptome changes and elevated levels of mucosal MPO in the ileum, but no increased bacterial translocation to the organs. REG3γ was equally distributed throughout the mucus of wild type (wt) mice and its absence results in an altered distribution of the ileal mucus. REG3γ deficiency resulted in increased bacterial contact with the epithelium and heightened inflammatory responses in the ileal mucosa. We showed that REG3γ binds pathogens and thus may contribute to mucus barrier function by ensnaring bacteria. Compared to wt mice, REG3γ -/- mice infected with *S. enteritidis* and *L. monocytogenes* show an increase of mucosal inflammatory markers indicating protective, anti-microbial roles of REG3γ in defence against both Gram-positive and -negative bacteria.

# *EXPLORING BIFIDOBACTERIUM BIFIDUM S17 FOR POTENTIAL PLAYERS IN HOST-MICROBE INTERACTIONS BY GENOMIC AND PROTEOMIC APPROACHES*

Daria Zhurina[1], Julia Schützner[1], Paul Walter[2], Christian Riedel[1]

[1] *Institute of Microbiology and Biotechnology, Ulm Germany*

[2] *Central Electron Microscopy Facility, University of Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany*

*Bifidobacterium bifidum* S17 is a promising candidate able to tightly adhere to intestinal epithelial cells (IEC) and showing potent anti-inflammatory activity in vitro and effect in several murine models of chronic intestinal inflammation. To further elucidate the mechanisms contributing to host-colonization and inhibition of inflammation by *B. bifidum* S17, we have sequenced and annotated the genome of this strain and set up 2D total proteome maps for bacteria grown *in vitro*. Several surface proteins, including the *B. bifidum*-specific adhesin BopA, were identified in the genome and shown to be expressed *in vitro*, with higher expression during exponential growth phase. This correlated with increased adhesion of *B. bifidum* S17 to Caco-2 cells in exponential phase. *B. bifidum* S17 possesses four putative pili-encoding gene clusters, which are expressed *in vitro* as well. Moreover, genes that potentially encode the biosynthetic pathway for extracellular polysaccharides were identified and scanning and transmission electron microscopy images provided evidence for the presence acidic polysaccharides on the bacterial cell surface. Furthermore, the effect of *B. bifidum* S17 on various subsets of $CD4^+$ T-cells and the cytokine milieu in the intestinal mucosa was investigated in two models of colitis (DSS-induced colitis and T-cell transfer model in $Rag^{-/-}$ mice). The results indicate that reduction of clinical symptoms of colitis upon pre-treatment of mice with *B. bifidum* S17 is associated with a reduction in tissue levels of pro-inflammatory cytokines (TNF-a, IL-6, IFN-g), reduced numbers of total CD4+ T cells and increased frequencies of $FoxP3^+$ regulatory T cells.

# INTERACTIONS OF SEGMENTED FILAMENTOUS BACTERIUM WITH THE HOST IMMUNE SYSTEM: LESSONS FROM GNOTOBIOTIC MICE

Valérie Gaboriau-Routhiau, Emelyne Lécuyer and Nadine Cerf-Bensussan

*INSERM U989, Université Paris Descartes-Sorbonne Centre and Institut IMAGINE, Paris, France*

In mammals, mutualistic interactions with the microbiota are maintained by a complex network of innate and adaptive immune mechanisms, which form the gut-associated lymhoid system (GALT). GALT development is initiated before birth but depends on post-natal interactions with the commensal microbiota for full maturation. How individual members of the microbiota can influence GALT maturation is an outstanding question with wide implications in physiology and immune pathology. Using gnotobiotic C3H/HeNmice, we have shown that the post-natal expansion of mucosal helper T cells is driven by a restricted number of host-specific and unculturable species, and more specifically by an unusual symbiont called Segmented Filamentous Bacterium (SFB). While colonisation by a complete human microbiota or by the culturable fraction of the mouse microbiota drove minimal and mainly regulatory mucosal T cell responses, monocolonisation by SFB largely recapitulated the coordinated expansion of pro-inflammatory and regulatory mucosal T cell responses induced by a complete mouse microbiota. These results extend and complete other studies highlighting the remarkable immunostimulatory property of SFB. That SFB is not yet culturable is major drawback to elucidate the pathway(s) activated by SFB. Yet, one hallmark of SFB is a host-specific adherence to the ileal mucosa, which may be necessary either to trigger innate signals fostering adaptive responses and/or to facilitate uptake in ileal Peyer's paches, a major site of induction of intestinal adaptive immune responses. The two hypotheses have been tested in gnotobiotic mice lacking Peyer’s patches and/or isolated follicles and in mice lacking a functional TLR pathway. We will discuss how results provide new insight into the immunostimulatory properties of SFB and unexpected results on the pathways governing IgA responses to the microbiota.

# MECHANISMS OF INFLAMMATION

$14^{00}$ – $15^{30}$ Chair: M. Heimesaat, Microbiology, Charité Berlin

C. Günther, Medical Clinic 1, Friedrich-Alexander-University Erlangen
*Caspase 8 in intestinal epithelial cells regulates immune homeostasis in the gut*

T. Kruis, Campus Benjamin Franklin, Charité Universitätsmedizin Berlin
*The interaction of macrophages and the microbiota –*
*a modulator of the intestinal barrier?*

C. Kunst, Department of Internal Medicine, University of Regensburg
*Physiologic TLR9-CpG-DNA interaction is essential for the homeostasis of the intestinal immune system*

S. Lipinski, Institute of Clinical Molecular Biology, CAU Kiel
*RNAi screening identifies FRMPD2: a scaffolding protein controlling NOD2-mediated immune responses*

B. Sovran, Host-Microbe Interactomics Group, Wageningen University
*Homeostatic mechanisms preventing ileitis in mice with absent or deficient Muc2 production*

A. Wittmann, Institute of Medical Microbiology and Hygiene, University of Tübingen
*TLR2- and TLR4-Mediated Amelioration of DSS-Colitis by Induction of CD103-Expressing Dendritic Cells*

# *CASPASE 8 IN INTESTINAL EPITHELIAL CELLS REGULATES IMMUNE HOMEOSTASIS IN THE GUT*

Claudia Günther [1], Stefan Wirtz[1], Markus F. Neurath [1] and Christoph Becker [1]

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

The intestinal epithelium is an important barrier of our body against the external environment characterized by the bacterial flora and food antigens present in the gut lumen. Excessive infiltration of bacteria through this cell layer is believed to result in deregulated intestinal immune response and to the pathogenesis of inflammatory bowel disease. However the regulation of epithelial cell death and its role in intestinal homeostasis remains poorly understood.

We demonstrate that deletion of Caspase8 in intestinal epithelial cells results in spontaneous inflammatory lesions in the terminal ileum and that conditional knock-out mice for Caspase8 are highly susceptible to experimental colitis. Caspase8 deficiency results in an impaired expression of antimicrobial peptides. This leads to an attachment of bacteria to the epithelial cell surface which causes a destroyed barrier function and consequently the translocation of bacteria into the lamina propria. Treating Caspase8$^{\Delta IEC}$ mice with antibiotics to decrease the bacterial concentration in the gut partially rescued the severe course of the colitis.

Taken together, our data demonstrate for the first time a critical role of Caspase8 in regulating the epithelial integrity and intestinal homeostasis, and have important implications for the understanding the mechanism controlling the pathogenesis of human inflammatory bowel disease.

# THE INTERACTION OF MACROPHAGES AND THE MICROBIOTA – A MODULATOR OF THE INTESTINAL BARRIER?

Tassilo Kruis[1], Donata Lissner[1], Michael Schumann[1], Arvind Batra[1], Lea Isabel Kredel[1], Anja Andrea Kühl[1], Laura Hanske[2], Michael Blaut[2], Britta Siegmund[1]

*[1]Charité Universitätsmedizin Berlin, Campus Benjamin Franklin, Medizinische Klinik I, Berlin, Germany*
*[2]Deutsches Institut für Ernährungsforschung, Gastrointestinale Mikrobiologie, Potsdam-Rehbrücke, Germany*

*Introduction:* In Crohn's disease a dysbiosis of the gut microbiome and increased bacterial translocation occurs. Both effects might be involved in the alterations of the intestinal immune system seen in affected persons, as for example the increase in pro-inflammatory M1 macrophages in the lamina propria. In this study we aimed to understand the effect of macrophage polarization on the epithelial barrier function and the impact of selected gut bacteria altered in Crohn's disease on macrophage polarization and activity.

*Methods:* Human CD14+ cells were isolated from peripheral blood by magnetic cell sorting. Cells were polarized in vitro into M1 or M2 macrophages by culture in GM-CSF or M-CSF, respectively. Polarization was verified assessing LPS induced cytokine production and cell surface marker expression by flow cytometry. Polarized macrophages were placed under epithelial cells and cultures were stimulated with LPS. Changes in the epithelial layer were investigated via confocal microscopy, local alterations in the tight junction protein composition by staining for ZO-1. Human CD14+ cells were stimulated with either inactivated cells or supernatants of selected bacterial strains (F. prausnitzii, B. adolescentis, E. coli, B. fragilis, R. gnavus). Cell viability was assessed by flow cytometry using an Annexin V assay.

*Results:* Biotin exposure of the epithelial layer revealed inhomogeneous paracellular barrier defects in LPS-stimulated co-cultures with M1 macrophages. Moreover, the ZO-1 expression was profoundly suppressed in the LPS-stimulated M1 group indicating altered tight junctions. No such effects were seen in epithelial cells cultured with M2 macrophages. Whereas high concentrations of inactivated cells of various bacteria affected cell viability negatively, in particular supernatants of cultures from F. prausnitzii and B. adolescentis favoured the survival of CD14+ monocytes in vitro.

*Discussion:* The data reveal that the presence of M1 macrophages results in a destruction of the epithelial layer not occurring with M2 macrophages, thus supporting the concept, that the local macrophages are eminent in the maintenance of epithelial integrity. Since supernatants and inactivated cells of the different bacterial strains tested exerted unequal effects on the cell viability of monocytes, changes in the gut microbiota might as well drive altered immune cell accumulation at mucosal sites.

# *PHYSIOLOGIC TLR9-CPG-DNA INTERACTION IS ESSENTIAL FOR THE HOMEOSTASIS OF THE INTESTINAL IMMUNE SYSTEM*

Claudia Kunst, Nadja Dunger, Kristina Doser, Elisabeth Lippert, Sebastian Siller, Matthias Edinger, Martina Müller, Werner Falk, Florian Obermeier

*Department of Internal Medicine I, Department of Internal Medicine III, University of Regensburg, Germany*

*Background:* Cytosine-guanosine dinucleotide (CpG) sequence motifs are the immunostimulatory components of bacterial DNA and potent activators of the innate immunity via Toll-like receptor 9 (TLR9) ligation. Administration of exogenous CpG oligodeoxynucleotides before the onset of experimental colitis prevents intestinal inflammation. We investigated whether physiologic CpG-TLR9 interactions are critical for the homeostasis of the intestinal immune system.

*Methods:* Mesenteric lymph node cells (MLC) and lamina propria mononuclear cells (LPMC) from BALB/c wildtype (wt) or TLR9-/- mice were assessed by flow cytometry and proteome profiling. Cytokine secretion was determined and nuclear extracts were analyzed for NF-κB and CREB activity. To assess the colitogenic potential of intestinal T cells CD4+CD62L+ cells from LPMC of wt and TLR9-/- donor mice were injected i.p. in recipient C.B.-17 SCID mice.

*Results:* TLR9 deficiency was accompanied by slight changes in cellular composition and phosphorylation of signalling proteins of MLC and LPMC. NF-κB activity in cells from TLR9-/- mice was enhanced, while CREB activity was reduced compared to wt. LPMC from TLR9-/- mice displayed an increased pro-inflammatory phenotype and CD4+ lamina propria T cells showed reduced features associated with regulatory capacities compared to wt. Lamina propria CD4+CD62L+ T cells from TLR9-/- mice induced severe colitis, whereas wt lamina propria CD4+CD62L+ T cells displayed an attenuated phenotype.

*Conclusion:* Lack of physiologic CpG-TLR9 interactions impairs the function of the intestinal immune system. Even under non-inflamed conditions TLR9-/- LPMC display an increased proinflammatory phenotype. Thus, physiologic CpG/TLR9 interaction is essential for homeostasis of the intestinal immune system as it is required for the induction of counter-regulating anti-inflammatory mechanisms.

# *RNAI SCREENING IDENTIFIES FRMPD2: A SCAFFOLDING PROTEIN CONTROLLING NOD2-MEDIATED IMMUNE RESPONSES*

Simone Lipinski, Nils Grabe, Gunnar Jacobs, Susanne Billmann-Born, Stefan Schreiber, Philip Rosenstiel

*Institute of Clinical Molecular Biology, Christian-Albrechts-University, Kiel, Germany*

The cytosolic pattern recognition receptor NOD2 is the prototypical member of the NOD-like receptor (NLR) family and a key player in host defense. NOD2 detects bacteria-derived muramyl dipeptide (MDP) and activates pro-inflammatory signaling cascades like the NF-□B pathway. In the present study, we used a systematic siRNA screen comprising 7784 genes (druggable genome), to uncover relevant modulators of NOD2-dependent NF-□B signaling. We identified a set of 20 positive NF-□B regulators including the known pathway members RIPK2, RELA and BIRC4 (XIAP) and the hitherto unknown activator FRMPD2 (FERM and PDZ domain containing 2). We found that FRMPD2 directs NOD2-mediated MDP recognition to the basolateral membrane of polarized intestinal epithelial cells (IEC) by physically interacting with leucine-rich repeats (LRR) of NOD2. In addition, FRMPD2 facilitates membrane recruitment of RIPK2, the adaptor kinase of NOD2, resulting in the formation of a functional multiprotein NOD2 signalosome. We show that genetic truncation of the NOD2 LRR domain (L1007insC), which is associated with Crohn's disease, impairs the interaction with FRMPD2, and that intestinal inflammation leads to down-regulation of FRMPD2. These results for the first time suggest a structural mechanism where FRMPD2 acts as a membrane scaffolding complex providing a spatial control mechanism for NOD2-mediated immune responses.

# HOMEOSTATIC MECHANISMS PREVENTING ILEITIS IN MICE WITH ABSENT OR DEFICIENT MUC2 PRODUCTION

Bruno Sovran[1,4], Linda Loonen[1,4], Ellen Kranenbarg[1], Clara Belzer[3], Ingrid Renes[6], Peng Lu[6], Mark Boekschoten[2], Peter van Baarlen[1], Michiel Kleerebezem[1,4], Paul de Vos[4,5], Jerry M. Wells[1,4] and Jan Dekker[4]

[1]*Host-Microbe Interactomics Group, Animal Sciences Department, Wageningen University and Research Center, Wageningen, The Netherlands;*
[2]*Division of Human Nutrition, Wageningen University and Research Center, Wageningen, The Netherlands;*
[3]*Laboratory of Microbiology, Wageningen University and Research Center, The Netherlands;*
[4]*Top Institute Food and Nutrition, Wageningen, The Netherlands;*
[5]*University Medical Center of Groningen, Groningen, The Netherlands;*
[6]*Department of Pediatrics, Division of Neonatology, Erasmus MC-Sophia, Rotterdam, The Netherlands*
*Corresponding author: bruno.sovran@wur.nl*

The gastrointestinal tract is lined with a single layer of epithelial cells, which works in concert with secreted mucus and antimicrobial factors to avoid excessive contact with intestinal bacteria and other luminal compounds that have the potential to induce inflammatory responses. Mucus-structure, and expression of its main constituents the Muc-glycoproteins, differs along the intestinal tract with a single and thin layer of loosely-attached mucus in the small intestine, and thick two-layered mucus structures with different physical properties in stomach and colon. Muc2 mucin, a glycoprotein that is assembled into enormous net-like polymers, is the main component of the mucus layer in small intestine and colon. Muc2 knockout (Muc2-/-) mice spontaneously develop colitis and the biological responses that play a role in development of colitis have been recently described. The aim of this study was to investigate the role of mucin barrier in the small intestine by comparing the age-dependent biological responses in mice which have a reduction or absence of Muc2. Wild-type (Muc2+/+), heterozygote (Muc2+/-) and knockout (Muc2-/-) mice were reared under SPF conditions and sacrificed at 2, 4 and 8 weeks of age. Total RNA from ileum and colon was purified, and analysed by Affymetrix GeneChips according to manufacturer's protocols, for full genome transcriptome analysis. For morphological studies segments of ileum and proximal colon were fixed in 4% PFA and stained with Haematoxylin/Eosin, PAS/Alcian blue, or Muc2-specific immunostaining.

Muc2-deficient mice develop colitis in the proximal colon after 4 weeks, which worsens by week 8. This was evident from the progressive thickening of the mucosa and morphological changes in the epithelium. In contrast, no overt morphological changes were observed in the ileum. Muc2 expression increased with age in wild type mice, but was not detected in KO mice. In Muc2-/+ mice, Muc2 expression was approximately halved. Innate immune responses, e.g., Reg3β and Reg3γ, were elevated in the ileum of both Muc2-/- and Muc2-/+ at week 4 and week 8. By week 4 and 8, adaptive immune responses and inflammatory signalling pathways, including NF-κB activation, were down-regulated in KO vs. WT mice. Lipid metabolism and fatty acid biosynthesis pathways were down-regulated in the ileum at week 4 and week 8 (KO vs. WT) in suggesting altered digestive functions.

In the colon Muc2 deficiency leads to inflammation, associated with tissue damage. In contrast there are no apparent histological changes in the ileum despite increased cytokine and chemokine expression, and decreased expression of inflammatory pathway genes. In the small intestine the lower numbers of bacteria and/or intrinsic homeostatic mechanisms appear to prevent tissue damage in the absence of Muc2. Muc2+/- heterozygote mice might be a good model to study the role of mucus in intestinal health and is relevant to IBD where mucus levels are reduced during active disease and remission.

# *TLR2- AND TLR4-MEDIATED AMELIORATION OF DSS-COLITIS BY INDUCTION OF CD103-EXPRESSING DENDRITIC CELLS*

Alexandra Wittmann[1], Richard P. Darveau[2], Peter A. Bron[3], Iris I. van Swam[3], Michiel Kleerebezem[3], Ingo B. Autenrieth[1] and Julia-Stefanie Frick[1]

[1] *Interfacultary Institute for Microbiology and Infection Medicine Tübingen*

[2] *Department of Periodontics, University of Washington*

[3] *NIZO, the Netherlands*

In order to investigate the function of dendritic cells and their TLR2 and TLR4 expression during acute-phase inflammation the dextran sulfate sodium salt (DSS) model was used. BL/6 mice were DSS only administered or DSS administered and bacteria fed. Employed strains were *E. coli* JM83 wild-type, *E. coli* JM83 Δ*htrB htrB*$_{Pg}$ mutant, featuring an altered Lipid A structure resulting in a weaker TLR4 signal induction, *L. plantarum* WCSF1 wild-type, as well as *L. plantarum* Δ*dltX-D* mutant, lacking D-alanylation at its lipoteichoic acids leading to a decreased induction of TLR2 cascade. Feeding of wild-type bacteria, capable of a potent TLR signaling induction, during DSS administration was able to prevent weight loss, disease activity index, and colons shrinking, whereas feeding of mutant bacteria was not able to prevent from disease. TLR2 and TLR4 signaling mediated protection owing to bacteria feeding was independent of activation and maturation state of lamina propria dendritic cells (LPDCs). However, significantly increased levels of CD103-positive DC are associated with prevention from inflammation. In order to elucidate which cells are responsible for TLR-mediated protection Bone marrow-chimeric mice were generated. TLR2 or TLR4 deficiency of intestinal epithelial cells prevented from weight loss, disease activity index, and colons shrinking. In contrast, mice lacking TLR2 or TLR4 expression on hematopoietic cells exhibited a comparable inflammation as DSS treated wild-type mice did.

# REGULATION OF CHRONIC INFLAMMATORY DISEASES BY THE MICROBIOTA

Nicola Harris

*Global Health Institute, Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland*

Over recent decades there have been clear lifestyle changes in affluent countries that have been associated with the increased prevalence of certain inflammatory diseases (1). Host-microbial interactions are a well known to regulate intestinal diseases and a number of epidemiological studies indicate that the exact composition of the intestinal microbiota of children can be linked to the development, or lack, of atopy (2, 3). Thus microbial colonization following birth may be an important determinant in the development of respiratory diseases such as asthma. We have addressed this hypothesis using germ-free mice, which lack exposure to environmental microbes including intestinal bacteria. Germ-free mice exhibited an increased susceptibility to allergic airway inflammation indicating that microbial colonization plays a protective role against disease development (4), an observation that is supported by the subsequent findings of Olszak and colleagues (5) and Hill and colleagues (6). These findings have important therapeutic implications and indicate that the delivery of specific microbes could act in a probiotic fashion to reduce the development of allergic asthma.

Alternatively, components that act as prebiotics could be included in the diet and provide a substrate for the outgrowth of beneficial intestinal bacteria. Within the context of fiber, a study by De Fillippo and colleagues (7) compared child cohorts from Burkina Faso and Europe where dietary fiber intake is either high or low, respectively. The children with high fiber diets had a significant enrichment of the Bacteroidetes phyla while Firmicutes were reduced; similarly, increased short-chain fatty acids (SCFA) were found in the Burkina Faso cohort, thought to be a consequence of Bacteroidetes-mediated fermentation of the fiber. This diet-SCFA link could have relevance when placed within the context of the increased level of allergy in Europe as compared to rural Africa. Indeed SCFA's such as butyrate and acetate have been shown to exhibit anti-inflammatory actions within the context of inflammatory bowel disease, and more recently in mouse models of arthritis (8, 9). We recently generated data demonstrating a protective role for high fiber diet, or administration of purified SCFA's, in experimental models of allergic asthma.

In summary, we provide evidence supporting the assertion that microbes influence the development of allergic asthma. We additionally show that dietary intervention, or administration of bacterial products, represent-promising strategies for the development of novel therapeutics.

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# PROGRAM
# Sunday,
# June 30

# H. HEPATICUS COLITIS MODELS AS AN EXAMPLE FOR INTERPLAY OF BACTERIAL COLITOGENIC FACTORS, MICROBIOTA AND THE HOST DEFENCE

Christine Josenhans

*Institute for Medical Microbiology and Hospital Epidemiology, Hannover Medical School, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany*

*Helicobacter hepaticus* is a model organism and intestinal pathobiont, a bacterium that can cause intestinal inflammatory disease in a susceptible mouse host. The bacteria persistently colonize the intestinal tract, in particular the caecum of mice, and still today, is a very prevalent bacterium in many mouse houses world wide, where it may represent an important factor of variation in immunological parameters between research labs. In many mouse strains, the bacteria do not cause overt disease but colonize asymptomatically. In some immunocompromised mice such as IL-10$^{-/-}$, RAG$^{-/-}$ or TLR-negative animals, or in T-celll transfer models, the bacteria can lead to quite extensive and chronic intestinal disease which resembles human inflammatory bowel disease (IBD)(Nell et al., 2010). Since the whole genome sequence of *H. hepaticus* has been clarified about 10 years ago (Suerbaum et al., 2003), researchers have been investigating the causal contribution of *H. hepaticus* infection to intestinal disease and coinfections, and specific bacterial factors to influence the interplay of host immune system, microbiota, pathobionts and chronic inflammatory disease have been studied.

We have asked different questions concerning *H. hepaticus* bacterial factors, their effect on infection outcome and the resident microbiota over the last 10 years. Three prominent factors that we have been investigating are bacterial lipopolysaccharide (Sterzenbach et al., 2007), flagellar motility (Sterzenbach et al., 2008), and a large pathogenicity island (HHGI1) expressing a type 6 secretion system (Bartonickova et al., 2013). We suggest that the immunoevasive properties or immunomodulating function of several of these bacterial factors, in combination with dysbalanced and chronic immune reactions to other *H. hepaticus* factors and to the resident microbiota, contribute to the chronic inflammation caused by the bacteria in susceptible hosts. In this overview, these current concepts, some new approaches, and the potential impact on human disease will be discussed.

References:

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# MECHANISMS OF INFECTIONS

$10^{00}$ – $11^{30}$ Chair: G. Loh, Gastrointestinal Microbiology, DIFE

S. Brugiroux, Max von Pettenkofer Institut, LMU Munich
*Generation of an Oligo-Mouse Microbiota to study colonization resistance against enteropathogens*

M.M. Heimesaat, Inst. for Microbiology + Hygiene, Charité – University Med. Berlin
*Campylobacter jejuni induces acute non-self-limiting enterocolitis in gnotobiotic IL-10-/- mice via Toll-like-receptor-2 and -4 signaling*

R. Lakra, Medicine Clinic I, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU)
*The C.rodentium T6SS (Type 6 Secretion System) is Important for the Effective Gut Colonization*

L. Maier, ETH Zurich, Institute of Microbiology, Switzerland
*Multiple factors influence the occurrence of population bottle neck(s) during Salmonella Typhimurium colitis*

S. Ocvirk, Chair of Nutrition and Immunology, Technische Universität München
*Enterococcus faecalis polysaccharide antigen and lipoproteins mediate virulence in chronic inflammation and in infection models*

N. Steck, Inst. for Experimental Med., University of Kiel / Research Center Borstel
*Complex role of proteases in Salmonella-induced intestinal fibrosis*

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

Sandrine Brugiroux[1], Yvonne Lötscher[2], Manuel Diehl[1], David Berry[3], Alexander Loy[3] and Bärbel Stecher[1]*

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

[2]*Institute of Microbiology, ETH Zurich. current address: Inst. f. Agrarwissenschaften ETH Zurich, SWITZERLAND*

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

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

The intestinal microbiota diversity in humans and mice is high ranging between an estimated 200 to 1,000 different species. The majority being strictly anaerobic and has not been cultured yet. The high complexity of the intestinal ecosystem precludes investigating the contribution of individual strains to host-bacterial interactions as well as studying their individual role within the intestinal ecosystem. To this end, gnotobiotic mice harboring a microbiota of known composition have proven highly useful. The majority of current gnotobiotic mouse models are based on using a selection of human gut isolates. The "Altered Schaedler Flora", a collection of 8 murine isolates which had been widely used in the past, is currently not available in public strain collections. For this reason we isolated a collection of bacterial strains, the Oligo-Mouse Microbiota (Oligo-MM), abundant in the mouse intestinal tract. The Oligo-MM so far comprises 15 isolates derived from 6 eubacterial phyla (*Firmicutes*, *Bacteroidetes*, *Deferribacteres*, *Actinobacteria*, *Verrucomicrobia* and *Proteobacteria*). We established a protocol using cryo-preserved mixtures which can be used to reproducibly colonize germfree and gnotobiotic mice with the Oligo-MM. Further, we established molecular tools to detect these strains in this mouse model (FISH, qPCR, 16S amplicon sequencing, draft genome sequencing). Eventually, we showed that this Oligo-MM restores colonization-resistance against *Salmonella enterica* serovar Typhimurium to germfree mice. We believe that this collection of publicly available and well characterized strains will be an useful tool for the scientific community in the future in order to understand the role of single species in a complex microbial consortium and address specific aspects of microbiota-host mutualism.

# *CAMPYLOBACTER JEJUNI INDUCES ACUTE NON-SELF-LIMITING ENTEROCOLITIS IN GNOTOBIOTIC IL-10-/- MICE VIA TOLL-LIKE-RECEPTOR-2 AND -4 SIGNALING*

Stefan Bereswill, Lea-Maxie Haag, André Fischer, Bettina Otto, Rita Plickert, Anja A. Kühl, Ulf B. Göbel, Markus M. Heimesaat

*Institute for Microbiology and Hygiene, Charité – University Medicine Berlin, Campy Benjamin Franklin, Hindenburgdamm 27, 12203 Berlin*

*Aim:* To present a murine C. jejuni infection model displaying acute non-self-limiting enterocolitis mimicking severe episodes of human campylobacteriosis and to elucidate potential Toll like receptor (TLR) -2 and/or –4 dependence of immunopathology.

*Methods and Major Findings:* Gnotobiotic IL-10-/- mice were generated by quintuple antibiotic treatment starting right after weaning, thereby preventing animals from commensal bacteria induced colitis. Following oral infection, C. jejuni B2 strain readily colonized the gastrointestinal tract of gnotobiotic IL-10-/- mice and induced acute non-self-limiting ulcerative enterocolitis within 7 days. Immunopathology was further characterized by increased numbers of apoptotic cells, T- and B-lymphocytes as well as regulatory T-cells and elevated TNF-$\alpha$, IFN-$\gamma$, and MCP-1 concentrations in the inflamed colon. Infection of gnotobiotic IL-10-/- mice with a commensal E. coli strain, however, did not induce disease indicating a C. jejuni-specific induction of disease. C. jejuni infection of gnotobiotic IL-10-/- mice additionally lacking TLR-4 or -2 revealed that immunopathology is mediated by TLR-4- and, less distinctly, by TLR-2- dependent signalling of C. jejuni-LPS and -lipoprotein, respectively.

*Main Conclusion and Impact of the research:* The presented murine C. jejuni infection model displaying acute non-self-limiting enterocolitis mimicks severe episodes of human campylobacteriosis as in immuno-compromized patients. This acute model proves useful for further dissecting the immunopathological mechanisms underlying Campylobacter infections in vivo and to elucidate the interplay between intestinal pathogens, the commensal intestinal microbiota and the innate as well as adaptive immune system of the host.

# THE C.RODENTIUM T6SS (TYPE 6 SECRETION SYSTEM) IS IMPORTANT FOR THE EFFECTIVE GUT COLONIZATION

Roshni Lakra[1], Markus F. Neurath[1], Christian Riedel[2] and Stefan Wirtz[1]

[1]*Medicine Clinic I, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU)*
[2]*Institute of Microbiology and Biotechnology, Ulm*

*Citrobacter rodentium* is a gram negative enteric pathogen causing acute colitis and transmissible colonic hyperplasia in mice, by inducing "attaching and effacing" type lesions in intestinal epithelial cells, similar as *Enterohemorrhagic E.coli* (EHEC) and *Enteropathogenic E.coli* (EPEC) in humans. A recently discovered protein secretion system in gram negative bacteria called Type 6 Secretion System (T6SS) has been shown to play an important role in inter-bacterial competition or to elicit response in eukaryotic cells. Although *C.rodentium* genome sequence has revealed 2 clusters of T6SS, carrying all conserved genes such as *hcp*, *vgrg* and *icmf*, however much of their functions are unknown. *In-vivo* infection of B6 and Rag1-/- mice with wild-type (WT ICC-169) and generated T6SS mutant *Citrobacter* (Δ*hcp*, Δ*vgrg* and Δ*icmf*), suggests that T6SS mutants cause lesser bacterial burden than WT *Citrobacter* which is measured by bioluminescent intensity of *Citrobacte*r under the IVIS *in-vivo* imaging system. Furthermore, we found that T6SS mutants exhibit significantly decreased crypt hyperplasia, a hallmark of *Citrobacte*r infection in mice and reduced colonization of mice gut as compared to WT *Citrobacter.* In order to study the cause of less intestinal colonization of T6SS mutants we performed, predator vs prey assay with WT and T6SS mutants vs bacteria isolated from mice stool result of which strongly suggests that T6SS in *Citrobacter* is required for competition with other bacteria. Additionally, 16s metagenomic analysis of gut microflora of mice infected with WT and T6SS mutant *Citrobacter* clearly indicated that *Citrobacter* utilizes T6SS to target bacterial competitors. Overall, our study establishes the important role of T6SS in *C. rodentium* virulence and this is the first evidence of differential community wide response of mice gut microflora with regard to presence or absence of *Citrobacter* T6SS.

# MULTIPLE FACTORS INFLUENCE THE OCCURRENCE OF POPULATION BOTTLE NECK(S) DURING SALMONELLA TYPHIMURIUM COLITIS

Lisa Maier[1], Médéric Diard[1], Balamurugan Periaswamy[1], Bärbel Stecher[2] and Wolf-Dietrich Hardt[1]

[1] *ETH Zurich, Institute of Microbiology, Wolfgang-Pauli-Str.10, 8093 Zurich, Switzerland*

[2] *Max von Pettenkofer-Institut, Pettenkoferstr. 9a, 80336 München, Germany*

Several protective barriers prevent infection of a healthy individual by enteric bacterial pathogens, like the acidity of the stomach, the need to occupy a niche in the already densely colonized intestine and innate and adaptive immune mechanisms. During the process of overcoming these obstacles population bottle necks occur for the pathogen in which a proportion of a population is killed or prevented from reproducing. This stochastic process might result in a wide variability in spatio-temporal representation of bacterial subpopulations.

This study aimed at identifying and elucidating the mechanisms which lead to such population bottle necks in a mouse model of *Salmonella* Typhimurium (*S*. Tm) -induced colitis. C57Bl/6 mice were infected orally with mixtures of differentially tagged (WITS, wild-type isogenic tagged strains) but phenotypically identical *S*. Tm strains which can be individually tracked using real-time PCR-based quantification. Systematic dilution experiments of these tagged strains revealed severe bottle neck effects in mice harboring a complex microbiota, which were less pronounced in mice with a low complex microbiota. Furthermore, the bottle neck phenomenon was shown to be inflammation-dependent: Triggering inflammation depends on two main virulence factors of *S*. Tm, namely type three secretion systems TTSS-1 and TTSS-2. By gradually decreasing inflammation severity by infection with different *S*. Tm mutants in the type three secretion systems and the associated effector proteins, the observed bottle neck effect gets less prominent and is completely absent in case of infection with an avirulent mutant.

An implication of this finding is that genetic screening approaches (i.e. signature-tagged mutagenesis) for bacterial genes required for infection and *in vivo* fitness should be accompanied by empirical characterization of bottle neck effects, since population bottle necks will cause loss of mutants and therefore increase the ratio of false-positives. Our results indicate that effects of population bottle necks in a model for oral *S*. Tm infection can be minimized by switching from mouse models with a complex microbiota to a defined and low complex microbiota model system. This will permit screening for *Salmonella* mutants with altered fitness *in vivo* without the need to compromise on inflammatory conditions.

# *ENTEROCOCCUS FAECALIS POLYSACCHARIDE ANTIGEN AND LIPOPROTEINS MEDIATE VIRULENCE IN CHRONIC INFLAMMATION AND IN INFECTION MODELS*

Irina G. Sava[1]*, Soeren Ocvirk[1]*, Natalie Steck[1], Yinyin Bao[3], Johannes Huebner[3], Barbara E. Murray[4], Balfour R. Sartor[5], Dirk Haller[1,2]

*[1]Chair of Nutrition and Immunology, [2]ZIEL – Research Centre for Nutrition and Food Science, Biofunctionality Unit, Technische Universität München, Gregor-Mendel-Str. 2, 85350 Freising, GERMANY*

*[3]Division of Infectious Diseases, Department of Medicine, University Medical Center, Freiburg, GERMANY*

*[4]Division of Infectious Diseases, Department of Internal Medicine, The University of Texas, Medical School, Houston, Texas, USA*

*[5]Department of Medicine, Division of Gastroenterology and Hepatology, University of North Carolina at Chapel Hill, USA*

**Both authors equally contributed*

*Introduction: Enterococcus faecalis* (*E. faecalis*) is an ubiquitous bacterium occurring as member of the human core microbiota but also as nosocomial pathogen. It has been shown that IL10-/- mice are susceptible to *Enterococcus faecalis*, developing chronic colitis after monocolonization with this microorganism. In addition, both enterococcal polysaccharide antigen (Epa) and lipoproteins (highly conserved bacterial structures recognized by Toll-like receptor 2) have been previously described to impact *E. faecalis* virulence.

*Methods:* We investigated the role of the two above-mentioned *E. faecalis* structures by deleting two genes: *epaB* (part of *epaBCD* operon, involved in Epa biosynthesis) and *lgt* (involved in lipoproteins synthesis). Wild-type strain OG1RF and the mutants epaB and Δlgt were compared regarding development of colitis in IL10-/- mice and virulence in invertebrate models - *Galleria mellonella, Caenorhabditis elegans* and *Manduca sexta*. Additionally, the impact of *epaB* and *lgt* deletion was investigated with regard to biofilm formation and innate immune response in bone marrow-derived dendritic cells

*Results:* In a mono-association experiment, IL-10-/- mice associated with *E. faecalis* ΔepaB and Δlgt mutant strains showed significantly reduced intestinal pathology compared to OG1RF-associated mice.

*E. faecalis* ΔepaB and Δlgt mutant strains showed impaired virulence in both an insect (*Galleria mellonella)* and a nematode (*Caenorhabditis elegans*) model of infection, while the ΔepaB mutant revealed altered adhesion to intestinal epithelium of mono-associated *Manduca sexta* larvae and diminished *in vitro* biofilm formation.

As expected, inactivation of *lgt* led to reduced Toll-like receptor 2-mediated activation of bone marrow-derived dendritic cells *in vitro*.

*Conclusions:* Our results reveal an important role of Epa and lipoproteins as virulence factors mediating the interaction of *E. faecalis* with the host in multiple ways. In germ-free Il-10-/- mice *epaB*- or *lgt*-deficient *E. faecalis* caused attenuated intestinal pathology. This indicates to mechanisms related to altered adhesion (ΔepaB) or diminished innate immune recognition (Δlgt) and may disclose a track of how these two bacterial structures contribute to *E. faecalis*-host interaction.

# *COMPLEX ROLE OF PROTEASES IN SALMONELLA-INDUCED INTESTINAL FIBROSIS*

Natalie Steck[1,2], Reinhild Kappelhoff[3], Christopher M. Overall[3], B. Brett Finlay[4], Guntram A. Grassl[1,2]

[1]*Institute for Experimental Medicine, University of Kiel, Kiel Germany*
[2]*Research Center Borstel, Borstel, Germany*
[3]*Centre for Blood Research, Oral Biological and Medical Sciences, University of British Columbia, Vancouver, BC, Canada*
[4]*Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada*

Intestinal fibrosis, a common complication of Crohn's disease, is characterized by the accumulation of fibroblasts, deposition of extracellular matrix and formation of scar tissue. Although many factors including cytokines and proteases contribute to the development of intestinal fibrosis, the initiating mechanisms and the complex interplay of these factors remain unclear. Here, we show protease and protease inhibitor involvement in intestinal fibrosis by using a mouse model of chronic enteric *Salmonella* infection. Mice developed severe and persistent fibrosis in the cecum after oral infection with *Salmonella* enterica serovar Typhimurium (*S*.T.). A protease and protease inhibitor specific gene expression CLIP-CHIP microarray analysis revealed 141 up- or down-regulated proteases or protease inhibitors. Various matrix metalloproteases (MMP-3, -7, -8, -10, -13), serine proteases (kallikreins, trypsin, tryptase, neutrophil elastase) and cysteine proteases (cathepsins, calpains) as well as protease inhibitors (TIMP-1, cystatins, elafin) were regulated in the fibrotic tissue and we confirmed 13 up- and 6 down-regulated proteases by quantitative RT-PCR analysis. By immunohistochemical staining, we showed that MMP-8 and MMP-10 expression was induced in the mucosa of fibrotic cecal tissue, while MMP-13 could only be detectable submucosal regions. Expression kinetics of intestinal epithelial MMP-7 and meprin-β in the development of *S*.T.-induced fibrosis were converse suggesting a potential unexpected relationship between those two metalloproteases. In summary, we could show that proteases and protease inhibitors play a complex role in *S*.T.-induced intestinal fibrosis. Further studies aim to evaluate expression kinetics of the identified proteases/protease inhibitors in humans and to elucidate specific functions in the development of intestinal fibrosis.

POSTER

# 1 TLR4 CO-RECEPTOR CD14 PLAYS AN IMPORTANT ROLE IN EPITHELIAL BARRIER FUNCTION

Marijana Basic[1], Lydia M. Keubler[1], Anna Smoczek[1], Andrea Liese[1], Andre Bleich[1]

[1]*Institute of Laboratory Animal Science, Hannover Medical School, Hannover, Germany*

*Background:* CD14, a co-receptor in TLR4 signaling pathway, was demonstrated to have a protective mechanism in experimental IBD development. *E. coli* Nissle, a probiotic bacterium, was demonstrated to induce severe and lethal inflammation in C3H/HeJZtm (Tlr4-defective) monoassociated mice. Thereby this monoassociation study was used to elucidate the *Cd14* impact on intestinal homeostasis.

*Material and methods:* Germfree *Tlr4*-defective, *Tlr4*-intact (C3H/HeOuJ) and *Cd14*$^{-/-}$ (C3H/HeN.129S1-*Cd14*$^{tm1Smg}$) mice received $10^9$ CFU of *E. coli* Nissle 1917 by oral gavage. 72 h p.i. CFU were determined in liver, spleen, cecum and colon. Expression of tight junction proteins was assessed by qRT-PCR and immunofluorescence. Apoptosis rate was determined by TUNEL staining. cDNA generated from small intestines was used for microarray analyses.

*Results:* In spleens and livers of *Tlr4*-intact mice no CFU were observed. *Cd14*$^{-/-}$ mice displayed up to $10^3$ CFU in organs and *Tlr4*-defective mice had even more pronounced numbers. Apoptosis rate was markedly increased and expression of tight junction proteins ZO-1, Occludin, Claudin -2, -7, -8, and E-cadherin were reduced in the small intestine of *Tlr4*-defective and *Cd14*$^{-/-}$ mice. Microarray analysis demonstrated an upregulation of genes associated with inflammatory response in *Tlr4*-defective and *Cd14*$^{-/-}$ mice.

*Conclusion: E.coli* Nissle disseminates in *Cd14*$^{-/-}$ mice beyond intestinal barrier as well as by *Tlr4*-defective mice. Reduced expression of tight junction proteins and increased apoptosis in *Tlr4*-defective and *Cd14*$^{-/-}$ mice indicates a relevant role of TLR4 and CD14 for the function of the epithelial barrier in this model, and demonstrate the important role of CD14 in intestinal homeostasis.

# 2 EFFECTS OF RETINOID TREATMENT ON MURINE GUT MICROBIOTA AND EXPERIMENTAL COLITIS

Becker Eugenia[1], Frey-Wagner Isabelle[1], Stanzel Claudia[1], Atrott Kirstin[1], Biedermann Luc[1], Rehman Ateequr[2], Jonas Daniel[2], Rogler Gerhard[1]

[1]*Division of Gastroenterology and Hepatology, University Hospital Zurich, Zurich, Switzerland*
[2]*Department of Environmental Health Sciences, University Medical Center, Freiburg, Germany*

*Background: In-vitro* and *in-vivo* data have shown that retinoid treatment promotes an anti-inflammatory milieu with few adverse effects towards the gastrointestinal (GI) tract, yet there is current debate about a causal relation between retinoid treatment and inflammatory bowel disease (IBD). Here we studied the effects of retinoid treatment on murine gut microbiota and on intestinal inflammation in two mouse models of IBD.
*Design:* Animals were treated with isotretinoin (30mg/kg) for 2 weeks daily by oral gavage. Faecal samples were collected before, directly after and 4 weeks after the treatment period. The composition of the murine gut microbiota was analysed by 16S rRNA gene sequencing. Chronic DSS colitis was induced by four cycles of 2.5 % DSS in the drinking water with retinoid treatment starting with the last DSS cycle and continuing until the end of the study 5 weeks later.Transfer colitis was induced by transfer of CD4+CD62L+ T cells (naïve T cells) and treated by transfer of CD4+CD25+ Treg cells. Naïve T cells and Treg were isolated from isotretinoin or vehicle treated donors. Assessments included endoscopic/histological scores, colon length, spleen weight, myeloperoxidase (MPO) activity, serum cytokines, and plasma isotretinoin levels.
*Results:* Retinoid treatment did not influence the course of colitis in both IBD mouse models. Preliminary results of the microbiota analysis show that retinoid treatment has no significant effects on the composition of murine microbiota.
*Conclusion:* Retinoid treatment has no adverse effects on experimental colitis and leads to no fundamental changes in murine gut microbiota composition.

# 3 ESTABLISHMENT OF A SPECIFIC HYDROLYSIS PROBE QUANTIFICATION METHOD FOR THE GNOTO OLIGO-MOUSE MICROBIOTA MODEL

Markus Beutler[1], Sandrine Brugiroux[1], Manuel Diehl[1] and Bärbel Stecher[1]*

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

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

The gastrointestinal (GI) microbiota is indispensable for human health, nutrient degradation and the development of a functional immune system. So far, targeted microbiota manipulation has been attributed to disease such as obesity, multiple sclerosis and autoimmune diseases.

Since there is a high abundance of different bacterial species that compose a very complex intestinal ecosystem, gnotobiotic mouse models have emerged in the last years in order to investigate host-microbial interactions involved in microbiota-dependent host phenotypes. The bacteria used in those mouse models are either based on human bacterial isolates or on a mixture of mouse-derived bacteria, the “Altered Schaedler Flora”, which is currently not accessible to the public.

Recently, we have developed a novel type of model microbiota which can be readily used in gnotobiotic mouse models. This oligo-mouse microbiota (Oligo-MM) is derived from murine GI bacteria and comprises 15 isolates from 6 eubacterial phyla (*Firmicutes*, *Bacteroidetes*, *Deferribacteres*, *Actinobacteria*, *Verrucomicrobia* and *Proteobacteria*). We show that the consortium stably colonizes the mouse GI tract over time. To do so, we have established a collection of molecular tools to detect the strains of the Oligo-MM (FISH, 454-sequencing, draft genome sequencing) as well as a highly sensitive realtime-PCR assay. This assay allows us to reproducibly detect and follow the bacterial consortium in the murine GI tract over time as well as in response to perturbations, and thereby enables us to assess Oligo-MM stability.

# 4 WHY ARE AGR2 KO MICE RESISTANT AGAINST SALMONELLA-INDUCED COLITIS ?

Sandrine Brugiroux[1], Diana Kaiser[1], David Berry[2], Alexander Loy[2], David J. Erle[3] and Bärbel Stecher[1]*

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

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

[3] *University of California, San Francisco, USA.*

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

*Salmonella enterica* serovar Typhimurium (*S.* Tm)-induced colitis is the result of the interplay of microbiota, the mucosal immune system and *S.* Tm virulence factors. As previously shown, chemotaxis is required for *S.* Tm to efficiently colonize the inflamed, but not the non-inflamed gut in streptomycin (sm)-pretreated mice (Stecher *et al.,* Cell. Microbiol. 2008). We hypothesized that increased mucus secretion upon inflammation contributes to this phenotype. To test this, we performed a competitive infection experiment of *S.* Tm wildtype (WT) and an isogenic chemotaxis mutant (CheY$^-$) in mice lacking anterior gradient 2 (AGR2). AGR2-deficient mice exhibit severe defects in the mucosal barrier including attenuated mucin (Muc2) secretion but do not display overt intestinal pathology (Park *et al.,* PNAS 2009). In line with our hypothesis, we found that *S.* Tm WT out-competed the CheY$^-$in AGR2$^{+/-}$ mice (mucin secretion$^+$) but not in their AGR2-deficient littermates (mucin secretion$^-$). When performing WT/CheY$^-$ competition in an avirulent *S.* Tm strain background (*invG ssaV*; defective in inducing inflammation) WT and CheY$^-$ colonized at equal levels. At a late time point post infection (day 4 p.i.), AGR2$^{-/-}$ and AGR2$^{+/-}$ littermates both exhibited comparably severe colitis. Surprisingly, AGR2$^{-/-}$ showed no overt inflammatory symptoms in the cecum at early time point (24h p.i.) contrary to littermate controls. Thus, the effect observed in the WT/CheY$^-$ competition experiment may also be caused by attenuated inflammation in AGR2$^{-/-}$ mice. Interestingly, we found that AGR2$^{-/-}$ mice exhibit an altered microbiota composition as compared to AGR2-proficient littermate control animals. Further, they exhibit a 20-fold increase in microbiota density 24h after sm-treatment. This increased microbiota density may explain the enhanced resistance of AGR2$^{-/-}$ mice to *S.* Tm-induced colitis. In summary, our results suggest that AGR2$^{-/-}$ mice exhibit an altered microbiota composition (*i.e.* harboring commensals with increased resistance to sm) compared to wildtype animals. Since this microbiota difference is apparent between AGR2$^{-/-}$ and AGR2$^{+/-}$ mice from the same litter it may be caused by impaired mucosal homeostasis of AGR2$^{-/-}$ mice.

# 5 AKKERMANSIA MUCINIPHILA EXACERBATES GUT INFLAMMATION FOR SALMONELLA TYPHIMURIUM INDUCED ACUTE ENTEROCOLITIS IN GNOTOBIOTIC MOUSE MODEL

Bhanu Priya Ganesh[1], Gunnar Loh[1] and Michael Blaut[1]

[1]*The German Institute of Human Nutrition (DIfE) Potsdam-Rehbrücke*

This study investigated how the presence of a mucin degrading commensal bacterium *Akkermansia muciniphila (Amuc)* affects the out-come of an intestinal *S.* Typhimurium (*STm*)-induced gut inflammation. Using a gnotobiotic mouse model with a background microbiota of 8 bacterial species (SIHUMI) we investigated the impact of *Amuc* (SIHUMI-A) on inflammation and infectious symptoms caused by *STm*. Presence of *Amuc* in *STm*-infected mice caused significantly increased histopathology scores and elevated mRNA levels of IFN-γ, IP-10, TNF-α, IL-12, IL-17 and IL-6 in cecal and colonic tissue. This increase in pro-inflammatory cytokines was accompanied by increased macrophage recruitment and by 1 $\log_{10}$ higher *STm* cell numbers in mesenteric lymph nodes of SIHUMI mice associated concomitantly with *Amuc* and *STm* (SIHUMI-AS) compared to SIHUMI mice with only *STm* (SIHUMI-S). Mucin filled goblet cell numbers were 2 to 3 fold lower in cecal tissue of SIHUMI-AS mice compared to SIHUMI-S, SIHUMI-A or SIHUMI mice. Reduced goblet cell numbers significantly correlated with increased IFN-γ ($r^2$ = 0.8618, ****P<0.001*) mRNA levels of the mice. Independent of *STm* infection, concentrations of N-acetylneuraminic acid (NANA) of cecal mucosa was significantly increased in the presence of *Amuc*. Concomitant presence of *Amuc* and *STm* resulted in a drastic change in existing microbiota composition. This was not observed in SIHUMI mice with either one of the two organisms being present. The proportion of *B. thetaiotaomicron* decreased from 88% to 0.02% while *STm* increased from 2.2% to 94% in SIHUMI-AS mice compared to other groups. We propose that *Amuc* exacerbates *STm*-induced intestinal inflammation by its ability to disturb host mucus homeostasis thereby turning into a pathobiont.

# 6 ACTIVATION OF RESIDENT LAMINA PROPRIA CELLS IN RESPONSE TO EPITHELIAL LAYER DAMAGE

Judith Gras[1], Serin Schiessling[1,2], Juliane Ilse[1], Timea Szikszai[1], Felix Lasitschka[3], Mohammed AlSaeedi[2], Thomas Giese[1], Stefan Meuer[1], Jutta Schröder-Braunstein[1]

*[1]Institute for Immunology, [2]Department of Surgery, [3]Institute of Pathology, University Hospital Heidelberg*

Epithelial layer damage represents an early hallmark of acute and chronic intestinal inflammation. It is associated with the induction of an inflammatory response in resident lamina propria cells including myeloid cells (LPMO) as demonstrated using a human organ culture model. In particular, an up-regulation of pattern recognition receptors, co-stimulatory receptors as well as soluble inflammatory mediators (e.g. IL-1b, IL-8, MIP-1b) can be observed in LPMO emigrated from mucosal specimens in response to loss of epithelial cells (LEL). In order to further characterize the inflammatory response of LPMO, global gene expression profiles were obtained from these cells. In comparison to peripheral blood monocytes, LPMO expressed genes characteristic of dendritic cells such as FLT3, LAMP3, CD80, CD86, CCR7, CD83. Furthermore, high expression of CCL19, CCL22, Il-23p19, EBI3 as well as matrix metalloproteinases such as MMP12 and MMP9 suggest an important role of these cells in the regulation of an intestinal immune response. Preliminary signaling pathway analysis suggests a potential role of the OX40/OX40L pathway in the activation of LPMO in response to LEL. (Supported by DFG/SFB 938.)

# 7 MOLECULAR TOOL BOX FOR FUNCTIONAL ANALYSIS OF BIFIDOBACTERIA

Verena Grimm, Marita Gleinser, Daria Zhurina, and Christian U. Riedel

*Institute of Microbiology and Biotechnology, University of Ulm, D-89081 Ulm, Germany*

Bifidobacteria are commensals of the lower human gastrointestinal tract and some strains, have been shown to exert a range of beneficial health properties. For example, *Bifidobacterium bifidum* S17 was shown to tightly adhere to intestinal epithelial cells and have potent anti-inflammatory activity *in vitro* and in several murine models of colitis. However, due to the lack of appropriate tools for genetic modification of bifidobacteria the exact mechanisms contributing to these effects still remain to be uncovered.

Hence, a range of *E.coli-Bifidobacterium* shuttle vectors with different antibiotic resistance were generated and tested for effects on growth and plasmid stability in the absence of antibiotics. Plasmids pMGC and pMGE conferring either chloramphenicol or erythromycin resistance were stably maintained over at least 100 generations and did not affect growth rate of bacteria *in vitro*. Use of these two plasmids allowed the investigation of gastrointestinal transit times and colonization of *B. bifidum* in C57BL/6J mice.

The *gusA* reporter system of pMDY23 was used to screen the range of bifidobacterial and synthetic promoters. The *gap* promoter ($P_{gap}$) of *B. bifidum* S17 was shown to drive high level expression of the reporter and was cloned into pMGS resulting in a vector for the stable and strong homologous and heterologous gene expression for bifidobacteria as shown by. This system was validated by cloning the genes for different fluorescent proteins under control of $P_{gap}$.

# 8 SPECIES-SPECIFIC IDENTIFICATION AND QUANTIFICATION OF PROBIOTIC LACTOBACILLI IN YOGHURT BY REAL-TIME PCR

Stefan R. Herbel[1], Markus von Nickisch-Rosenegk[2], Matthias Kuhn[3], Lothar H. Wieler[1], Sebastian Guenther[1]

[1]*Institute of Microbiology and Epizootics, Freie Universität Berlin, Philippstr. 13, 10115 Berlin, Germany. Tel.: +49 (0)30-2093 6030; E-mail: herbel.stefan@vetmed.fu-berlin.de*
[2]*Fraunhofer Institute, IBMT Potsdam-Golm, Am Mühlenberg 13, 14476 Potsdam, Germany*
[3]*CONGEN Biotechnologie GmbH, Robert-Rössle-Straße 10, 13125 Berlin, Germany*

Probiotics are believed to promote beneficial influences on the gastrointestinal tract and thereby on health in general. They are therefore often used in dairy and non-dairy products in human nutrition. Besides that feeding of animals with probiotics has increased, in particular since the European Union approved a prohibition to use antibiotics for growth promotion.

Classic diagnostic methods for the identification of strains of the genera Lactobacillus and Bifidobacterium included phenotypic comparison with reference strains and polymerase chain reaction (PCR). These present methods for the isolation of probiotic bacteria were using selective media and utilizing different growth conditions resulting in time-consuming, labor-intensive diagnostic schemes. Furthermore this phenotypic characterization and the species differentiation is error-prone.

As probiotic action depends both on the quality and quantity of the probiotic strains a conventional PCR-detection method is not feasible, as it does not allows a quantification of strains in the samples. Thus there is a need to establish a real time PCR (qPCR) method to comply with the requirements to identify and quantify probiotic strains in food without a prior cultivation step.

Screening of different target-sequences for a species-specific identification of probiotic strains ruled out classic targets like the 23s-5s rRNA (intergenic spacer) because of a lack species- specificity other targets like the heat shock proteins (hsp60) were chosen for the specific identification of different species of the genus Lactobacillus. The ATPase subunit of the ATP-dependent clpC-gene was selected for the species-specific identification of members of the genus Bifidobacterium.

The validation of primers targeting these genes is still ongoing with the final goal to establishing a qPCR method for the detection of Lactobacilli-species in different products like yoghurts directly.

The main advantage of a qPCR method based on the same annealing conditions is a rapid and species-specific detection, identification and quantification of different probiotic strains within one single qPCR run.

# 9 IMPACT OF GUT MICROBIAL ANTIGENS IN EXPERIMENTAL ILEITIS

Sarah Just[1,2], George Kollias[3], Dirk Haller[2] and Thomas Clavel[1,2]

[1]*Junior Research Group Intestinal Microbiome,*

[2]*Chair of Nutrition and Immunology, ZIEL – Research Centre for Nutrition and Food Science, Biofunctionality Unit, Technische Universität München, Gregor-Mendel-Str. 2, 85350 Freising, Germany,*

[3]*Institute of Immunology, Biomedical Sciences Research Center Alexander Fleming, Vari 16672, Greece*

*Introduction:* Inflammatory bowel diseases are characterized by the loss of immunological tolerance towards commensal microorganisms and are associated with dysbiosis of the gut microbiota. However, knowledge of molecular mechanisms underlying microbiota/host interactions in ileal inflammation is scarce. Sydora *et al.* (2011) showed in the IL-10$^{-/-}$ mouse model of colitis that neonatal intraperitoneal injection of fecal antigens reduced the severity of inflammation. Furthermore, bacterial heat-shock proteins (HSP) were shown to play an important role in various bacteria-driven infections. Additionally, they are dominant peptides in the gut and can mimic the actions of host molecular chaperones. Wieten *et al.* (2009) recently used bacterial HSP 70 for the prevention of arthritis in mice. The aim of the work was to analyze the effect of HSP70 and caecal bacterial lysates on the onset of inflammation in the Tnf$^{\Delta ARE/wt}$ mouse model of Crohn's disease-like ileitis.

*Methods & Results:* Mice (n = 6 per group) were challenged by intraperitoneal injections of either a recombinant streptococcal HSP70 or caecal bacterial lysates (CBL) from 13-week-old Tnf$^{\Delta ARE/wt}$ mice (ARE-CBL) (one injection of 20 µg on each day 4, 11, 18 and 28 after birth). The challenge of Tnf$^{\Delta ARE/wt}$ mice with bacterial HSP70 did not result in significant differences in body weight development, spleen and caecum weight. T-cell populations and histological scores in the distal ileum and proximal colon at the age of 8 weeks were not changed. The analysis of plasma samples revealed marked anti-HSP70-IgG and -IgM responses in the treatment *vs.* control group. High-throughput 16S rRNA gene sequencing (V4 region) of caecal microbiota revealed changes in bacterial diversity. HSP70 challenge was also associated with a significant decrease in the proportion of *Firmicutes* (55.7 ± 10.6 % *vs.* 69.7 ± 10.9 %; $p < 0.05$), while the proportion of *Bacteroidetes* (26.7 ± 4.9 % *vs.* 19.9 ± 9.9 %) and *Proteobacteria* (6.4 ± 2.7 % *vs.* 4.0 ± 1.4 %) was slightly increased. The challenge of Tnf$^{\Delta ARE/wt}$ mice with ARE-CBL significantly reduced spleen to body weight ratio when compared with sham-injected mice and a trend towards lower histological scores in the distal ileum was observed.

*Conclusion:* Treatment of Tnf$^{\Delta ARE/wt}$ mice with bacterial HSP70 and ARE-CBL did not result in reduced severity of intestinal inflammation but seems to alter bacterial diversity and composition in the caecum.

# 10 INFLUENCE OF LACTOBACILLI EXTRACELLULAR FACTORS UPON EPEC ADHERENCE TO INTESTINAL EPITHELIAL CELLS

Kempf, M., Cichon, C. and Schmidt, M.A.

*Department of Infectiology, Centre for Molecular Biology of Inflammation (ZMBE), Westfaelische Wilhelms-Universitaet Muenster, Muenster, Germany*

Infections with the Gram-negative enteropathogenic Escherichia coli (EPEC) are a main cause of diarrhea in infants. The adhesion of the pathogen to intestinal epithelial 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 these pathogenic effects. To achieve this we refined a quantitative adhesion assay employing different Lactobacillus strains (L. acidophilus, L. fermentum, L. gasseri and L. rhamnosus), which boost a reduced binding of EPEC E2348/69 to intestinal epithelial cells. We were able to show that culture supernatants of these strains are sufficient to decrease the adhesion of EPEC to T84 monolayers significantly. In addition, measurements of the transepithelial resistance (TER) during EPEC infection confirmed these findings. We detected a dose dependent reduction of epithelial barrier disruption after applying increasing amounts of bacterial supernatant. Adhesion assays performed with heat inactivated as well as Proteinase K digested culture supernatants revealed for some of the Lactobacillus stains proteinogenic factors as causative agents for the reduction of EPEC binding.

The definitive nature of the potential Lactobacillus factors and the underlying molecular mechanisms, as well as the cellular target structures are currently under investigation. New regulatory factors will foster the development of advanced strategies for treatment of EPEC infections.

# 11 COMPETITION OF COMMENSAL LIPOPOLYSACCHARIDES FOR BINDING TOLL-LIKE-RECEPTOR 4 – ROLE IN MAINTENANCE OF INTESTINAL HOMOEOSTASIS

T. Kliem, A. Molinero, A. Steimle, A. Schäfer, I.B. Autenrieth and J.S. Frick

Many types of LPS, e.g. enterobacterial LPS, are potent stimulators of TLR4, inducing proinflammatory responses. However, there are certain types of LPS having no or very low biological activity. The endotoxic potential is determined by the phosphorylation and acylation pattern as well as differences in the structure of the fatty acids chains. This led to the hypothesis that LPS or the endotoxic part of LPS, Lipid A, of commensal bacteria might be a key factor in the maintenance of intestinal homoeostasis. It is supposed that the different Lipid A structures of Bacteroidetes and Enterobacteriaceae may account for the different anti- or pro-inflammatory potential.

In previous work it was shown that the commensal strain B. vulgatus is able to prevent and even more strikingly to heal inflammatory bowel disease (IBD). The bacterial factor promoting prevention or induction of IBD seems to be the bioactive part of LPS, Lipid A.

The aim of this project is to find out whether therapeutic LPS can block the binding of pro-inflammatory LPS to TLR4. To elucidate the molecular mechanisms leading to prevention of IBD upon administration of B. vulgatus LPS ($LPS_{BV}$) or, respectively, to induction of IBD upon feeding of E. coli LPS ($LPS_{EC}$) in vitro as well as in vivo competition assays of $LPS_{EC}$ and $LPS_{BV}$ for binding to the TLR4/MD2 Heterotetramer will be performed. TLR4 over-expressing HEK cells and immature dendritic cells will be used for the in vitro competition assays.

# 12 THE ROLE OF DENDRITIC CELLS IN INTESTINAL INFLAMMATION

A.G. Korkmaz[1], K. Gronbach[1], S. Autenrieth[2], J.S. Frick[1]

[1]*Institute of Medical Microbiology and Hygiene, University of Tübingen, Germany*
[2]*Interfaculty Institute for Cell Biology, University of Tübingen, Germany*

Dendritic cells (DCs) are an integral component of innate and acquired immunity in the gut. DCs continuously interact with microbial antigens in the intestine and have the potential to induce both tolerogenic and immunogenic T cell responses. In accordance with their crucial role in intestinal immunity, previous work has shown the involvement of DCs in inflammatory bowel diseases. However, the precise role of DCs and the extend of their involvement in inflammation is controversially discussed. In our current study we address the question whether dendritic cells are vital for mediating the pro- and anti-inflammatory effects of the intestinal microbiota. To be able to study DC function *in vivo* we will employ Rag1$^{-/-}$ CD11c-DTR mice. These mice further give the advantage of depleting dendritic cells conditionally, upon administration of diphtheria toxin. After DC depletion, the mice hosting specific pro-inflammatory and anti-inflammatory intestinal microflora will be transplanted with naïve CD4 T-cells, thereby modelling T cell transfer colitis in a DC deficient background. The disease occurrence and the course of disease are to be monitored by PET/MRT, and the activation and polarization of lamina propria/mesenteric lymph node T-cells will be investigated by analyzing cell surface markers and cytokine secretion profiles. The results of this study should provide more information on whether DCs are necessary to mediate pro-inflammatory and anti-inflammatory effects of the microbiota, or whether other cell types are at play in mediating the microbial effects in the intestine.

# 13 DIFFERENT SURVIVAL STRATEGIES OF GUT COMMENSAL BACTERIA IN IBD

A. Lange, S. Beier, D. Huson, S. Henz, K. Hantke, C. Lanz, I.B. Autenrieth and J.S. Frick

The gut microbiota plays an important role in development of inflammatory bowel diseases (IBD). The commensal bacterium *E. coli mpk* can cause colitis in *IL-2*$^{-/-}$ mice due to a yet unknown mechanism. In contrast *B. vulgatus mpk* – another gut commensal – prevents induction of colitis by *E. coli mpk* in *IL-2*$^{-/-}$ mice. To identify potential inflammatory or anti-inflammatory bacterial compounds we performed whole genome sequencing of both microbes.

*E. coli* IBD isolates are reported to represent a heterogeneous population and it is not possible to screen for a specific IBD marker gene. But *E. coli mpk* genome harbours a set of common genes shared with other IBD-related *E. coli* isolates like genes for adhesion and biofilm formation, propanediol utilization, and haemolysis. Furthermore *E. coli mpk* encodes a complete operon for a functional type VI secretion system (T6SS). The system is particularly interesting as it can be involved either in interspecies competition or in mediating a pathogenic or symbiotic relationship with the host. T6SS knockout strains should be used to dissect its role during development of colitis.

The genome of *B. vulgatus mpk* harbours a large number of genes related to polysaccharide metabolism, different LPS clusters, and sensory proteins. Here we give a first overview of the factors of commensal bacteria that might induce or prevent colitis in genetically predisposed hosts.

# 14 INFLAMMATION TRIGGERS COLICIN IB-DEPENDENT COMPETITION OF SALMONELLA SEROVAR TYPHIMURIUM AND COLICIN-SENSITIVE E. COLI IN ENTEROBACTERIAL BLOOMS

Lubov Nedialkova[1,2*], Rémy Denzler[3,4*], Martin Koeppel[1,2], Manuel Diehl[1,2], Diana Ring[1,2], Roman Gerlach[5] and Bärbel Stecher[1,2†]

[1]*Max-von-Pettenkofer Institute, LMU Munich, Pettenkoferstr. 9a, 80336 Munich, Germany*
[2]*German Center for Infection Research (DZIF)*
[3]*Institute of Microbiology, ETH Zürich, Wolfgang-Pauli Str. 10, 8093 Zürich, Switzerland*
[4]*Present address: Institute of Molecular Systems Biology, ETH Zürich. Wolfgang-Pauli-Str. 16, 8093 Zürich, Switzerland*
[5]*Robert Koch-Institut, Burgstraße 37, 38855 Wernigerode, Germany*
** These authors contributed equally*

The host's immune system plays a key role in modulating growth of pathogens and the intestinal microbiota in the gut. In particular, inflammatory bowel disorders and pathogen infections induce shifts of the resident commensal microbiota and results in overgrowth of *Enterobacteriaceae* ("*Enterobacterial* blooms"). Here, we investigated competition pathogenic *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) and commensal *E. coli* in such inflammation-induced blooms. *S.* Typhimurium produces colicin Ib, which is a narrow-spectrum protein toxin active against related *Enterobacteriaceae*. Colicin Ib production conferred a competitive advantage to *S.* Typhimurium over sensitive *E. coli* strains in the inflamed gut. In contrast, an avirulent *S.* Typhimurium mutant strain defective in triggering gut inflammation did not benefit from colicin Ib. Expression of colicin Ib is regulated by iron limitation and the SOS-response and its cognate outer membrane receptor CirA on colicin sensitive *E. coli* is induced upon iron limitations. We show that conditions prevailing in inflammation-induced blooms induce expression of both, *S.* Typhimurium colicin Ib and the receptor CirA, thus fuelling colicin Ib dependent competition of *S.* Typhimurium and commensal *E. coli*. This reveals how bacterial competition in the gut can be modulated by the host's immune response and sheds new light on the importance of enterobacterial colicins as fitness factors in *Enterobacteriaceae* blooms.

# 15 PROBIOTIC ESCHERICHIA COLI STRAIN NISSLE 1917 (ECN) BINDS TO GLUCONAT OF MUCIN2/HUMAN MUCUS VIA ITS FLAGELLUM

Anja Troge[a], Wolfgang Scheppach[b], Bjoern O. Schroeder[c], Stefan A. Rund[a], Jan Wehkamp[c], Tobias A. Oelschlaeger[a]

[a]*Institut für Molekulare Infektionsbiologie, University of Würzburg, Josef-Schneider-Str. 2/D15, 97080 Wuerzburg*

[b]*Department of Medicine, Division of Gastroenterology/Rheumatology, Juliusspital Wuerzburg, Juliuspromenade 19, 97070 Wuerzburg*

[c]*Dr. Margarete Fischer Bosch Institute of Clinical Pharmacology and Internal Medicine I, Robert Bosch Hospital, Auerbachstraße 112, 70376 Stuttgart*

Efficient adherence to host cells is viewed as an important feature of probiotics. However, in the healthy gut commensal and probiotic bacteria do not reach the enterocytes but reside in the gut lumen or the distal layer of the mucus. Therefore, it was no surprise to find EcN to adhere efficiently to porcine mucin2, human mucus, the mucus producing cell line LS174T and cryosetions of human gut biopsies but not to mouse mucus. The responsible adhesin was none of the suspected adhesins (type 1, F1C, curli) but EcN's flagellum. Neither these adhesins nor the flagellum were necessary for efficient adherence to non-mucus producing cell lines. Of several tested mucus components only gluconate was able to interfere with EcN's flagellum-mediated adherence to mucin2/human mucus indicating gluconate to be an important (part of the) receptor structure. The FliC domain D3, the most surface exposed one in the assembled flagellum, was not the ligand for mucus/gluconate. Flagella composed of a FliC mutant lacking domain D3 bound ten-fold more efficiently to mucin2/human mucus and this adherence was still inhibitable by gluconate. The construction of a FliC mutant lacking domain D2 is in progress to test D2 for functioning as the adhesive part of FliC binding gluconate of mucin2/human mucus.

# 16 IMPACT OF NUTRITIONAL FACTORS ON THE PROTEOME OF INTESTINAL BACTERIA: NOVEL INSIGHTS INTO E. COLI'S HEXURONATE METABOLISM UNDER OSMOTIC STRESS CONDITIONS

Monique Rothe[1], Gunnar Loh[1], Wolfram Engst[2] and Michael Blaut[1]

*German Institute of Human Nutrition, Department of Gastrointestinal Microbiology[1], Analytics Group[2], Arthur-Scheunert-Allee 114-116, 14558 Nuthetal, Germany*

Diet is a major force influencing the intestinal microbiota, but little is known about the bacterial response at the cellular level. To study the impact of nutritional factors on the protein expression of intestinal bacteria, gnotobiotic mice monoassociated with *Escherichia coli* K-12 were fed three different diets: a carbohydrate (lactose)-rich diet, a protein-rich diet and a diet rich in starch. Two-dimensional difference gel electrophoresis followed by electro-spray ionization-tandem mass spectrometry was used to identify proteins differentially expressed in *E. coli* cells recovered from the mouse intestinal tract.

The function of two so far poorly characterised *E. coli* proteins was further analysed *in vitro* for their possible roles in bacterial adaptation to the various diets: the 2-deoxy-D-gluconate-3-dehydrogenase (KduD) was upregulated in intestinal *E. coli* of mice fed the lactose-rich diet and this enzyme and the 5-keto-4-deoxyuronate-isomerase (KduI) were downregulated on the casein-rich diet. Reporter gene analysis identified galacturonate and glucuronate as inducers of the *kduD* and *kduI* gene expression. KduI was shown to facilitate the breakdown of these hexuronates, which are normally degraded by the uronate isomerase (UxaC), the altronate oxidoreductase (UxaB), the altronate dehydratase (UxaA), the mannonate oxidoreductase (UxuB) and the mannonate dehydratase (UxuA), whose expression was repressed by osmotic stress. The growth of *kduID* deficient *E. coli* on galacturonate or glucuronate was impaired in the presence of osmotic stress, suggesting KduI and KduD to compensate for the function of the regular hexuronate degrading enzymes under such conditions. Growth on lactose promoted the intracellular formation of hexuronates, which possibly explain the induction of KduD on a lactose-rich diet. These results indicate a novel function of KduI and KduD in *E. coli* and demonstrate the crucial influence of osmotic stress on the gene expression of hexuronate degrading enzymes.

# 17 ANTAGONISTIC EFFECTS OF PROBIOTIC E. COLI NISSLE 1917 ON VARIOUS EHEC STRAINS

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

[1]*Institute of Molecular Infection Biology, University of Wuerzburg, Germany*

*Objective*: The aim of this study was to investigate potential antagonistic effects of EcN on adhesion, growth, and Shiga toxin production of EHEC strains, such as the O157:H7 strain EDL933 or EHEC O104:H4 (clinical isolates) from the 2011 outbreak in Germany, *in vitro*.
*Methods*: 24-well-plates were coated with the human gut epithelial cell lines Caco2 or LS174T, to determine the adhesion of living bacteria to those cells. Growth of the bacteria in each well was determined at t=0h, t=2h, t=5h and t=24h by plating of serial dilutions on agar plates. Shiga toxin production of single cultures of EcN, EHEC, and co-cultures at ratios of 1:1 and 10:1 (EcN:EHEC) was analyzed after 24h incubation via Verotoxin ELISA. Shiga toxin expression was investigated in EHEC (O157:H7, EDL933) with a biolumincescent reporter system in real time.
*Results:* In our experiments EcN significantly reduced the adhesion of all tested pEc strains to Caco2 and LS174T cells by up to 99 % for EAEC O104:H4, EHEC O157:H7 and to >80 % for O104:H4 EHEC, in a co-culture with 10-fold EcN. Shiga toxin production of EHEC O104:H4 (TY3730) and EHEC EDL933 in co-culture with EcN in DMEM medium at a 10:1 ratio (EcN:EHEC) decreased by ~80%. Microcins were identified as the first factor contributing to the antagonistic effects of EcN on EHEC O104:H4. EcN and the isogenic microcin-negative mutant SK22D strongly inhibited the Shiga toxin expression in bioluminescent reporter strains (O157:H7, EDL933).
*Outlook*: We are currently investigating the mechanisms responsible for the observed antagonistic effects.

# 18 IDENTIFICATION AND CHARACTERIZATION OF MICRORNA INVOLVED IN THE MECHANISM OF ACTION OF PROBIOTIC BACTERIA E. COLI NISSLE 1917

Sabharwal, H., Cichon, C. and Schmidt, M.A.

*Department of Infectiology, Centre for Molecular Biology of Inflammation (ZMBE), Westfaelische Wilhelms-Universitaet Muenster, Muenster, Germany*

E.coli Nissle 1917 (EcN) is a widely used probiotic for the treatment of inflammatory intestinal disorders. However the regulatory mechanisms underlying its probiotic effects remain enigmatic. We are engaging in an alternative approach of studying the probiotic effect of EcN by analyzing the role of induced microRNAs in target cells. In general microRNAs have been shown to regulate intestinal architecture, inflammation and response to many bacteria.

Within our project we use human intestinal epithelial cell culture models to study miRNA responses in the presence of EcN. Based on miRNA microarray and qRT-PCR data we have identified microRNAs that are differentially regulated in the presence of EcN. These miRNA are predicted to regulate inflammation, cytokine activation and intestinal permeability. Furthermore we performed mRNA profiling by microarray to identify candidate miRNA target genes that are downregulated or upregulated at transcriptional level and are inversely co-related with the expression of miRNA. Currently we are validating the miRNA expression profiles and their putative targets and the according bacterial factors that trigger the miRNA responses will be determined.

Unraveling the role of probiotics-induced, miRNA-mediated alterations of mammalian host signaling will foster the development of new strategies for the treatment of gastrointestinal diseases.

# 19 THE EFFECT OF INTESTINAL INFLAMMATION ON E. COLI'S PROTEIN EXPRESSION IN THE MURINE GUT

S. Schumann[1], C. Alpert[1], W. Engst[1], R. Klopfleisch[2], A. Bleich[3], G. Loh[1], M. Blaut[1]

[1] *German Institute of Human Nutrition Potsdam Rehbrücke, Nuthetal*

[2] *Freie Universität Berlin, Berlin*

[3] *Hannover Medical School, Hannover*

Crohn's disease and ulcerative colitis are associated with a reduced microbial diversity and increased numbers of *Escherichia coli*. However, the reasons for the proliferation and its exact role in intestinal inflammation are unclear. We previously demonstrated that *E. coli* adapts to a severe DSS-induced intestinal inflammation by expressing various proteins that improve its ability to cope with oxidative stress caused by the inflammatory conditions. Here we report how a mild intestinal inflammation affects the proteome of *E. coli* in interleukin 10-deficient (IL-$10^{-/-}$) mice. We also investigated the molecular basis for strain-specific differences between probiotic and harmful *E. coli* in their response to gut inflammation.

The IL-$10^{-/-}$ mice were monoassociated either with the colitogenic *E. coli* UNC or with the probiotic *E. coli* Nissle (EcN). Differentially expressed proteins in *E. coli* strains collected from caecal contents were identified by 2-dimensional difference gel electrophoresis.

Histology and cytokine expression data of UNC associated IL-$10^{-/-}$ mice displayed a mild caecal inflammation in comparison to wildtype mice. Accordingly, changes in the bacterial proteome were only minimal. As previously observed in the DSS-experiment, proteins involved in the central energy metabolism were downregulated in *E. coli* from the inflamed mice. We did not observe an upregulation of bacterial chaperone proteins and the Fe/S biogenesis protein NfuA in the state of mild inflammation as detected previously in *E. coli* from DSS-treated mice. Interestingly, the inhibitor of vertebrate C-type lysozyme Ivy was 2- to 3-fold upregulated on mRNA and protein level in *E. coli* Nissle compared to *E. coli* UNC isolated from IL-$10^{-/-}$ animals. Therefore we investigated the role of Ivy and its function under the inflammatory conditions in more detail.

# 20 IMMUNOGENICITY OF DIFFERENT LIPID A STRUCTURES – THE IMPACT OF DENDRITIC CELL SEMI-MATURATION ON INTESTINAL HOMEOSTASIS

A. Steimle [1], R. Harmening[1], A. Schäfer [1], K. Gronbach [1], J. Müller [1], , T. Reinheckel [3], M. Jucker [4], H. Kalbacher [2], I. B. Autenrieth [1], J.-S. Frick [1]

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

[2] *University of Tübingen, Interfakultäres Institut für Biochemie, Tübingen, Germany*

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

[4] *Hertie-Institut für klinische Hirnforschung, Tübingen, Germany*

Monocolonization of T cell transplanted *Rag1*$^{-/-}$ mice with *Escherichia coli* mpk results in induction of colitis whereas *Bacteroides vulgatus* mpk does not induce and even prevents *E. coli* induced colitis. The protective effect of *Bacteroides 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.

# 21 THE ROLE OF E. COLI NISSLE 1917 MICROCINS IN PREVENTING ADHESION TO EPITHELIAL CELLS AND GROWTH OF EHEC AND EAEC STRAINS

K. Stelzner[1], S. A. Rund[1], T. A. Oelschlaeger[1]

[1]*Institute of Molecular Infection Biology, University of Wuerzburg, Germany*

The probiotic strain *E. coli* Nissle 1917 (EcN) is able to reduce adhesion to epithelial cells and inhibits growth of various pathogenic *E. coli* strains (pEc), e.g. EHEC strains from the outbreak 2011 in Germany. We hypothesized that EcN's antimicrobial peptides, microcin M and H47, are involved in this antagonistic effect. To test this theory we used the isogenic microcin-negative EcN mutant SK22D. Adhesion to Caco-2 epithelial cells after 2 h and growth after 0, 2, 5 and 24 h were determined for single cultures and co-cultures. SK22D was co-incubated with EAEC (O104:H4), EHEC (O157:H7) and EHEC (O104:H4) strains in ratios of 1:1 and 10:1 (SK22D:pEc). The relative adhesion of pEc strains in co-culture with SK22D was compared with the results which were obtained with EcN. We observed a reduction of adhesion of pEc strains in co-culture with SK22D (EAEC: 75%, EHEC O157:H7: 97% and EHEC O104:H4: 81%), but adhesion in 10:1 co-cultures was significantly less reduced compared to the 10:1 co-culture with EcN (EAEC: 99.5%, EHEC O157:H7: 99% and EHEC O104:H4: 95%). EHEC O104:H4 also exhibited significantly less reduction of adherence to Caco-2 cells in a 1:1 co-culture with SK22D (72%) compared to the co-culture with EcN (35%). Furthermore, co-incubation of these pathogenic *E. coli* strains with SK22D compared to EcN showed minor inhibitory effects on growth. We conclude that microcins lead to an inhibition of growth, but play only a minor role in the anti-adhesive effect of EcN on these pEc strains as co-incubation with SK22D still showed a significantly reduced adherence. Other factors involved in this effect are currently under investigation.

# 22 FAECALIBACTERIUM PRAUSNITZII STRAIN HTF-F AND ITS EXTRACELLULAR POLYMERIC MATRIX ATTENUATE CLINICAL PARAMETERS IN DSS-INDUCED COLITIS

Oriana Rossi[1], M. Tanweer Khan[2], Martin Schwarzer[3], Tomas Hudcovic[3], Dagmar Srutkova[3], Sylvia H. Duncan[4], Ellen H. Stolte[1], Hana Kozakova[3], Harry Flint[4], Janneke N. Samsom[5], Hermie J. M. Harmsen[2] and Jerry M. Wells[1]

*[1]Host-Microbe Interactomics Group, ASG, University of Wageningen, NL. [2]Department of Medical Microbiology, UMCG, Groningen, NL.*

*[3]Institute of Microbiology of the Academy of Sciences of the Czech Republic, Prague, Czech Republic.*

*[4]Microbial Ecology Group, Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, UK.*

*[5]Department of Pediatrics, Division of Gastroenterology and Nutrition, Erasmus Medical Center - Sophia Children's Hospital, Rotterdam, NL.*

A decrease in the abundance and biodiversity of intestinal bacteria within the Firmicutes phylum has been associated with inflammatory bowel disease (IBD). In particular, the anti-inflammatory bacterium *Faecalibacterium prausnitzii*, member of the Firmicutes phylum and one of the most abundant species in healthy human colon, is underrepresented in the microbiota of IBD patients. In this study we investigated the capacity of *F. prausnitzii* strain A2-165, the biofilm forming strain HTF-F and the extracellular polymeric matrix (EPM) isolated from strain HTF-F, to suppress inflammation in the mouse dextran sodium sulphate (DSS) colitis model. The two *F. prausnitzii* strains have anti-inflammatory effects in the DSS colitis model. *F. prausnitzii* HTF-F is more effective than A2-165 partly because of the immune-regulating properties of the EPM. The immunomodulatory effects of the EPM are mediated through the TLR2-dependent modulation of IL-12 and IL-10 cytokine production in antigen presenting cells. However, the precise anti-inflammatory mechanism of EPM awaits identification of the active component. Both *F. prausnitzii* HTF-F and the EPM may have a therapeutic use in IBD.

# Participants

# PARTICIPANTS 2013


**Prof. Dr. Ingo Autenrieth**
Universität Tübingen
Institut für Med. Mikrobiologie + Hygiene
Elfriede-Aulhorn-Str. 6
72076 Tübingen
Ingo.Autenrieth@med.uni-tuebingen.de

**Prof. Dr. John Baines**
Christian-Albrechts-University Kiel
AG Evolutionary Genomics - Institute for Experimental Medicine
Arnold-Heller-Str. 3, House 17
D-24105 Kiel
j.baines@iem.uni-kiel.de

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

**Prof. Dr. Christoph Becker**
Universitätsklinikum Erlangen
Medical Department 1
Hartmannstrasse 14
91052 Erlangen
Christoph.Becker@uk-erlangen.de

**Eugenia Becker**
University Hospital Zurich
Gastroenterology
Sternwartstr. 14; LAB H14
8091 Zurich
eugenia.becker@usz.ch

**Markus Beutler**
Max von Pettenkofer-Institut
Bakteriologie
Pettenkoferstrasse 9 a
80336 München
Beutler@mvp.uni-muenchen.de

**Prof. Dr. Michael Blaut**
German Institute of Human Nutrition
Gastrointestinal Microbiology
Arthur-Scheunert-Allee 114-116
14558 Nuthetal
blaut@dife.de

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

**Stefanie Bollmann**
Max von Pettenkofer-Institut
Bakteriologie
Pettenkoferstrasse 9 a
80336 München
bollmann@mvp.uni-muenchen.de

**Sandrine Brugiroux**
Max von Pettenkofer-Institut
Bakteriologie
Pettenkoferstrasse 9 a
80336 München
Brugiroux@mvp.uni-muenchen.de

**Prof. Dr. Nadine Cerf-Bensussan**
INSERM U989
Université Paris Descartes-Sorbonne Paris Centre, Institut Imagine
98 rue Didot
75014 Paris
nadine.cerf-bensussan@inserm.fr

**Dr. Christoph Cichon**
Centre for Molecular Biol. of Inflammation
Institut for Infectiology
Von-Esmarch-Str. 53
48149 Münster
cichon@uni-muenster.de

**Dr. Tom Clavel**
TU München
Lehrstuhl für Ernährung und Immunologie
Gregor-Mendel-Str. 2
85350 Freising
thomas.clavel@tum.de

**Lioba Courth**
IKP Stuttgart

Auerbachstr. 112
70376 Stuttgart
Liobacourth@ikp-stuttgart.de

**Prof. Dr. Andreas Diefenbach**
Uniklinik Freiburg
Microbiology & Molecular Immunology
Hermann-Herder-Str. 11
79104 Freiburg
andreas.diefenbach@uniklinik-freiburg.de

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

**Isabell Flade**
Universität Tübingen
Institut für Med. Mikrobiologie und Hygiene
Elfriede-Aulhorn-Str. 6
72076 Tübingen
isabell.flade@med.uni-tuebingen.de

**Dr. Isabelle Frey-Wagner**
Universitätsspital Zürich
Klinik für Gastroenterologie + Hepatologie
Rämistr. 100
8091 Zurich
Isabelle.Frey@usc.ch

**Prof. Dr. Julia Frick**
Universität Tübingen
Institut für Med. Mikrobiologie und Hygiene
Elfriede-Aulhorn-Str. 6
72076 Tübingen
julia-stefanie.frick@med.uni-tuebingen.de

**Bhanu Priya Ganesh**
German Institute of Human Nutrition
Gastrointestinal Microbiology
Arthur-Scheunert-Allee 114-116
14558 Nuthetal
Bhanu_Priya.Ganesh@dife.de

**Dr. Georg Gradl**
Eurofins MWG Operon
Sales/ NGS
Anzinger Str. 7A
85560 Ebersberg
georggradl@eurofins.com

**Judith Gras**
Uniklinikum Heidelberg
Institut für Immunologie
Im Neuenheimer Feld 305
69120 Heidelberg
Judith.Gras@immu.uni-heidelberg.de

**Prof. Dr. Guntram Graßl**
Universität Kiel/FZ Borstel
Institute for Experimental Medicine
Parkallee 29
23845 Borstel
ggrassl@fz-borstel.de

**Verena Grimm**
University of Ulm
Institute for Microbiology and Biotechnology
Albert-Einstein-Allee 11
89069 Ulm
verena.grimm@uni-ulm.de

**Dr. Hans-Dieter Grimmecke**
Laves Arzneimittel GmbH
Leiter F&E
Lavesstraße
CH-6247 Schötz
d.grimmecke@laves-pharma.ch

**Dr. Kerstin Gronbach**
Universität Tübingen
Institut für Med. Mikrobiologie und Hygiene
Elfriede-Aulhorn-Str. 6
72076 Tübingen
kerstin.gronbach@med.uni-tuebingen.de

**Dr. Claudia Günther**
Universitätsklinikum Erlangen
Medical Department 1
Hartmannstrasse 14
91052 Erlangen
c.guenther@uk-erlangen.de

**Prof. Dr. Dirk Haller**
TU München
Lehrstuhl für Ernährung und Immunologie
Gregor-Mendel-Str. 2
85350 Freising
dirk.haller@tum.de

**Prof. Dr. Nicola Harris**
EPFL-SV-GHI-UPHARRIS
Laboratory of Intestinal Immunology
Station 19
CH - 1015 Lausanne
nicola.harris@epfl.ch

**Prof. Dr. Anton Hartmann**
Helmholtz Zentrum München
Department Microbe-Plant Interactions
Ingolstädter Landstr. 1
85764 Neuherberg
anton.hartmann@helmholtz-muenchen.de

**Dr. Markus M. Heimesaat**
Charité Berlin
Microbiology
Hindenburgdamm 27
12203 Berlin
markus.heimesaat@charite.de

**Stefan Herbel**
Free University Berlin, Veterinary Faculty
Institute of Microbiology and Epizootics
Philippstr. 13
10115 Berlin
Stefan.Herbel@fu-berlin.de

**Dr. Dirk Hofreuter**
Medizinische Hochschule Hannover
Medizinische Mikrobiologie
Carl-Neuberg-Str. 1
30625 Hannover
hofreuter.dirk@mh-hannover.de

**Prof. Dr. Mathias Hornef**
Hannover Medical School
Medical Microbiology
Carl-Neuberg-Str. 1
30625 Hannover
hornef.mathias@mh-hannover.de

**Dr. Nico Jehmlich**
Helmholtz Centre for Environmental Research - UFZ
Department for Proteomics
Permoserstr. 15
04318 Leipzig
nico.jehmlich@ufz.de

**Prof. Dr. Christine Josenhans**
Hannover Medical School
Medical Microbiology and Hygiene
Carl-Neuberg-Str. 1
30625 Hannover
Josenhans.Christine@mh-hannover.de

**Sarah Just**
TU München
Lehrstuhl für Ernährung und Immunologie
Gregor-Mendel-Str. 2
85350 Freising
sarah.just@tum.de

**Melanie Kempf**
ZMBE
Institute of Infectiology
Von-Esmarch-Str. 56
48149 Münster
K_Melanie@gmx.de

**Thorsten Kliem**
Universität Tübingen
Institut für Med. Mikrobiologie und Hygiene
Elfriede-Aulhorn-Str. 6
72070 Tübingen
thorsten.kliem@gmail.com;
Thorsten.Kliem@med.uni-tuebingen.de

**Dr. Martin Koeppel**
Ludwig Maximilians Universität Munich
Max von Pettenkofer-Institut
Pettenkoferstrasse 9 a
80336 München
koeppel@mvp.uni-muenchen.de

**Giray Korkmaz**
Universität Tübingen
Institut für Med. Mikrobiologie und Hygiene
Elfriede-Aulhorn-Str. 6
72076 Tübingen
aligiraykorkmaz@gmail.com

**Prof. Dr. Karsten Kristiansen**
University of Copenhagen
Department of Biology
Ole Maaløes Vej 5
DK-2200 Copenhagen N
kk@bio.ku.dk

**Dr. Tassilo Kruis**
Charité - Med. Klinik I
Gastroenterologie
Hindenburgdamm 30
D - 12200 Berlin
tassilo.kruis@charite.de

**Dr. Claudia Kunst**
University Hospital Regensburg
Internal Medicine 1
Franz-Josef-Strauss Allee 11
93053 Regensburg
claudia.kunst@ukr.de

**Roshni Lakra**
Universität Erlangen/Nürnberg
Medicine Clinic 1
Hartmannstrasse 14
91054 Erlangen
lakra.roshnii@gmail.com

**Anna Lange**
University of Tübingen
Institut für Med. Mikrobiologie und Hygiene
Elfriede-Aulhorn-Str. 6
72076 Tübingen
anna.lange@med.uni-tuebingen.de

**Dr. Simone Lipinski**
CAU Kiel
Institute of Clinical Molecular Biology
Am Botanischen Garten 11
24118 Kiel
S.Lipinski@ikmb.uni-kiel.de

**Dr. Gunnar Loh**
German Institute of Human Nutrition
Gastrointestinal Microbiology
Arthur-Scheunert-Allee 114-116
14558 Nuthetal
loh@dife.de

**Prof. Dr. Alexander Loy**
University of Vienna, Faculty of Life Sciences
Department of Microbial Ecology (DOME)
Althanstrasse 14
A-1090 Wien
loy@microbial-ecology.net

**Lisa Maier**
ETH Zürich
Institute of Microbiology
Wolfgang-Pauli-Str. 10
CH-8093 Zürich
lisa.maier@micro.biol.ethz.ch

**Prof. Dr. Jochen Mattner**
Universitätsklinikum Erlangen
Klinische Mikrobiologie
Wasserturmstraße 3/5
D-91054 Erlangen
jochen.mattner@uk-erlangen.de

**Dr. Marjolein Meijerink**
University Wageningen
Host-Microbe-Interactomics Group
De Elst 1
6708 WD Wageningen, Niederlande
marjolein.meijerink@wur.nl

**Sarah Menz**
Universität Tübingen
Institut für Med. Mikrobiologie und Hygiene
Elfriede-Aulhorn-Str. 6
72076 Tübingen
sarah.menz@med.uni-tuebingen.de

**Prof. Dr. Annick Mercenier**
University Wageningen / Nestle
Host-Microbe-Interactomics Group
De Elst 1
6708 WD Wageningen, Niederlande
annick.mercenier@rdls.nestle.com

**Lubov Nedialkova**
Max von Pettenkofer-Institut
Bakteriologie
Pettenkoferstrasse 9 a
80336 München
Nedialkova@mvp.uni-muenchen.de

**Sören Ocvirk**
TU München
Lehrstuhl für Ernährung und Immunologie
Gregor-Mendel-Str. 2
85350 Freising
soeren.ocvirk@tum.de

**Dr. Tobias Ölschläger**
University Würzburg
Institute for Molecular Infection Biology - IMIB
Josef-Schneider-Str. 2; Bau D15
97080 Würzburg
t.oelschlaeger@uni-wuerzburg.de

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

**Monique Rothe**
German Institute of Human Nutrition
Gastrointestinal Microbiology
Arthur-Scheunert-Allee 114-116
14558 Nuthetal
Monique.Rothe@dife.de

**Harshana Sabharwal**
ZMBE
Institut for Infectiology
Von-Esmarch-Str. 56
48149 Münster
harshana.s@uni-muenster.de

**Sara Schumann**
German Institute of Human Nutrition
Gastrointestinal Microbiology
Arthur-Scheunert-Allee 114-116
14558 Nuthetal
Sara.Schumann@dife.de

**Bruno Sovran**
University Wageningen
Host-Microbe-Interactomics Group
De Elst 1
6708 WD Wageningen, Niederlande
bruno.sovran@wur.nl

**Prof. Dr. Bärbel Stecher**
Max von Pettenkofer-Institut
Bakteriologie
Pettenkoferstrasse 9 a
80336 München
Stecher@mvp.uni-muenchen.de

**Dr. Maureen Ostaff**
IKP Stuttgart

Auerbachstr. 112
70376 Stuttgart
maureen.ostaff@ikp-stuttgart.de

**Dr. Christian Riedel**
Universität Ulm
Institut für Mikrobiologie und Biotechnologie
Albert-Einstein-Allee 11
89081 Ulm
christian.riedel@uni-ulm.de

**Stefan Rund**
University Würzburg
Institute for Molecular Infection Biology - IMIB
Josef-Schneider-Str. 2; Bau D15
97080 Würzburg
stefan.rund@web.de

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

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

**Sabrina Stebe**
IKP Stuttgart

Auerbachstr. 112
70376 Stuttgart
Sabrina.stebe@ikp-stuttgart.de

**Dr. Natalie Steck**
Christian-Albrechts-University Kiel +
Research Center Borstel
Entzündungsmodelle
Parkallee 29
23845 Borstel
nsteck@fz-borstel.de

**Alexander Steimle**
Universität Tübingen
Institut für Med. Mikrobiologie und Hygiene
Elfriede-Aulhorn-Str. 6
72076 Tübingen
alexander.steimle@med.uni-tuebingen.de

**PD Dr. Till Strowig**
Helmholtz-Zentrum für Infektionsforschung

Inhoffenstr. 7
38124 Braunschweig
ab 1.6. till.strowig@helmholtz-hzi.de;
tst13@helmholtz-hzi.de

**Monika Weiher**
TU München
Lehrstuhl für Ernährung und Immunologie
Gregor-Mendel-Str. 2
85350 Freising
monika.weiher@tum.de

**Dr. Stefan Wirtz**
University of Erlangen
Medical Department 1
Hartmannstrasse 14
91052 Erlangen
Stefan.Wirtz@uk-erlangen.de

**Dr. Daria Zhurina**
Universität Ulm
Institut für Mikrobiologie und Biotechnologie
Albert-Einstein-Allee 11
89069 Ulm
daria.zhurina@uni-ulm.de

**Kathrin Stelzner**
University Würzburg
Institute for Molecular Infection Biology - IMIB
Josef-Schneider-Str. 2; Bau D15
97080 Würzburg
kathrin.stelzner@stud-mail.uni-wuerzburg.de

**Alesia Walker**
Helmholtz Zentrum München
Dep. of Environmental Sciences
Ingolstädter Landstr. 1
85764 Neuherberg
alesia.walker@helmholtz-muenchen.de

**Prof. Dr. Jerry Wells**
University Wageningen
Host-Microbe-Interactomics Group
De Elst 1
6708 WD Wageningen, Niederlande
jerry.wells@wur.nl

**Alexandra Wittmann**
Universität Tübingen
Institut für Med. Mikrobiologie und Hygiene
Elfriede-Aulhorn-Str. 6
72076 Tübingen
alexandra.wittmann@med.uni-tuebingen.de