4th Seeon Conference

Microbiota, Probiota and Host
Mikrobiota, Probiota und Wirt

15.- 17. April 2011
Conference Center
Monastery Seeon / Chiemsee

For more information:
DGHM@wzw.tum.de
http://www.dghm.org/red/fachgruppen/

April 15, 2011

Dear Participant,

On behalf of the German Society of Hygiene and Microbiology (DGHM) and the Organizing Committee, welcome to the 4th 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.

The past few years 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. The “Seeon Conference” has become a known platform to critically discuss the role of microbe-host interactions in health and disease sharing cutting-edge science and technologies. Basis mechanisms of the host’s microbiome are discussed at the interface of metabolic and immune functions aiming to be implemented in therapy and prevention of chronic inflammatory, atopic and metabolic diseases.

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

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PROGRAM Friday, April 15

15^{00} - 17^{00}	Registration
17^{00} - 17^{15}	Welcoming (D. Haller, Biofunctionality, TU Munich)

17^{15} – 18^{00}	Keynote Lecture: **A. Walker**, Sanger Institute, Cambridge, UK ***Targeted alteration of the intestinal microbiota: Implications and future prospects for dietary and therapeutic interventions***

18^{15}	Dinner

19^{15}	DGHM Section Meeting

GUT MICROBIOME AND HOST

19^{45}– 21^{00} Chair: M. Blaut, Gastrointestinal Microbiology, DIFE, Nuthetal

N. Steck, Biofunctionality, TU Munich
Bacterial proteases contribute to intestinal inflammation by impairing mucosal barrier function in the susceptible host

E. von Mutius, Dr. von Haunersches Kinderspital, Munich
Protection from childhood asthma and allergies in farming environments

M. Rothe, Gastrointestinal Microbiology, DIFE, Nuthetal
Response of intestinal bacteria to dietary factors in the mouse intestine

F.-A. Heinsen, Institute of Clinical Molecular Biology, University Kiel
Dynamic changes of the human intestinal microbiota during antibiotic perturbation and resilience using sanger- and pyrosequencing

J.F. Baines, Max-Planck-Institute for Evolutionary Biology, Plön
Expression of the glycosyltransferase B4galnt2 influences the intestinal microbiota in mice

21^{00}	Drink at the Bar – An Invitation!

PROGRAM Saturday, April 16

08^{30} – 09^{15} Keynote Lecture: **G. Hansson**, University Gothenburg, Sweden
The two colon mucus layers are organized by the MUC2 mucin and the bacteria has the outer as their habitat

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

PROBIOTIC MECHANISMS

09^{45} – 11^{15} Chair: G. Hörmannsperger, Biofunctionality, TU Munich

M. Meijerink, Host-Microbe Interactomics, Animal Sciences, Wageningen University
Identification of genetic loci in Lactobacillus plantarum that modulate the immune response of dendritic cells using comparative genome hybridization

P. Veiga, Danone Research, Palaiseau, France
Bifidobacterium animalis subsp. lactis fermented milk product reduces inflammation by altering a niche for colitogenic microbes

S. Menz, Institute of Medical Microbiology and Hygiene, University of Tuebingen
Flagellin and tcpC are essential factors of the protective effect of E. coli Nissle strain 1917 in DSS- induced colitis

M. Gleinser, Institute of Microbiology and Biotechnology, University of Ulm
BopA, an adhesin specific for B. bifidum strains

C.-A. Alpert, German Institute of Human Nutrition, Nuthetal
Protection mechanisms in Escherichia coli during gut inflammation

R. Albesharat, Technical Microbiology, TU Munich
Comparative analysis of Lactobacillus plantarum strains isolated from mothers, their milk and their babies

11^{15} – 12^{00} Keynote Lecture: **W. Verstraete**, University Ghent, Belgium
Mucosal and luminal associations of co-evolved gut microbes Beneficially interact with the host

PROGRAM Saturday, April 16

12^{00} - 14^{00} Lunch and Guided Tour through the Church

MECHANISMS OF INFLAMMATION

14^{00} – 15^{30} Chair: J. Wells, Wageningen University, The Netherlands

S. Ocvirk, Biofunctionality, TU Munich
Critical role of ER chaperone Grp78 for the maintenance of intestinal CD8αβ+ T cell homeostasis in chronic intestinal inflammation

A. Wullaert, Institute for Genetics, University of Cologne
Role of epithelial NF-κB signalling in intestinal immune homeostasis

C. Vonarbourg, IMMH, University of Freiburg
Continous loss of RORt expression confers distinct functional fates to lymphoid tissue inducer cell-derived NK cell receptor-expressing lymphocytes

A. Wittmann, Inst. of Microbiology and Infection Medicine, University of Tuebingen
Role of dendritic cell activation as well as Toll-like receptor 2 and 4 expression while DSS colitis

J.H. Niess, Department of Internal Medicine I, University of Ulm
CD69 mediates type I interferon-induced tolerogenic signals to mucosal CD4 T cells that attenuate their colitogenic potential

D. Lissner, Gastroenterologie, Charité Berlin
Adipokines of the mesenteric fat tissue modulate subtype and function of resident macrophages

15^{30} – 16^{00} Coffee Break

16^{00} – 18^{00} **Poster Slam** (2minutes/2slides) **and Posterdiscussion** (J. Frick, Institute for Medical Microbiology + Hygiene, University of Tuebingen)

18^{00} – 18^{45} Keynote Lecture: **A. Kaser**, Cambridge, UK
Endoplasmatic reticulum stress, intestinal inflammation & beyond

18^{45} Dinner

20^{30} Bowling at the Bar

PROGRAM Sunday, April 17

08^{30} – 09^{15} Keynote Lecture: **G. Krishnamoorthy**, Max-Planck-Institute of Neurobiology, Martinsried
Coordinated regulation of pathogenic autoimmune T and B cell Responses by commensal microbiota

09^{15} – 09^{45} Coffee Break

09^{45} – 10^{00} **Poster Awards**

MECHANISMS OF INFECTIONS

10^{00} – 11^{30} Chair: M. Hornef, Med. Microbiology, Hannover Medical School

B. Stecher, Max-von-Pettenkofer Institute, LMU Munich
The microbiota influences horizontal gene transfer between Salmonella commensal and *E. coli*

B.O. Schröder, Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart
Reduction of disulphide bonds unmasks potent antimicrobial activity of hBD-1

A. Hartmann, Department Microbe-Plant Interactions, Helmholtz Center Munich
The Pseudomonas aeruginosa Autoinducer N-(3-Oxododecanoyl) Homoserine Lactone Inhibits Dendritic Cell Functions

U. Grundmann, Institute of Microbiology + Hygiene, Charité Berlin
Analysis of IL-10-/Nod2-double-deficient mice reveals contributions of Muramyl-Dipeptide signalling in chronic colitis and Campylobacter jejuni infections

A.-K. Claes, Institute for Experimental Medicine, Universtity of Kiel
Role of Nod-like receptors in intestinal inflammation

J. Wells, University Wageningen, The Netherlands
The Role of Intestinal RegIIIβ in Bacterial Infection

11^{30} Lunch and Departure

PROGRAM
Friday,
April 15

TARGETED ALTERATION OF THE INTESTINAL MICROBIOTA: IMPLICATIONS AND FUTURE PROSPECTS FOR DIETARY AND THERAPEUTIC INTERVENTION

A.W. Walker

Pathogen Genomics Group, The Wellcome Trust Sanger Institute, Hinxton, UK, aw6@sanger.ac.uk, http://www.sanger.ac.uk/research/projects/pathogengenomics

The human large intestine plays host to an extremely abundant and diverse collection of microbes, which are collectively termed the intestinal microbiota. During the long co-evolution of humans and their intestinal microbiota a generally mutualistic relationship has developed and under normal circumstances our resident microbes are considered to play a number of key roles in the maintenance of human health. However, imbalances or disruption in the composition and/or activity of the intestinal microbiota have been implicated in a number of disorders.

Given the role that the microbiota plays in the maintenance of host health a clear goal for researchers in this area must therefore be to address imbalances in species composition/metabolite production in order to prevent or ameliorate disease. Diet is a potentially important modulating factor and non-digestible carbohydrates provide the major energy source for many intestinal bacteria. However, in vivo studies characterising the response of the intestinal microbiota to strictly controlled dietary changes are lacking. I will therefore discuss recent research that aims to better characterise the response of the microbiota to dietary manipulation and that has identified key bacterial groups that appear to be involved in insoluble substrate degradation.

Recent years have also seen novel insights into the previously underappreciated role the microbiota can play in preventing and suppressing disease caused by both facultatively- and obligately-anaerobic gastrointestinal pathogens. Indeed, pathogens such as Salmonella spp. and Clostridium difficile appear to have developed behavioural strategies in order to outcompete indigenous gut microbes, further highlighting the tripartite nature of gastrointestinal infectious disease, involving the host, the pathogen and the microbiota. I will therefore describe more novel means of restoring bacterial diversity in order to clear anaerobic infection and bring about tangible therapeutic benefits.

GUT MICROBIOME AND HOST

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Expression of the glycosyltransferase B4galnt2 influences the intestinal microbiota in mice

BACTERIAL PROTEASES CONTRIBUTE TO INTESTINAL INFLAMMATION BY IMPAIRING MUCOSAL BARRIER FUNCTION IN THE SUSCEPTIBLE HOST

Steck N.[1], Hoffmann M.[1], Sava I.[1], Kim S.C.[2], Hahne H.[3], Mair K.[4], Vogelmann R.[4], Schemann M.[5], Sartor R.B.[2], and Haller D.[1]

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[2]*Center for Gastrointestinal Biology and Disease, University of North Carolina, Chapel Hill, NC, USA*

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[4]*Technische Universität München, Klinikum rechts der Isar, II. Medizinische Klinik und Poliklinik, Ismaninger Straße 22, 81675 München*

[5]*Technische Universität München, Chair for Humanbiology, Liesel-Beckmann-Straße 2, 85350 Freising-Weihenstephan, Germany*

Background: Proteolytic degradation of extracellular matrix by matrix metalloproteases is a serious consequence of intestinal inflammation. The aim of our study was to investigate whether proteases produced by commensal gut bacteria contribute to the pathogenesis of IBD. For this purpose we focused on the zinc dependent metalloprotease Gelatinase (GelE) from *Enterococcus faecalis* (*E. faecalis*).

Results: Histological analysis (score 0-4) of *E. faecalis*-monoassociated IL-10$^{-/-}$ mice revealed a significant reduction of colonic tissue inflammation in the absence of bacteria-derived GelE. Extracellular E-Cadherin proteindomain was degraded in the presence of GelE. Furthermore, we could indentify cleavage sites for GelE in the sequence of recombinant murine E-Cadherin suggesting the possibility for a GelE- mediated degradation. Ussing chamber experiments with purified GelE revealed the loss of barrier function and extracellular E-Cadherin in mice susceptible to intestinal inflammation (IL-10$^{-/-}$ and TNF$^{\Delta ARE/Wt}$) before tissue pathology has developed. Finally, Ptk6 cells exhibited decreased TER and increased translocation of permeability markers after stimulation with GelE from OG1RF background. We could show specificity for the GelE-mediated loss of barrier function by using another *E. faecalis* protein as unrelated control and the broad spectrum MMP inhibitor Marimastat to block GelE activity.

Conclusion: E. faecalis GelE impairs intestinal mucosal barrier function associated with the degradation of extracellular E-Cadherin proteindomain. Our data clearly demonstrates the role of GelE, as a commensal-derived protease, in the development of intestinal inflammation in the disease-susceptible host.

PROTECTION FROM CHILDHOOD ASTHMA AND ALLERGIES IN FARMING ENVIRONMENTS

E. von Mutius

Dr. von Haunersche Kinderklinik der Universität, Lindwurmstr. 4; D 80337 München

Background: Exposure to farming environments protects from respiratory allergy. This has been shown in a large number of epidemiological studies across the world among children and adults. The timing and duration of exposure seem to play a critical role. The protective factors in these farming environments have not been completely unraveled. Increased levels of microbial substances may at least in part contribute to the protective effects. In prior studies markers of microbial exposure such as endotoxin, muramic acid (peptidoglycan) and extracellular polysaccharides from *Penicillium spp.* and *Aspergillus spp.* have been inversely related to these conditions.

Methods: In two cross-sectional studies farm and reference children were compared with respect the diversity of their microbial exposure. In the PARSIFAL study, mattress dust samples were screened for bacterial exposure using single strand conformation polymorphism (SSCP), a DNA based technique detecting environmental bacteria not accessible by culture techniques. In the GABRIEL Advanced study (GABRIELA), samples of settled dust were evaluated for bacterial and fungal taxa by culture methods.

Results: In both studies, farm children were exposed to a greater variety of environmental microorganisms, which in turn was inversely related to the asthma risk. Within the microbial exposure spectrum the presence of certain more circumscribed exposures were inversely related to asthma; these included exposure to the fungal taxon *Eurotium* sp. and exposure to a variety of bacterial species including *Listeria monocytogenes, Bacillus* sp., *Corynebacterium* sp. and others.

Conclusions: Farm children were exposed to a wider range of microbial exposure than control children; this exposure can explain a substantial fraction of the inverse relation of asthma status and farming. Within the broad microbial exposure spectrum several hot spots have been identified.

RESPONSE OF INTESTINAL BACTERIA TO DIETARY FACTORS IN THE MOUSE INTESTINE

M. Rothe, C. A. Alpert, W. Engst, G. Loh, M. Blaut

German Institute of Human Nutrition, Department of Gastrointestinal Microbiology, Arthur-Scheunert-Allee 114-116, 14558 Nuthetal, Germany

Diet is one of the dominant factors influencing the gut microbiota, but little is known on how dietary composition influences bacterial activities in the intestine and how this in turn affects the host. We used mice monoassociated with *Escherichia coli* MG1655 as a simplified model for host-microbiota interaction to investigate the influence of dietary factors on bacterial protein expression in the intestine. The mice 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. One of the *E. coli* proteins induced on the lactose-rich diet and down-regulated on the casein diet was the 2-deoxy-D-gluconate 3-dehydrogenase (KduD). Luciferase reporter gene assays revealed that the growth on glucuronic acid or galacturonic acid leads to an induction of *kduD*. Therefore, we hypothesize that KduD plays a role in the degradation of uronic acids. Galactose induced *kduD* only under aerobic conditions, while there was no growth under anaerobic conditions. We assume that galactose is oxidized to galacturonate which is then further converted by KduD to 2-keto-3-deoxygluconate. To test this hypothesis we presently generate kduD knock out mutants for further analysis. The lactose-rich diet also led to an induction of proteins involved in *E. coli*'s oxidative stress response (FUR, AhpCF, DPS). The corresponding genes are under control of the OxyR transcriptional dual regulator which is activated by oxidative and other forms of stress. Using luciferase reporter gene assays we demonstrate that osmotic stress exerted by various sugars such as glucose, lactose, sucrose and sorbitol as well as by sodium chloride activates genes of the oxyR regulon.

DYNAMIC CHANGES OF THE HUMAN INTESTINAL MICROBIOTA DURING ANTIBIOTIC PERTURBATION AND RESILIENCE USING SANGER- AND PYROSEQUENCING

F.-A. Heinsen, A. Rehman, S. Schreiber, S. Ott

Institute of Clinical Molecular Biology (ICMB), Christian-Albrechts-University of Kiel, Schittenhelmstr. 12, 24105 Kiel, Germany, f.heinsen@ikmb.uni-kiel.de

The microbial community in the normal human gut is individual specific, remains stable over a period of time and usually exerts a high self-regeneratory capacity (resilience phenomenon) after external perturbation. Compositional changes can be observed during/after antibiotic treatment although the resilience phenomenon persists. The aim of the current study is to investigate the self-regeneratory capacity of the colonic microbiota. Perturbation of the microbiota was carried out by an antibiotic agent (Humatin®) and the regeneration was monitored with and without probiotic (VSL#3®) administration. 16S rRNA gene (sanger-sequencing from stool samples and pyrosequencing from sigmoid biopsy samples) and 16S rRNA (pyrosequencing from sigmoid biopsy samples) based culture independent molecular techniques were applied. Antibiotic dosage resulted in a significant increase of *Bacteroides* and a significant decrease of *Ruminococcus*, *Dialister* and *Faecalibacterium* in fecal microbiota. A significant decrease in present *Faecalibacterium* could be detected in the mucosa-attached microbiota. After allocation to probiotics/placebo, we observed a significantly lower amount of *Clostridium* and a significantly higher amount of *Streptococci* in the probiotic group compared to placebo in the fecal microbiota. However, in biopsies there could not be detected any significant differences between placebo and probiotics on genus level so far.

EXPRESSION OF THE GLYCOSYLTRANSFERASE B4GALNT2 INFLUENCES THE INTESTINAL MICROBIOTA IN MICE

J.F. Baines[1,2], F. Staubach[1,3], S. Künzel[1], F. Bäckhed[4], J.M. Johnsen[5]

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[2] *Institute for Experimental Medicine Christian-Albrechts-University of Kiel, Germany*

[3] *Department of Biology, Stanford University, Stanford CA, 94305-5020; USA*

[4] *Department of Molecular and Clinical Medicine, Sahlgrenska Center for Cardiovascular and Metabolic Research/Wallenberg Laboratory, University of Gothenburg; Sweden*

[5] *Puget Sound Blood Center and Department of Internal Medicine, University of Seattle, WA, USA*

Glycans on mucosal surfaces play an important role in host-microbe interactions. The locus encoding the glycosyltransferase β-1,4-N-acetylgalactosaminyltransferase 2 (B4galnt2) is subject to strong selective forces in natural house mouse populations which contain a common allelic variant which specifically turns off gene expression in bowel. We reasoned that altered glycan-dependent intestinal host-microbe interactions may underlie these signatures of selection. To determine if B4galnt2 influences the intestinal microbial ecology, we profiled wild type and B4galnt2-deficient siblings throughout the GI tract using 16S rRNA gene pyrosequencing. This revealed both distinct communities at different anatomic sites and significant changes in composition with respect to genotype, indicating a role for B4galnt2 in host-microbial homeostasis. Interestingly, in contrast to other glycosyltransferases, B4galnt2 expression is not dependent on presence of the microbiota. Our data suggest that variation in B4galnt2 gastrointestinal expression affects fitness in natural populations, likely by altering susceptibility to gastrointestinal disease, such as infectious gastroenteritis.

PROGRAM
Saturday, April 16

THE TWO COLON MUCUS LAYERS ARE ORGANIZED BY THE MUC2 MUCIN AND THE BACTERIA HAS THE OUTER AS THEIR HABITAT

G.C. Hansson

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Colon is covered with a single layer of active cells that have to have additional protection systems. The large intestine is especially difficult to protect as it harbors an enormous amount of bacteria. This was difficult to understand until we recently could show that the inner of the two mucus layers of colon are impermeable to bacteria. These mucus layers are built around the large MUC2 mucin, a protein that is assembled into enormous net-like polymers that can form a gel. These net-like polymers are secreted by the goblet cells together with a number of other components that we have discovered by proteomics. Several of these are likely to be important for building a stable inner mucus layer that is attached to the epithelia. The inner mucus layer is renewed in 1-2 hours and converted to an outer layer that is expanded in volume four times. This outer layer is the habitat for the commensal bacterial flora.

Colon bacteria are typical for the host species, something that is not understood today. Our observation of a uniform glycosylation of the MUC2 mucin among humans may provide a first clue to how this can work. If one assumes that the commensal flora typical for their host carries adhesins specific for the host glycans.

Bacteria are using the mucins as a food source, typically removing one monosaccharide at a time. In mice with truncated mucin O-glycans, the mucins is less efficient in protecting the epithelium. If the MUC2 mucin is less glycosylated of totally absent, the inner mucus layer is defective or absent. If this is the case, bacteria come in contact with the epithelia, penetrate into the crypts and into the cells. This causes inflammation, bloody diarrhea and later on colon cancer, identical to what is observed in the disease ulcerative colitis. The concept and implications of the mucus layers for the protection of colon and the bacterial habitat will be discussed.

PROBIOTIC MECHANISMS

09^{45} – 11^{15} Chair: G. Hörmannsperger, Biofunctionality, TU Munich

M. Meijerink, Host-Microbe Interactomics, Animal Sciences, Wageningen University
Identification of genetic loci in Lactobacillus plantarum that modulate the immune response of dendritic cells using comparative genome hybridization

P. Veiga, Danone Research, Palaiseau, France
Bifidobacterium animalis subsp. lactis fermented milk product reduces inflammation by altering a niche for colitogenic microbes

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Flagellin and tcpC are essential factors of the protective effect of E. coli Nissle strain 1917 in DSS- induced colitis

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BopA, an adhesin specific for B. bifidum strains

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Protection mechanisms in Escherichia coli during gut inflammation

R. Albesharat, Technical Microbiology, TU Munich
Comparative analysis of Lactobacillus plantarum strains isolated from mothers, their milk and their babies

IDENTIFICATION OF GENETIC LOCI IN LACTOBACILLUS PLANTARUM THAT MODULATE THE IMMUNE RESPONSE OF DENDRITIC CELLS USING COMPARATIVE GENOME HYBRIDIZATION

M. Meijerink[1,2], S. van Hemert[1,3,a], N. Taverne[1,2], M. Wels[1,3], P. de Vos[1,4], P.A. Bron[1,3], H.F. Savelkoul[1,5], J. van Bilsen[1,6], M. Kleerebezem[1,3,7], J.M. Wells[1,2,*]

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Background: Probiotics can be used to stimulate or regulate epithelial and immune cells of the intestinal mucosa and generate beneficial mucosal immunomodulatory effects. Beneficial effects of specific strains of probiotics have been established in the treatment and prevention of various intestinal disorders, including allergic diseases and diarrhea. However, the precise molecular mechanisms and the strain-dependent factors involved are poorly understood. Dendritic cells (DCs) play a major role in orchestrating the responses of innate and adaptive cells to control tolerance and immunity to microbes encountered at mucosal surfaces. *Lactobacillus* are naturally present in the human intestinal tract and several species and strains have been evaluated for their probiotic activity. Conclusive evidence for the mechanisms underlying the beneficial properties of probiotics is lacking but results obtained from *in vitro* studies and animal intervention models indicate a strong role for immunomodulation and enhancement of the epithelial barrier functions. In the small intestine DCs are known to sample microbes that gain access to the Peyer's Patches via M-cells but

also directly across the epithelium by opening tight junctions and sending dendrites to the luminal side.

Methods: DC cytokine responses to several species of probiotics can be strikingly different and significant variation is also seen at the strain level. This could account for the strain-dependent properties of probiotics reported in different clinical trials and animal models. In this study, we aimed to identify gene loci in the model probiotic organism *Lactobacillus plantarum* WCFS1 that modulate the immune response of host dendritic cells. The amounts of IL-10 and IL-12 secreted by dendritic cells (DCs) after stimulation with 42 individual *L. plantarum* strains were measured and correlated with the strain-specific genomic composition using comparative genome hybridisation and the Random Forest algorithm.

Results: This *in silico* “gene-trait matching” approach led to the identification of eight candidate genes in the *L. plantarum* genome that might modulate the DC cytokine response to *L. plantarum*. Six of these genes were involved in bacteriocin production or secretion, one encoded a bile salt hydrolase and one encoded a transcription regulator of which the exact function is unknown. Subsequently, gene deletions mutants were constructed in *L. plantarum* WCFS1 and compared to the wild-type strain in DC stimulation assays. All three bacteriocin mutants as well as the transcription regulator (lp_2991) had the predicted effect on cytokine production confirming their immunomodulatory effect on the DC response to *L. plantarum.* Transcriptome analysis and qPCR data showed that transcript level of *gtcA3,* which is predicted to be involved in glycosylation of cell wall teichoic acids, was substantially increased in the *lp_2991* deletion mutant (44 and 29 fold respectively).

Discussion: In silico gene-trait matching can be used in assessing the role of specific bacterial genes in the interaction with the host immune system, an approach that is fully supported by the recent availability of full genome sequences for some lactobacilli. In the future this knowledge may be useful to select probiotic strains with anti-inflammatory or immune stimulatory properties.

BIFIDOBACTERIUM ANIMALIS SUBSP. LACTIS FERMENTED MILK PRODUCT REDUCES INFLAMMATION BY ALTERING A NICHE FOR COLITOGENIC MICROBES

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Intestinal health requires the coexistence of eukaryotic self with the gut microbiota and dysregulated host-microbial interactions can result in intestinal inflammation. Here, we show that colitis improved in T-bet$^{-/-}$Rag2$^{-/-}$ mice that consumed a fermented milk product containing *Bifidobacterium animalis* subsp. *lactis* DN-173 010 strain. A decrease in cecal pH and alterations in short chain fatty acid profiles occurred with consumption, and there were concomitant increases in the abundance of select lactate-consuming and butyrate-producing bacteria. These metabolic shifts created a non permissive environment for the *Enterobacteriaceae* recently identified as colitogenic in a T-bet$^{-/-}$Rag2$^{-/-}$ ulcerative colitis mouse model. In addition, 16S rRNA-based analysis of the T-bet$^{-/-}$Rag2$^{-/-}$ fecal microbiota suggest that the structure of the endogenous gut microbiota played a key role in shaping the host response to the bacterial strains studied herein. We have identified features of the gut microbiota, at the membership and functional level, associated with response to this *B. lactis*-containing fermented milk product, and therefore this model provides a framework for evaluating and optimizing probiotic-based functional foods.

FLAGELLIN AND TCPC ARE ESSENTIAL FACTORS OF THE PROTECTIVE EFFECT OF E. COLI NISSLE STRAIN 1917 IN DSS- INDUCED COLITIS

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

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Background: 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. We studied in a preclinical model of acute colitis whether EcN protects from disease and analysed the bacterial mechanism underlying the anti-inflammatory capacity.

Methods: C57BL/6 and *TLR5*$^{-/-}$ mice were fed with either EcN or EcNΔfliC or EcNΔtcpC and treated with 3, 5% DSS. Body weight and disease activity index were assessed daily. At the end of the experiment the colon length and weight was measured and inflammation was determined by histological analyses of the colon. Furthermore activation and maturation of lamina propria and mesenteric lymph node dendritic cells and T cells was analysed.

Results: In wild type mice *E. coli* Nissle protects from DSS induced colitis whereas the protection is reduced in *TLR5*$^{-/-}$ mice. In line with this the ΔfliC mutant strain was less effective in protecting the host from disease as compared to the EcN wild type strain. However a second bacterial factor *tcpC* also contributes to the protective effect of EcN as the ΔtcpC mutant strain was not able to protect from disease. Administration of the double mutant ΔfliCΔtcpC of EcN evidences this conclusion.

Conclusions: EcN ameliorates a DSS induced acute colitis via flagellin and the secreted protein *tcpC*. However contribution of further bacterial factors to the anti-inflammatory effect of EcN can not be excluded.

BopA, an adhesin specific for B. bifidum strains

M. Gleinser, C.U. Riedel

Institute of Microbiology and Biotechnology, University of Ulm

Bifidobacteria represent an important group of the human gut microbiota. Various strains have been shown to have probiotic properties including inhibition of pathogens, reinforcement of intestinal barrier function and anti-inflammatory effects. Adhesion to intestinal epithelial cells could provide these bacteria with the capacity to colonize the host for prolonged periods. However, the molecular mechanisms of adhesion are largely unknown.

Here, we report the analysis of BopA, a cell surface protein and its role in adhesion to intestinal epithelial cells of different strains of *B. bifidum*. Our results indicate that *bopA* is exclusively present in strains of *B. bifidum* and is absent in all other species of bifidobacteria tested. Interestingly, strains of *B. bifidum* show consistently higher adhesion to cultured intestinal epithelial cells (IECs). Furthermore, the *bopA* is expressed under the conditions used to cultivate bacteria for adhesion experiments. We cloned the gene encoding BopA with a His-tag fused to its C-terminus (BopA-His) and successfully expressed the fusion protein in *E. coli* BL21(DE3). Subsequently, BopA-His was purified by Ni-NTA affinity chromatography. Purified BopA-His protein was able to reduce adhesion of intact bacteria of *B. bifidum* S17 to HT-29, Caco-2 and T84 IECs.

PROTECTION MECHANISMS IN ESCHERICHIA COLI DURING GUT INFLAMMATION

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Increased numbers of *Escherichia coli* are observed in Crohn's disease and ulcerative colitis. We aim to identify how *E. coli* adapts to intestinal inflammation and how this in turn affects the host.

In our model system mice were monoassociated either with the colitogenic *E. coli* UNC or with the probiotic *E. coli* Nissle (EcN) and inflammation was induced by dextran sodium sulfate (DSS). Differentially expressed bacterial proteins were identified by 2D-difference gel electrophoresis followed by ESI-MS/MS.

Most of the proteins down-regulated after DSS-treatment belong to the central energy metabolism. Despite similar carbohydrate concentrations in the caecal water, the concentrations of the bacterial fermentation products succinate, formate and lactate were reduced in the inflamed animals.

Interestingly the Fe/S biogenesis protein NfuA was 3.75-fold (EcN) and 3.13-fold (UNC) up-regulated in both strains after DSS-administration. It plays a crucial role in the maturation of iron-sulfur proteins. Reactive oxygen species produced during inflammation damage Fe/S clusters and thereby inactivate the corresponding proteins. Our results indicate that the maturation of iron-sulfur proteins by NfuA is a central mechanism in *E. coli* to repair proteins under inflammatory conditions.

A potential fitness factor for EcN is the uncharacterized protein YggE, which was up-regulated in EcN vs. UNC both under DSS-treatment and control conditions. Previous publications revealed that YggE is capable of reducing the intracellular level of reactive oxygen species. We now investigate the molecular mechanism underlying these observations.

COMPARATIVE ANALYSIS OF LACTOBACILLUS PLANTARUM STRAINS ISOLATED FROM MOTHERS, THEIR MILK AND THEIR BABIES

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The large intestine is sterile at birth, but becomes rapidly colonized with bacteria. As it is likely influenced by the microbiota of the mother, we comparatively typed lactic acid bacteria in breast milk and faeces of healthy mothers, in her infants faeces, and in local fermented food. A total of 300 Isolates were genotypically typed by RAPD-PCR and identified by 16S rDNA sequence analyses revealing 36 different species of *Lactobacillus*, *Enterococcus, Weissella, Streptococcus and Pediococcus. L. plantarum* strains from mother/baby-pairs were characterized towards their growth behaviour on different media presenting different stresses (acid or bile salt, H_2O_2 and Paraquat), the presence of *kat* and *nox* genes, and expression of superoxide dismutase, α-galactosidase and β-galactosidase activities, and H_2O_2 production. Some species were unique for one of the three sources, while others were found in all sources. Identical genotypes of *L. plantarum, L. fermentum, L. brevis, E. faecium* and *P. pentosaceus* were found in the mother's faeces, her milk and the corresponding baby faeces. *L. plantarum* strains isolated from all three sources of the same mother/baby-pairs show similar growth behavior under different stress conditions, gene presence and enzymatic settings. All mother/baby-pair strains have high tolerance for oxidative and acid stress. Strains from one mother/baby-pair shared some special traits delineating them from other mother/baby-pairs. Identical RAPD-genotypes of *L. plantarum* strains isolated from fermented food *versus* from mother/baby-pairs were rare.

We suggest that such *L. plantarum* strains are candidates for a possible transfer from a mother to her baby through breastfeeding or intestinal contamination and are rare different from the majority of strains conducting food fermentation.

MUCOSAL AND LUMINAL ASSOCIATIONS OF CO-EVOLVED GUT MICROBES BENEFICIALLY INTERACT WITH THE HOST

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Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Coupure Links 653, B-9000 Ghent, Belgium (www.labmet.ugent.be)

Along the human gastrointestinal tract, microbes are confronted with multiple barriers. Besides selective physical conditions, the epithelium is regularly replaced and covered with a protective mucus layer trapping immune molecules, so that the host particularly selects the mucosa-associated microbiota. Moreover, microbes themselves differ in their adhesion capacity to mucus, resulting in a unique mucosal microbiota with a great potential to interact with the host. In this context, humans co-evolved with thousands of microbial species that have adapted to provide host benefits, while avoiding pathogenic behavior that might destabilize their host interaction. While mucosal microbes would be crucial for immunological priming, luminal microbes would be important for nutrient digestion. We hypothesize that the intestinal microbes also co-evolved with each other leading to coherently organized, resilient microbial associations. During disturbances, functionally redundant members become more abundant and are crucial for preserving community functionality. The outside of the mucus layer, where host defense molecules are more diluted, could serve as an environment where microbes are protected from disturbances in the lumen and from where they can re-colonize after perturbations. This might explain the marked temporal stability of microbial communities. Further, commensals that become renegade or a decreased exposure to essential co-evolved microbes may cause health problems such as inflammatory bowel diseases, obesity or allergies.

Our lab has developed a dynamic multi-compartment *in vitro* simulator to monitor gut microbial dynamics and activity, the Simulator of the Human Intestinal Microbial Ecosystem (SHIME). We demonstrated that the different colon regions harbour a microbial community that upon inoculation evolves in a diverse and dynamic way. This community is colon-region specific and relevant compared to *in vivo* conditions. Moreover, we incorporated a mucosal environment within this model and demonstrated its importance for the colonization of lactobacilli. Incorporation of a mucosal environment allowed colonization of specific microbes, in correspondence with the *in vivo* situation. It will be interesting to unravel how other microbial group colonize this *in vitro* model in order to obtain a more *in vivo*-like luminal and mucosal microbial community composition and activity.

MECHANISMS OF INFLAMMATION

14^{00} – 15^{30} Chair: J. Wells, Wageningen University, The Netherlands

S. Ocvirk, Biofunctionality, TU Munich
Critical role of ER chaperone Grp78 for the maintenance of intestinal CD8αβ+ T cell homeostasis in chronic intestinal inflammation

A. Wullaert, Institute for Genetics, University of Cologne
Role of epithelial NF-κB signalling in intestinal immune homeostasis

C. Vonarbourg, IMMH, University of Freiburg
Continous loss of RORt expression confers distinct functional fates to lymphoid tissue inducer cell-derived NK cell receptor-expressing lymphocytes

A. Wittmann, Inst. of Microbiology and Infection Medicine, University of Tuebingen
Role of dendritic cell activation as well as Toll-like receptor 2 and 4 expression while DSS colitis

J.H. Niess, Department of Internal Medicine I, University of Ulm
CD69 mediates type I interferon-induced tolerogenic signals to mucosal CD4 T cells that attenuate their colitogenic potential

D. Lissner, Gastroenterologie, Charité Berlin
Adipokines of the mesenteric fat tissue modulate subtype and function of resident macrophages

CRITICAL ROLE OF ER CHAPERONE GRP78 FOR THE MAINTENANCE OF INTESTINAL CD8AB+ T CELL HOMEOSTASIS IN CHRONIC INTESTINAL INFLAMMATION

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Defects in the endoplasmic reticulum (ER) stress associated mechanisms were recently implicated in the development of inflammatory bowel disease (IBD). We previously demonstrated in the $TNF^{\Delta ARE/+}$ mouse model for Crohn's disease-like ileitis that aberrant expression of ER chaperone Grp78 sensitizes intestinal epithelial cell (IEC) death. This is attributed to an aberrant cytotoxic CD8αβ+ IEL phenotype preferentially accumulating in the epithelium. In this study, we characterized the role of ER stress associated mechanisms that contribute to intestinal T cell homeostasis. We identified a critical activity of Grp78 as T cell intrinsic factor that mediated CD8αβ+IEL homeostasis in $TNF^{\Delta ARE/+}$ mice.

An increased expression of Grp78 and the proximal ER stress transducers ATF6, ATF4 and spliced XBP1 was identified at the transcript level in CD8αβ+ but not CD8αα+ IEL or lamina propria lymphocytes from inflamed $TNF^{\Delta ARE/+}$ mice. Subsequent promoter analysis in CD8αβ+T cells showed selective recruitment of ER stress transducers to the gene promoter of granzyme B. Providing evidence for a critical activity of Grp78 in maintaining a cytotoxic phenotype, heterozygous $Grp78^{-/+}$ mice revealed an attenuated granzyme B-dependent cytotoxicity of CD8αβ+ T cells against IEC. A deficient granzyme B production was associated with a defect in IL2-mediated proliferation of CD8αβ+ T cells. Adoptively transferred $Grp78^{-/+}$ CD8αβ+ T cells showed a decreased frequency to accumulate in the intestine of $RAG2^{-/-}$ recipient mice. This suggests that Grp78 intrinsically controls intestinal T cell homeostasis and promotes uncontrolled CD8αβ+IEL cytolytic activity against IEC that may further exacerbate disease manifestation in chronic intestinal inflammation.

ROLE OF EPITHELIAL NF-κB SIGNALLING IN INTESTINAL IMMUNE HOMEOSTASIS

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Although the intestine contains trillions of bacteria that are recognised by Toll-like Receptors (TLRs), the mucosal immune system stays hyporesponsive towards the gut microflora. Intestinal epithelial cells (IEC) form a physical barrier between the gut lumen and the mucosa, preventing the interaction of microflora with mucosal immune cells. Epithelial barrier disruption and immune responses to the microflora are thought to be key factors in the development of Inflammatory Bowel Diseases (IBD).

We are investigating bacterial-induced NF-κB activation in intestinal immune homeostasis by means of conditional gene targeting in mice. We have shown that NF-κB activation is essential for maintaining intestinal immune homeostasis, as mice lacking NEMO, an essential molecule for activating NF-κB, in IECs ($NEMO^{IEC\text{-}KO}$) mice spontaneously develop severe colitis. Germfree conditions rescue $NEMO^{IEC\text{-}KO}$ mice from colonic inflammation, indicating that commensals are colitogenic in these mice. However, studies in the DSS-induced colitis model suggested that TLR signaling initiated by intestinal microbiota is protective in mice. In order to specifically investigate the role of the microflora-induced NF-κB signalling in the intestine, we generated mice that allow cell type specific inactivation of TLR signalling. We used these mice to disable TLR-induced NF-κB signalling specifically in IECs and thus to elucidate the role of IEC-specific TLR signalling in intestinal immune homeostasis and the DSS-induced model of colitis.

CONTINOUS LOSS OF RORγT EXPRESSION CONFERS DISTINCT FUNCTIONAL FATES TO LYMPHOID TISSUE INDUCER CELL-DERIVED NK CELL RECEPTOR-EXPRESSING LYMPHOCYTES

C. Vonarbourg[1], A. Mortha[1], V.L. Bui[1,2], P. Hernandez[1,3], T. Hoyler[1], M. Flach[1], B. Bengsch[4], R. Thimme[4], M. Hönig[5], D. Finke[6] and A. Diefenbach[1,2,3]

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Natural killer (NK) cells and lymphoid tissue inducer (LTi) cells are two principal lymphocyte subsets within the innate immune system. Recently, a novel lymphocyte population expressing NK cell receptors (NKR) and RORγt has been identified in the intestinal mucosa. Using genetic lineage tracing of RORγt-expressing cells, adoptive transfer and *in vitro* differentiation assays, we demonstrate that LTi cells exclusively differentiate into NKR^+ cells that we have tentatively named NKR-LTi cells. LTi cells differentiated in a two-step program from $ROR\gamma t^+$ LTi cells into $ROR\gamma t^+$ NKR-LTi cells that consecutively turned off RORγt to become $ROR\gamma t^-$ NKR-LTi cells. While $ROR\gamma t^+$ LTi cells and $ROR\gamma t^+$ NKR-LTi cells were producers of IL-22 and IL-17A, $ROR\gamma t^-$ NKR-LTi cells did not produce either of these cytokines but expressed IFN-γ. Importantly, IFN-γ-producing $ROR\gamma t^-$ NKR-LTi cells but not NK cells were central mediators of experimental colitis. These data reveal a previously unappreciated plasticity within the LTi cell lineage.

ROLE OF DENDRITIC CELL ACTIVATION AS WELL AS TOLL-LIKE RECEPTOR 2 AND 4 EXPRESSION WHILE DSS COLITIS

A. Wittmann, I.B. Autenrieth and J.-S. Frick

Institute of Microbiology and Infection Medicine, University of Tübingen

Hosts live normally in symbiosis with their intestinal microbiota due to a long coevolution. Somehow this tolerance is abolished in IBD patients, which leads to a strong and long lasting inflammation irregularly interrupted by short remission phases. In order to investigate the role of dendritic cells and their TLR2 and TLR4 expression while acute phase inflammation the Dextran sodium sulfate (DSS) model was employed. Therefore DSS was administered to C57BL/6 mice for 7 days. Mice displayed significant weight loss and an increasing disease activity index. Lamina propria (LP) and mesenteric lymph node (MLN) DCs were isolated of untreated mock and DSS treated mice. LP DCs of DSS treated mice owned significantly increased levels of MHCII, CD40, CD80 and CD86 representing a more activated DC state as compared to DCs of mock mice, whereas MLN DCs did not differ concerning their activation state. Furthermore DSS mice featured higher levels of myeloid DCs in the LP as well as in the MLN compared to mock mice. As well had DSS mice a significantly increased percentage of CD103+ DCs in the LP in comparison to mock mice, whereas amounts of CD103+ DCs in MLN were vice versa. Surprisingly LP and MLN DCs of DSS mice expressed significantly higher amounts of TLR2 and TLR4 in comparison to mock mice. Increased appearance of CD103+ DCs and higher amounts of TLR2 and TLR4 are likely to be a counter regulation of the host in order to suppress developing inflammation.

CD69 MEDIATES TYPE I INTERFERON-INDUCED TOLEROGENIC SIGNALS TO MUCOSAL CD4 T CELLS THAT ATTENUATE THEIR COLITOGENIC POTENTIAL

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Background and Aims: The early activation antigen CD69 is highly expressed by CD4 T cells isolated from the intestinal tissues. Type I IFN promote CD69 surface expression by lymphocytes. Protective effects of type I IFN have been shown in colitis models. We aimed to investigate the role of CD69 in mucosal immune responses.

Methods: The expression of CD69 by CD4 T cells isolated from the small intestinal (siLP) and colonic lamina propria (cLP) was determined in specific pathogen free (SPF), germ-free (GF) B6 mice and T cell receptor (TCR) transgenic animals. The CD69 dependent pathways were mapped by microarray data set analysis.

Results: Highest CD69 expression by CD4 T cells was observed in siLP and cLP as compared to mesenteric lymph nodes (MLN) or spleen. In GF mice the absence of the intestinal microflora is associated with reduced CD69 expression. Oral challenge of OT-II x $RAG^{-/-}$ animals with OVA induced CD69 expression by CD4 T cells. $CD69^{+}$ CD4 T cells are characterized by LAP/TGF-ß expression. The TGF-ß pathway was identified as CD69-dependent pathway by analysis for enrichment of the microarray data sets in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database. The transfer of $CD69^{-/-}$ $CD45RB^{high}$ CD4 T cells into $RAG^{-/-}$ hosts induced an accelerated colitis as compared to transfer of B6 $CD45RB^{high}$ CD4 T cells. The treatment of B6 and OT-II x $RAG^{-/-}$ animals with the type I IFN inducer, poly (I:C), induced CD69 expression on CD4 T cells but not in IFN-I receptor 1-deficient ($IFNAR^{-/-}$) animals. Oral tolerance is impaired in $CD69^{-/-}$ and $IFNAR^{-/-}$ mice when compared to B6 and OT-II x $RAG^{-/-}$ animals. Poly (I:C) treatment of $RAG^{-/-}$ mice transplanted with B6 but not $CD69^{-/-}$ or $IFNAR^{-/-}$ CD4 T cells attenuated transfer colitis.

Conclusions: The activation antigen CD69 plays an important role in regulating mucosal immune responses to the commensal microflora. CD69 activation induced the production of immunosuppressive cytokine TGF-β supporting the establishment of oral tolerance and attenuation of colitis and could contribute to the protective effects of type I IFN in colitis models.

ADIPOKINES OF THE MESENTERIC FAT TISSUE MODULATE SUBTYPE AND FUNCTION OF RESIDENT MACROPHAGES

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Aim: The important role of the mesenteric fat tissue in intestinal inflammation has been elucidated in recent years. Macrophage invasion into this fat tissue can be found in adipose patients as well as in patients with IBD. Thus, the aim of our study was to evaluate subtype and function of these cells.

Materials and Methods: M1- and M2-macrophages in the mesenteric fat tissue of patients with Crohn's disease (CD), ulcerative colitis (UC), colorectal carcinoma (CRC) and healthy controls were analyzed by immunohistochemistry. Peripheral blood monocytes were polarized into M1- and M2-macrophages and the effect of LPS and adipokines on cytokine expression, inflammasome activation and chemotactic potency was characterized using ELISA, RT-PCR and transmigration assays.

Results: In the mesenteric fat tissue of CD patients, a profoundly increased number of CD68+ macrophages was found. These showed a high CD163 and Stabilin1 expression, suggesting an accumulation of M2-macrophages. In vitro, both M1- and M2–macrophages showed a higher cytokine expression when stimulated with leptin (M1-macrophages upregulated TNFα and IL-8 levels, M2-macrophages IL-10 and IL-6, respectively). This enhancing effect of leptin was seen much stronger in M2-macrophages. These cells also significantly increased expression of inflammasome components (predominantly IL-1ß) after stimulation with LPS and, to a lesser extent, leptin, as seen on RNA- and protein-level. Both subpopulations were able to attract CD4+ cells in vitro, however, leptin additionally increased the chemotactic potency of M2-macrophages.

Conclusion: Our data provide strong evidence for a unique M2-macrophage subtype within the mesenteric fat tissue of CD patients, whose function is regulated by the local adipokine milieu.

ENDOPLASMIC RETICULUM STRESS, INFLAMMATION, AND BEYOND

A. Kaser

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Endoplasmic reticulum (ER) stress arises secondary to the presence of unfolded or misfolded proteins in the ER, and initiates and adaptive response, the Unfolded Protein Response (UPR), which temporarily halts protein translation and transactivates UPR target genes. The intestinal epithelium is a highly secretory organ, in particular Paneth cells, which produce antimicrobial peptides, and goblet cells, which produce mucin. Hypomorphic function of the evolutionary most conserved effector arm of the UPR, X-box binding protein-1 (XBP1) within the intestinal epithelium results in the spontaneous small intestinal inflammation closely resembling human inflammatory bowel disease (IBD). The *XBP1* locus and hypomorphic rare variants of this gene have been associated with genetic risk for both forms of IBD, Crohn's disease and ulcerative colitis. In addition, genome-wide association studies (GWAS) have discovered additional genetic IBD risk loci that functionally map to the ER stress response. A hallmark of hypomorphic or absent XBP1 function within the intestinal epithelium is a profound hyperreactivity toward microbial molecules (like TLR ligands) and toward inflammatory mediators secreted from mucosal cells. Deletion of *Xbp1* depletes Paneth cells, resulting in a functional impairment in handling orally infected bacterial model organisms, which allows to predict a structural alteration of the commensal microbiota in the presence of a hypomorphic UPR. The UPR hence may play a major role in affecting the intestinal bacterial constituents, while at the same time determining the inflammatory reactivity of the epithelium toward these constituents, and the capacity to regulate intestinal epithelial turnover.

PROGRAM
Sunday,
April 17

COORDINATED REGULATION OF PATHOGENIC AUTOIMMUNE T AND B CELL RESPONSES BY COMMENSAL MICROBIOTA

K. Berer[1], M. Mues[1], M. Koutrolos[1], Z. Al-Rasbi[1], M. Boziki[1], A. Imaoka[3], Y. Umesaki[3], C. Johner[2], H. Wekerle[1], G. Krishnamoorthy[1]

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[2]*Max-Planck Institute of Immunobiology, 79108 Freiburg, Germany;*
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The nature of the trigger(s) that initiate Multiple sclerosis (MS) in people with genetic disease susceptibility remains elusive. Environmental factors have been suspected to trigger disease, but neither a specific microbial agent nor a triggering mechanism has been clearly established. Here we show that, using a TCR transgenic model that develops spontaneous relapsing-remitting (RR) experimental autoimmune encephalomyelitis (EAE; RR mice[1]), commensal gut flora is critically required for the disease development. While RR mice housed under SPF-conditions developed EAE within 3-8 months, disease was absent in mice housed under germ-free (GF) conditions. First, the gut flora drives the differentiation of intestinal transgenic myelin oligodendrocyte glycoprotein (MOG)-specific T cells, into IL-17 producing T_H17 lineage. Surprisingly, however, segmented filamentous bacteria (SFB), which were demonstrated to efficiently induce T_H17 differentiation and trigger autoimmunity in other models, play only a minor role in RR mouse. Elimination of SFB by treating RR mice with vancomycin did neither have an effect on the frequency of T_H17 cells nor prevent spontaneous EAE development. In addition, mono-colonization of germfree RR mice with SFB failed to induce T_H17 cells and moreover, was not able to trigger EAE at high frequency. Second, activated T cells recruited autoreactive B cells in secondary lymphoid organs in the presence of CNS derived MOG antigen to produce high titers of anti-MOG autoantibodies. Together, these findings identify a key role for commensal flora in shaping early pathogenic events in spontaneous CNS autoimmunity.

[1]B. Pöllinger*, et al.*, "Spontaneous relapsing-remitting EAE in the SJL/J mouse: MOG-reactive transgenic T cells recruit endogenous MOG-specific B cells," J. Exp. Med. (2009).

MECHANISMS OF INFECTIONS

10^{00} – 11^{30} Chair: M. Hornef, Med. Microbiology, Hannover Medical School

B. Stecher, Max-von-Pettenkofer Institute, LMU Munich
The microbiota influences horizontal gene transfer between Salmonella commensal and *E. coli*

B.O. Schröder, Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart
Reduction of disulphide bonds unmasks potent antimicrobial activity of hBD-1

A. Hartmann, Department Microbe-Plant Interactions, Helmholtz Center Munich
The Pseudomonas aeruginosa Autoinducer N-(3-Oxododecanoyl) Homoserine Lactone Inhibits Dendritic Cell Functions

U. Grundmann, Institute of Microbiology + Hygiene, Charité Berlin
Analysis of IL-10-/Nod2-double-deficient mice reveals contributions of Muramyl-Dipeptide signalling in chronic colitis and Campylobacter jejuni infections

A.-K. Claes, Institute for Experimental Medicine, Universtity of Kiel
Role of Nod-like receptors in intestinal inflammation

J. Wells, University Wageningen, The Netherlands
The Role of Intestinal RegIIIβ in Bacterial Infection

THE MICROBIOTA INFLUENCES HORIZONTAL GENE TRANSFER BETWEEN *SALMONELLA COMMENSAL* AND *E. COLI*

B. Stecher[1,2], R. Denzler[1], F. Bernet[1], L. Maier[1], U. Dobrindt[3], N. Thomson[4] and W.-D. Hardt[1]

[1]*Institute of Microbiology, ETH Zürich, Wolfgang-Paulistr. 10, 8093 Zürich, Switzerland*

[2]*Present address: Max-von-Pettenkofer Institute, LMU Munich, Pettenkoferstr.9a, 80336 Munich, Germany*

[3]*Institute for Molecular Infectious Biology, University of Würzburg, 97080 Würzburg, Germany*

[4]*Welcome Trust Sanger Center, Genome Campus, Hinxton, Cambridge, UK*

The mammalian gastrointestinal tract harbors a complex and extremely dense microbial community. Evidence of horizontal gene transfer (HGT) between individual members of this ecosystem is manifold. In particular, sequenced genomes of Gram-negative Enterobacteriaceae indicate high genetic flux. Using a mouse colitis model we investigated the factors influencing HGT of a conjugative plasmid from *Salmonella* to resident *E. coli*. We found that the commensal flora is a key modulator affecting conjugative plasmid transfer. This data indicate that microbiota manipulation can affect the rate of horizontal transfer of 'fitness factors' and possibly also virulence factors among enteric pathogens and closely related commensal strains.

REDUCTION OF DISULPHIDE BONDS UNMASKS POTENT ANTIMICROBIAL ACTIVITY OF HBD-1

Schroeder B.O., Wu Z., Nuding S., Groscurth S., Marcinowski M., Beisner J., Buchner J., Schaller M., Stange E.F. and Wehkamp J.

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

Human surfaces are permanently challenged by commensal and pathogenic microbiota. In the intestine the fraction of strict anaerobes increases from proximal to distal, reaching 99% of bacterial species in the colon. At colonic mucosa, oxygen partial pressure is below 25% of airborne oxygen content, moreover microbial metabolism causes reduction to a low redox potential of –200 mV to –300 mV in the colon. Defensins are key effector molecules of innate immunity and characterised by three intramolecular disulfide-bridges. Human β-defensin 1 (hBD-1) is one of the most prominent peptides of its class but despite ubiquitous expression by all human epithelia, comparison with other defensins suggested only minor antibiotic killing activity. While much is known about the activity of antimicrobial peptides in aerobic environment, data about reducing environment are limited. Here we show that after reduction of disulphide bridges hBD-1 becomes a potent antimicrobial peptide against the opportunistic pathogenic fungus *Candida albicans* and against anaerobic, Gram-positive commensals of *Bifidobacterium* and *Lactobacillus* species. *In vitro* the thioredoxin (TRX) system is able to reduce hBD-1, moreover TRX co-localizes with linear hBD-1 in human epithelia. Our study indicates that reduced hBD-1 shields the healthy epithelium against opportunistic fungi and colonisation by commensal bacteria. Accordingly an intimate interplay between redox-regulation and innate immune defence appears crucial for an effective barrier protecting human epithelia.

THE PSEUDOMONAS AERUGINOSA AUTOINDUCER N-(3-OXODODECANOYL) HOMOSERINE LACTONE INHIBITS DENDRITIC CELL FUNCTIONS

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Background: Infection with *Pseudomonas aeruginosa* (*P.ae*) is a major reason for pulmonary deterioration in patients with cystic fibrosis. The *P.ae* virulence factor *N*-(3-oxododecanoyl) homoserine lactone (3oxoC12-HSL) is synthesized in a cell density dependent manner. Reported effects of 3oxoC12-HSL on the human immune system are contradictory. We hypothesized that 3oxoC12-HSL inhibits the function of human dendritic cells (DC) to weaken host immune response.

Methods: DC were generated from human monocytes, and matured with 0.1 µg/ml LPS (*E. coli*) in the presence or absence of 60 µM 3oxoC12-HSL. 3oxoC4-HSL and C12-HSL were used to control for oxo-substitution and chain length. Costimulatory molecules (CD80, CD83, CD86) and migration markers (CXCR4, CCR7) expressing DC were quantified by flow cytometry (FACS Canto). Cytokines were measured in supernatants with Multiplex assays (Bio-Rad Laboratories GmbH). The phagocytotic activity of DC was tested using FITC-labelled Pseudomonas sp. cells using cytometric measurements after quenching the extracellular fluorescence with trypan blue. The localization of the cells within DCs was performed using confocal laser scanning microscopy. The migration behaviour of DC was tested in Transwell^{R}-assays using a CXCL12 gradient. To demonstrate possible stimulatory effects on T-cells by 3oxoC12-HSL exposed DC, the excretion of IFN-gamma was measured with ELISA in the culture fluids of cytotoxic JB4 T-cells.

Results: 3oxoC12-HSL reduced both the percentages of CD80- ($p< 0.05$), CD83- ($p< 0.04$), CD86- ($p< 0.03$) expressing DC and the number of the receptors CD80 ($p< 0.004$), CD83 ($p< 0.006$), CD86 ($p< 0.001$) per LPS-stimulated DC. Upregulation of CXCR4 ($p< 0.188$) and CCR7 ($p< 0.02$) was partially suppressed. 3oxoC12-HSL inhibited selectively the synthesis of IL-1β and IL-12 (each $p< 0.05$). In addition, the phagocytotic activity of DC using Pseudomonas aeruginosa cells was also reduced in the presence of 3oxoC12-HSL; the FITC-labelled Pseudomonas cells could be localized within phagolysosomes of the DC. Furthermore, the 3oxoC12-HSL and Jonuleit-Cocktail treated DC showed decreased migration abilities. No enhanced T-cell activation could be demonstrated by 3oxoC12-HSL-treated DC cells as compared to the control.

Conclusions: 3oxoC12-HSL inhibits the upregulation of costimulatory molecules on DC as well as their cytokine synthesis. This might impair the signal delivery to T cells, needed to generate T cell responses. The reduced expression of CCR7 suggests a lower capacity of DC to migrate to lymph nodes. It needs to be verified whether these results can be transferred to the *in-vivo* situation.

ANALYSIS OF IL-10-/NOD2-DOUBLE-DEFICIENT MICE REVEALS CONTRIBUTIONS OF MURAMYL-DIPEPTIDE SIGNALLING IN CHRONIC COLITIS AND CAMPYLOBACTER JEJUNI INFECTIONS

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Abstract: Loss of function mutations in the nucleotide oligomerization domain containing 2 (Nod2)-gene are significantly correlated with Crohn's disease (CD) which is one of the most abundant inflammatory bowel diseases (IBDs) worldwide.

Nod2 displays an intracellular pattern recognition receptor for muramyl dipeptide, a proteoglycan found in the vast majority of commensal gut bacteria. Nod2 deficiency is known to result in less alpha-defensin production and disturbed autophagy of intestinal cells which is essentially involved in eliminating intracellular bacterial compounds. Thus, Nod2 has an impact in intestinal innate immune responses.

Interleukin (IL)-10-deficient mice spontaneously develop pan-colitis after 4-5 months of age and are well suited to study chronic inflammation of the large intestine. Induction and progression of chronic colitis are triggered by signals derived from the commensal gut microbiota, whereas disease development is completely abolished in gnotobiotic IL-$10^{-/-}$ mice generated by quintuple antibiotic treatment.

The gut pathogen *Campylobacter (C.) jejuni* is one of the most frequent agents of bacterial diarrhea worldwide and proposed as a potential "initial onset damage" in the development of IBD in humans.

The aim of the presented study was to investigate whether Nod2 deficiency alters intestinal immune responses against *C. jejuni* infections in our IL-10-/Nod2-double-deficient mouse model.

In the colon of naïve, uninfected IL-10-/Nod2-double deficient mice harboring a conventional gut flora higher Monocyte-Chemotactic-Protein-1, IL-6 and Interferon-gamma levels could be detected as compared to IL-10- or Nod2-deficient mice. Five days following peroral *C. jejuni* infection IL-10-/Nod2-double-deficient mice exhibited a higher disease activity index as compared to control animals of either genotype. These initial results provide evidence for a potential protective role of Nod2 in the large intestine.

Germfree IL-$10^{-/-}$ mice developed acute colitis within one week following oral *C. jejuni* infection. Data from ongoing *C. jejuni* infection experiments in gnotobiotic IL-10-/Nod2-deficient mice will be provided and the proposed role for Nod2 further discussed.

ROLE OF NOD-LIKE RECEPTORS IN INTESTINAL INFLAMMATION

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A serious complication in Crohn's disease patients is intestinal fibrosis and stricture formation, which often requires surgical intervention. The most common genetic risk factor of this disease is a mutation in the Nod-like receptor (NLR) Nod2, that recognizes the microbial structure muramyldipeptide (MDP).

Here, we investigated the role of Nod2 in the development of intestinal fibrosis and *Salmonella*-infection in C57BL/6 and Nod2$^{-/-}$ mice. Streptomycin-pretreated mice were orally infected with *S*. Typhimurium wild-type or the attenuated *Salmonella* strains *S.* Typhimurium ∆*aroA* or *S.* Typhimurium ∆*msbB*.

Infections with *S*. Typhimurium wild type or *S.* Typhimurium ∆*aroA* resulted in comparable colonization of C57BL/6 and Nod2$^{-/-}$ mice and excessive transmural inflammation in the ceca of C57BL/6 and Nod2$^{-/-}$ mice. Inflammatory changes were characterized by submucosal edema, destruction of crypt architecture, epithelial ulcerations and hyperplasia of the muscle layers. Gut colonization of Nod2$^{-/-}$ mice by *S.* Typhimurium∆*msbB* was higher compared to C57BL/6 mice. Furthermore, *S.* Typhimurium ∆*msbB* induced transmural inflammation and fibrosis in the ceca of C57BL/6 and Nod2$^{-/-}$ mice. However, *S.* Typhimurium ∆*msbB*-induced immuno-pathology in Nod2$^{-/-}$ mice was exacerbated. Expression of proinflammatory and profibrotic cytokines was significantly up-regulated in both mouse strains.

These results indicate that Nod2 plays an important role in controlling *Salmonella* colonization and *Salmonella*-induced inflammation, which is likely dependent on TLR4 signaling.

THE ROLE OF INTESTINAL REGIIIβ IN BACTERIAL INFECTION

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Background and aim: The RegIII protein family contains conserved C type lectin domains involved in carbohydrate recognition. In humans one member of this family designated HIP/PAP is over-expressed in pancreatitis as a result of injury to acinar cells. Subsequently, it was demonstrated that PAP expression is substantially elevated in intestinal tissue as a result of cellular damage and inflammation. In the mouse the RegIIIγ and RegIIIβ family members are induced in the small intestine during the colonization of germ-free mice or in response to infection. At micromolar concentrations RegIIIγ and HIP/PAP are directly bactericidal to Gram-positive bacteria but the role of murine RegIIIβin bacterial infection is unknown. To investigate the role of RegIIIβ in intestinal infection, RegIIIβ knockout mice were infected with the Gram-negative *Salmonella enteritidis* or Gram-positive *Listeria monocytogenes*.

Material and methods: Eight week old animals were housed individually and fed a humanized diet. The animals were infected orally by gavage with $5*10^8$ bacteria. The animals were sectioned after 2 (*Listeria*) or 4 (*Salmonella*) days.

Results: Recovery of *Salmonella* or *Listeria* in the feces of RegIIIβ $^{-/-}$ and wt mice was not significantly different during the first days of infection indicating that RegIIIβ did not influence colonization levels. Nevertheless, significantly higher numbers of viable *Salmonella* were recovered from the colon, mesenteric lymph nodes, spleen and liver of the RegIIIβ $^{-/-}$ mouse compared to the wt mice. In contrast translocation of *Listeria* into these tissues was not significantly different between RegIIIβ $^{-/-}$ and wt mice. To investigate intestinal RegIIIβprotein levels Western blotting was performed on protein extracts from the ileal mucosa. As expected RegIIIβwas not detected in the knockout mice. In infected wt mice the production of RegIIIβ was strongly induced, showing higher levels in Salmonella infected animals. On the same samples Western blot analysis was performed with antibodies specific to RegIIIγ to determine whether a lack of RegIIIβ in the knockout mouse strain was compensated by an increase in the expression of RegIIIγ. In non-infected mice RegIIIγ expression was barely detectable but in all infected mice it was highly induced.

Conclusion: The translocation of *Salmonella* but not *Listeria* was increased in infected RegIIIβ $^{-/-}$ mice compared to infected wt mice. This was not due to higher induction of RegIIIγ in infected mice which has been shown to be bactericidal to Gram-positive bacteria. Current research is aimed at elucidating the precise mechanisms linked to the effect of RegIIIβ on *Salmonella* infection.

POSTER

1 THE EFFECT OF HIGH-FAT FEEDING IN A MOUSE MODEL OF INFLAMMATORY BOWEL DISEASE

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The gut acts as a highly selective barrier and communication organ between the luminal environment such as food and bacterial components and the host and is responsible for the regulation of metabolic and immune functions. The aim of this study was to investigate the effect of high-fat feeding on the pathogenesis of inflammatory bowel disease (IBD) in the $TNF^{\Delta ARE}$ mouse model of crohn's like IBD.

Male C57BL/6 *w*ildtype and $TNF^{\Delta ARE}$ mice at 4 weeks of age were fed either a high-fat diet (48kcal% from fat) or a control diet until the ages of 8 and 12 weeks.

Both wildtype and $TNF^{\Delta ARE}$ mice gained significantly more weight with high-fat feeding compared to the control diet. We observed clear impact of the diet on adipose tissue morphology as well as metabonomic parameters in plasma measured by targeted LC-MS analysis at the age of 12 weeks. Caecum weight was strongly reduced in high-fat feeding, indicating an influence of the diet on the gut microbiota. The high-fat feeding lead to an earlier onset of inflammation of the terminal ileum in the $TNF^{\Delta ARE}$ mice and to an increased severity at the age of 12 weeks compared to control diet animals. First analyses of isolated intestinal epithelial cells point to an alteration of markers of epithelial barrier function and ER stress in the ileum.

High-fat feeding triggers pathogenensis of crohn's like IBD in the $TNF^{\Delta ARE}$ mouse model, probably by a mechanism related to loss of barrier function.

2 MICROBIAL TOLL-SIGNALING AT THE INTESTINAL EPITHELIAL SURFACE STIMULATES APICAL SECRETION OF CXCL8 AND PROTECTION VIA AUTOCRINE SIGNALING

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Toll-like receptors (TLRs) play a role in the defense against microbes by inducing the expression of pro-inflammatory cytokines, such as CXCL8. These receptors have also an important role in the maintenance of intestinal homeostasis. The aim of this study was to deepen our understanding of the role of TLRs and CXCL8 secretion by intestinal epithelial cells in homeostasis.

Stimulation of Caco-2 monolayers with specific TLR ligands and cytokines induced polarized secretion of CXCL8 (also known as IL-8) and the polarity of CXCL8 secretion correlated with the location of the stimulus. Basolaterally secreted CXCL8 is a known inflammatory initiator of leukocyte migration and activator of neutrophils. To investigate the possible effects of apically secreted CXCL8, the cellular localization of CXCR1, the major receptor for CXCL8, was visualized using confocal microscopy in Caco-2 monolayers and in human biopsies. CXCR1 was constitutively expressed and located at the apical surface in Caco-2 cells, villus enterocytes but not in the crypts of human duodenal biopsies. In the human colon epithelium, CXCR1 was expressed on both the apical and basolateral surface. The apical location of CXCR1 suggests that apically secreted CXCL8 may have an autocrine function on intestinal epithelial cells.

To investigate CXCL8 signaling in epithelial cells a transcriptome analysis was performed on CXCL8 treated Caco-2 cells. Transcriptome data confirmed the autocrine role of CXCL8 on Caco-2 cells and revealed that CXCL8 induced the expression of genes involved in cellular differentiation.

3 *Lactobacillus plantarum* enhances human intestinal barrier function via a TLR2 and PKCδ- mediated mechanism

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L. *plantarum (LP)*, a commensal bacterium of humans, is able to modify the intestinal barrier in of human volunteers and intestinal cell-lines via a Toll like receptor (TLR) 2 pathway. To study the mechanism by which TLR signaling can modify tight junction proteins a genomic study was performed. Caco-2 BBE cells were treated for 2 and 6 hrs with LP, and TLR2 ligands PAM_3CSK and lipoteichoic acid isolated from LP.

538 genes were differentially regulated after 6 hrs exposure to LP ($p \leq 0.01$). No unregulated expression of tight junction proteins or tight junction associated proteins was observed but network analysis indicated that epithelial cells remodel after TLR2 stimulation. Affected networks include: Cellular Development, Lipid Metabolism, Molecular Transport, Cellular Assembly and Organization, Post-Translational Modification and Lipid Metabolism.

Using confocal laser scanning microscopy, phosphorylation of ERK1/2 and p38 was observed in LP, LTA and PAM_3CSK -treated Caco-2 cells. Furthermore, we observed TLR2 induced translocation of cytosolic PKCδ towards the apical part of the cell and to the tight junctions. Analysis of human biopsies of the duodenum also showed a very strong apical and junctional staining of PKCδ in villus cells. Surprisingly, no PKCδ was observed in crypt cells of the duodenal biopsy. This suggests that PKCδ is associated with differentiation of crypt cells into villus epithelial cells. TLR2 signaling can enhance the activation of PKCδ which may play a role in the modification of tight junctions. We are now investigating the specific targets of PKCδ in TLR2 triggered intestinal epithelial cells

4 Gut microbiota influences ileitis in TNF^{deltaARE} mice

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The intestinal microbiota plays a crucial role in host physiology. Dysbiosis, *i.e.*, imbalance within the microbial ecosystem, has been associated with pathophysiologic situations like Crohn´s disease. However, little is known about how microorganisms are involved in the onset and maintenance of chronic ileitis. The aim of our study was to assess changes in intestinal microbiota under inflamed and non-inflamed conditions using the TNF^{deltaARE} mouse model of ileitis. Mice (n = 8 per genotype/treatment group) were treated with ampicillin (1 g/l) (Amp) or a combination of metronidazole (1 g/l) and vancomycin (0.25 g/l) (Van/Met). Antibiotics were given in drinking water for four weeks, starting at the age of 8 weeks. We observed a distinct improvement of histopathology (infiltration of immune cells and villus atrophy) in the distal ileum after antibiotic treatment. Histopathology scores were 5.6 ± 1.2 in control mice *vs.* 0.9 ± 0.2 and 1.6 ± 1.0 in the Van/Met and Amp group, respectively ($p < 0.001$). Total bacterial counts (log_{10} cell/g wet weight) in the distal ileum were significantly lower in Amp-treated mice (10.1 ± 0.2 *vs.* 10.6 ± 0.3 for the control group) ($p < 0,001$). We also observed differences in the density of viable bacteria in the distal ileum after Amp treatment. Counts were (Amp *vs.* control): 6.7 ± 0.1 *vs.* 7.9 ± 0.2 log_{10} (CFU/g) for lactobacilli and (7.0 ± 0.2 *vs.* 8.3 ± 0.2) for total aerobes ($p < 0.001$). In contrast, there was no difference in bacterial density in mice from the Van/Met group, emphasizing that there is a clear difference in the ileal bacteria density in those two treatment models. Thus, via the use of antibiotics, we propose that intestinal microorganisms are essential for the establishment of ileitis in TNF^{deltaARE} mice. We now intend to characterize bacterial taxa of relevance in our experimental model by combining comparative diversity analysis via high-throughput sequencing with culture techniques.

Keywords: intestinal microbiota, TNF^{deltaARE} mice, chronic intestinal inflammation, antibiotics

5 Probiotic-Derived Protease Lactocepin Degrades the Pro-Inflammatory Chemokine IP-10: Impact on Chronic Intestinal Inflammation

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Clinical studies revealed protective effects of the probiotic mixture VSL#3 in the context of IBD. We previously reported that VSL#3-derived *Lactobacillus paracasei* (L.p) expresses cell surface proteins that mediate post-translational loss of the pro-inflammatory chemokine IP-10 in intestinal epithelial cells (IEC). The aim of this study was to identify the active probiotic structure triggering the loss of IP-10.

Stimulation of TNF-activated IEC with conditioned media of L.p (CM L.p) revealed that secreted proteins of L.p analogously mediate loss of secreted and surface-bound IP-10 in IEC. Differential LC-ESI-MS/MS analysis of chromatographic fractions, active or inactive in mediating IP-10 loss, identified lactocepin to be the active probiotic structure of L.p. Indeed, Lactocepin, encoded by the prtP gene, is a cell wall associated and secreted serine protease. Cell-free incubation and PMSF-inhibitor-studies with CM L.p revealed that lactocepin directly degrades IP-10. Beside IP-10, lactocepin selectively targets an array of pro-inflammatory chemokines (eg. I-TAC, Fractalkine), whereas all tested cytokines were not cleaved. Finally, an isogenic prtP disruption mutant confirmed lactocepin as IP-10 degrading structure. Underlining our *in vitro* data, ileal explant culture experiments with ileal tissue from $TNF^{\Delta ARE/+}$-mice revealed that CM L.p degrades also tissue-associated IP-10. Furthermore intraperitoneal injection of sterile CM L.p into $TNF^{\Delta ARE/+}$-mice resulted in a significant reduction of ileal inflammation attended with reduced IP-10 tissue levels, thus demonstrating physiological relevance of lactocepin.

The identification of lactocepin as probiotic structure enables a structure-based evaluation of probiotic bacteria for therapeutical interventions in the context of IBD.

6 Impact of maternal gut inflammation on the gene expression profile of the offspring's intestinal epithelial cells

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Background: Maternal stress during pregnancy has long term consequences for disease risk of the offspring. In this study, the aim was to investigate the impact of maternal stress (ileal inflammation) on the gene expression profile of the offspring's intestinal epithelial cells (IEC), using the $TNF^{\Delta ARE/+}$ mouse as a model for Crohn's ileitis.

Methods: The experimental setup includes two different breeding situations. Female wildtype $TNF^{+/+}$ mice (WT dam) were bred with male $TNF^{\Delta ARE/+}$ mice (ARE sire) and vice versa. Thus, 4 groups of offspring were generated: WT and ARE offspring from healthy WT dams, and WT and ARE deriving from inflamed ARE dams. The offspring were sacrificed 17.5 days post conception (dpc), 1, 3 and 8 weeks postnatal. IEC from flash frozen ileal samples were captured by laser micro dissection. RNA was extracted for microarray-based gene expression analysis.

Results: Histopathological scoring reveals no inflammation and differences up to an age of 3 weeks, whereas 8 week old ARE mice show a mild ileal inflammation, compared to WT, but no changes due to maternal gut inflammation is observed within the groups of the same genotype (scores: F0(WT)F1(WT) 0.39+/-0.31; F0(ARE)F1(WT) 0.50+/-0.14 and F0(WT)F1(ARE) 4.08+/-0.85; F0(ARE)F1(ARE) 3.96+/-0.71). However, maternal gut inflammation during pregnancy leads to an altered gene expression profile of the ileum in 17.5 dpc fetuses, decreasing in 8 week old offspring. In total 705 genes in WT and 1790 genes in ARE offspring (FC of ±1.5, *p-value* <0.05) are significantly regulated under a maternal inflammatory environment in the fetuses, although there is no change in morphology (body size and body weight). In contrast, at 8 weeks postnatal only 222 genes in WT and 63 genes in ARE offspring are regulated.

Conclusion: A persistent maternal inflammatory exposure does not influence any ileal pathology of the offspring up to an age of 8 weeks. However, there are changes in the gene expression profile in 17.5 dpc fetal mice due to the inflammatory environment that might be overwritten by the ileal inflammation in the 8 week old offspring. This aspect might be responsible for an altered susceptibility to inflammatory bowel disease.

7 PKR links mitochondrial unfolded protein response in the epithelium to the pathogenesis of intestinal inflammation

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Background & Aim: Endoplasmic reticulum (ER) unfolded protein responses (UPR) in intestinal epithelial cells (IEC) contribute to the development of intestinal inflammation. In this study we characterized the role of mitochondrial (mt) UPR in the epithelium of patients with inflammatory bowel diseases (IBD) and murine models of colitis.

Methods: Protein expression profiling was performed in IEC from the murine models of colitis. Immunohistochemistry was used to quantify expression levels of glucose-regulated protein (GRP)78, chaperonin (CPN)60 and dsRNA-activated protein kinase (PKR) in mice and humans. Truncated ornithine transcarbamylase (OTCΔ) was used to selectively induce mtUPR *in vitro*. $Pkr^{-/-}$ mice were tested for their susceptibility to dextran sodium sulfate (DSS)-induced colitis.

Results: Proteome- as well as immunohistochemical and Western blot analysis of primary IEC from IBD patients and the murine models revealed strongly activated ER- and mtUPR as reflected by increased expression of the ER chaperone GRP78 and mitochondrial CPN60. This was associated with an induction of PKR. MtUPR-induction in Mode-K cells triggered the phosphorylation of eukaryotic translation initiation factor (eIF2)α through the recruitment of PKR. Using pharmacological inhibitors and siRNA, we identified the mtUPR-induced eIF2α phosphorylation and transcription factor activation (CHOP, cJun) to be dependent on the activity of the mitochondrial protease ClpP and the cytoplasmic kinase PKR. Moreover, Pkr-/- mice showed almost complete resistance to DSS-induced colitis accompanied by loss of epithelial CPN60 expression.

Conclusion: These results demonstrate a novel mechanism for mitochondrial stress-integration into the disease-relevant ER signaling cascade and suggest PKR to link metabolic-, inflammatory- and immune responses.

8 Regulation of goblet cell differentiation factors Hath1 and Hes1 in colonic mucin producing cells

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Backgrounds & Aims: The intestinal mucus layer is a part of the mucosal barrier protecting the epithelium from luminal microbes. The mucins forming the mucus are secreted from goblet cells. The differentiation from the intestinal stem cell to the goblet cell is controlled by several transcription factors like Hath1, Hes1 and KLF4. The pervious studies show an association of ulcerative colitis (UC) with a diminished mucus layer and reduced number of goblet cells. Moreover, it was shown that the induction of Hath1 and KLF4 is defective in inflamed vs. noninflamed UC (Gersemann et al., Differentiation 2009). The aim of the present study was to elucidate the regulation of Hath1 and KLF4 in colonic mucin producing cells.
Methods: The goblet cell like colon adenocarcinoma cells LS174T were incubated with IL-4, IL-13, TNFα, IL-22 and several heat killed bacteria strains (*E. coli, Lactobacilli, Bifidobacteria* and *Bacterioides*). Subsequently the expression of Hath1, Hes1, KLF4, and the two major colonic mucins Muc1 and Muc2 was determined by real-time PCR.
Results: The stimulation of LS174T cells with interleukin IL-4 and IL-13 resulted in a reduction of the Hath1- and Hes1- mRNA expression. TNFα and IL-22 treatment resulted in an upregulation of the Muc1 expression. Interestingly, the stimulation of LS174T cells with *E. coli K12* and *E. coli Nissle* leads to a significantly decreased expression of Hes1 and Hath1. In contrast, Muc1 mRNA was induced in these cells, whereas Muc2 seems to be constitutively expressed.
Conclusion: Various cytokines and also several luminal microbes are capable to influence goblet cell differentiation and mucin expression. The deeper understanding of the regulatory mechanisms of those goblet cell differentiation factors could contribute to a better understanding of the pathology of UC.

9 Biofilm formation of bifidobacteria is induced by bile

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One strategy of microorganisms to colonize the gastro-intestinal tract (GIT) is the formation of biofilms e.g. on the surface of food particles. In the present study we investigated the capacity of *B. bifidum* S17, a strain with good anti-inflammatory activity, to form biofilms under various conditions.

No formation of biofilm could be observed in standard polystyrene plates when bacteria were grown for up to 72 h in normal MRS medium supplemented with cysteine (MRSC). However, biofilm formation could be induce by adding a complex mixture of bile salts to the biofilm assay. Biofilm formation was first observed after 48 h in MRSC medium. The increase in biofilm formation was positively correlated with the concentration of bile added. However, at bile concentration above 5 % biofilm formation was completely abolished. Kill curve experiments showed that bile concentrations above 2.5 % are bactericidal to *B. bifidum* S17.

Our results demonstrate, that biofilm formation of *B. bifidum* S17 is induced at physiological concentrations of bile. This indicates a potential involvement of biofilm formation for host colonization by *B. bifidum* S17.

10 Lignan transformation by gut bacteria influences tumor growth in a gnotobiotic rat breast cancer model

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Intestinal bacteria convert dietary plant lignans to enterolignans. This conversion is thought to be important for the reported health benefits of lignans, particularly in hormone-dependent cancers. We used a gnotobiotic rat model associated with lignan-converting bacteria and germ-free controls to explore the exact role of bacterial lignan transformation in chemically induced breast cancer. Animals were fed a lignan-rich diet and killed 3 months after cancer induction. Enterolignans were only observed in the associated animals but their formation did not influence tumor incidence. However, more tumors per tumor-bearing animal were observed in the germ-free group. The tumors in these rats were bigger and had a higher proliferation activity in conjunction with a lower apoptotic index. Lignan transformation did not influence circulating estrogen concentrations or the expression of estrogen receptors or selected genes involved in cell growth. A higher plasma and liver catalse, superoxide dismutase, and glutathione-S-transferase activity in the associated rats indicated that bacterial enterolignan formation influences anti-oxidative enzyme systems. However, an improved “anti-oxidative status” of the organism was not supported by the measurement of reduced glutathione and malondialdehyde as oxidative stress markers. We conclude that bacterial lignan transformation can lower breast tumor burden possibly via an induction of anti-oxidative enzyme systems. The exact mechanisms require further investigations.

11 *S.* Typhimurium inflammation triggers pathogen elimination by commensal *E. coli*

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Gastrointestinal infections are a serious global health problem with 1.8 million victims per year according to the WHO. In addition to the innate and adaptive immune system, the commensal microflora populating the intestine at high density protects against those kinds of infections. This phenomenon is known as colonization resistance (CR). When the enteric pathogen *Salmonella enterica* spp. I serovar Typhimurium (*S*. Tm) overcomes CR, it out-competes most of the anaerobic commensal microflora by the induction of gut inflammation.
Although it has been assumed that only pathogens can profit from gut inflammation, it was recently observed in our lab that certain commensal *E. coli* strains can also benefit from this condition. Moreover, these *E. coli* strains (e.g. *E. coli* 8178) out-competed the pathogen *S*. Tm in a mouse model for acute *Salmonella*-induced colitis. Strikingly, *E. coli* 8178 was able to suppress intestinal growth of *S*. Tm more effectively than the current standard probiotic used for treatment of human Salmonellosis, *E. coli* Mutaflor (MF).
We are currently analyzing the mechanism and outcome of this pathogen-commensal competition and putative virulence factors which might be involved (e.g. *fim*). The data will be presented.

12 *Repair the barrier function of stress-induced damaged microbiota in IBD by the multispecies probiotic "Omni-Biotic Stress Repair"*

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In patients with IBD (CD, UC, Pouchitis) we find reduced levels of bifidobacteria, modified *E. coli* strains or larger amounts of bacteria attached to the epithelial surface. Based on this knowledge a specific multispecies probiotic mixture (containing 9 different strains) was designed to maintain the intestinal barrier function, even after exposure to stress factors. These strains were selected on their capacity to

1) inhibit various pathogens associated with IBD (e.g. *C. difficile, E. coli*)
2) improve barrier-function
3) produce anti-inflammatory cytokines.

All strains included in the probiotic product had to pass a "survival test" (developed by University of Maastricht): in an in vitro simulation of the gastro-intestinal tract the pH and the addition of pepsin, pancreatin and bile are regulated as under physiological conditions and the total cell count is measured at 3 different times (executed at 37^{o} C). Then we measured the production of lactic acid (as a parameter for metabolic activity), screened the bacteria on their ability to inhibit pathogens (diffusion test after Hechard) and tested against *E. coli* and examined inhibition of *C. difficile* growth and the production of toxins A & B, the barrier function was measured in an in vitro setting (Trans-epithelial electric resistance) and cytokine production focused on induction of IL-10 was measured (by the multiplex cytokine assay Luminex method), in a clinical trial could be shown a reduction of symptoms related to IBD (e.g. diarrhoea, constipation, abdominal pain, cramps or bloating).

13 *Functional studies and absolute quantification of NOD1 and NOD2 in murine preadipocytes*

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Hypertrophy of the mesenteric fat is a common phenomenon observed in Crohn's disease. Since the last few years the understanding of fat tissue has evolved from a storing site into a more functional tissue.Recent studies indicate that preadipocytes are linked to innate immunity since they express functional Toll-like receptors (TLR), and can act as phagocytes. With the present study the expression and function of nucleotide-binding site and leucine-rich repeat (NBS-LRR) proteins, a recently discovered family of intracellular pattern recognition receptors, in preadipocytes, was investigated.Preadipocytes were isolated from the abdominal fat of C57BL/10 (WT) mice and the expression profile for NOD1, NOD2 was evaluated in unstimulated as well as receptor-specific stimulated preadipocytes. Functionality of these receptors was characterized by subsequent evaluation of cytokine production. NOD-specific stimulation was confirmed by RNA interference (RNAi) strategies. For further characterisation, the absolute copy numbers for NOD1 and NOD2 were determined using plasmid standards. NOD1 could be detected in all samples. Copy number for WT was approx. 4 copies per cell, while expression of NOD2 was significant lower but could be up-regulated by pro-inflammatory cytokines. Stimulation with the NOD1 specific ligand Lactyl-Tetra-DAP (LT-DAP) induced a strong IL-6 production. Corresponding to the low NOD2 expression no IL-6 production could be observed after stimulation with its specific ligand muramyl dipeptid (MDP).
Our data provide evidence that functional NBS-LRR proteins are expressed in preadipocytes. These receptors could be activated by bacterial translocation during intestinal inflammation and contribute to hypertrophy of mesenteric fat observed in inflammatory bowel diseases.

14 A further function for the flagellum of *Escherichia coli* Nissle 1917: the major adhesin *in vivo*?

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E. coli Nissle 1917 is probably one of the best characterized probiotics, which is motile due to the expression of flagella. Additionally, it has been shown that flagellin induces the production of beta-defensin 2 by Caco2 cells. Here we report a further function of the flagellum.

In *ex vivo* studies we were able to show, that the flagellum of EcN is necessary for its efficient adhesion to cryosections of human gut biopsies. We isolated a hyper-flagellated variant of EcN, which, compared to the wildtype, adhered much more efficiently to the sections, whereas a non-flagellated mutant adhered barely. *In vitro* studies showed that neither overexpression nor the lack of flagella has a significant effect on the adhesion to the human epithelial cell lines Caco-2 and T24. This is probably due to the fact, that these cells are not able to produce any mucins, whereas mucosal structures might still be present in the cryosections. This is in agreement with our latest finding, that EcN shows a flagella-dependent adhesion to the human epithelial cell line LS174T, which produces mucins *in vitro*.

To elucidate the importance of the presence of mucin for successful adhesion of EcN, we preincubated flagellated EcN strains with mucin2, which resulted in reduced adhesion efficiency to the cryosections. In addition we were able to show that the hyper-flagellated variant adhered much more efficiently to mucin2 compared to the wildtype. However, the non-flagellated strain adhered barely. In contrast this effect could not be observed for *E. coli* CFT073, although this strain expresses the same serotype of flagella. The direct interaction of EcN flagella with mucin 2 was demonstrated by both, western blot and ELISA. Interestingly, we could observe by electron microscopy, that EcN wildtype is monotrichously flagellated. This is in contrast to the general assumption, that *E. coli* are peritrichous bacteria. All these results lead to the conclusion, that the flagellum of EcN is its major adhesin *in vivo*, which mediates adhesion to the mucus layer on top of the mucosa.

15 Molecular analysis of microbial communities during oral biofilm formation

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A more detailed knowledge of oral microbiota in biofilm formation is important for a better understanding of common diseases such as dental caries and periodontal disease. In the present pilot study the composition of human microbiota during intraoral biofilm formation was analysed in 7 healthy volunteers (females, aged 24 to 26) using intraoral bovine enamel slabs harvested after 3 and 30 minutes, 2, 6 and 24 hours. In parallel, interproximal dental plaque and saliva were analyzed. Following nucleic acid extraction the microbial communities were characterized by T-RFLP (Terminal Restriction Fragment Length Polymorphism) analysis of 16S rRNA genes applying three different restriction enzymes, *hae*III, *msp*I and *hha*I. T-RFLP signals of the phylogenetic groups were assigned to sequence data obtained by DGGE (Denaturing Gradient Gel Electrophoresis) analysis or in silico analysis of published sequence data, respectively.

T-RFLP using restriction enzyme *hae*III provided the best discrimination results for the microbial community; however, a number of species required additional restriction enzymes analysis for better discrimination (*msp*I, *hha*I). The microbial diversity of in situ oral biofilms was significantly lower compared to saliva and interproximal dental plaque. We could show that quantitative analysis of various phylogenetic groups was different between oral biofilms, saliva and interproximal dental plaque. The abundance of streptococci was highest in oral biofilms whereas *Bacteroidetes* were predominantly found in saliva. The relative abundance of *Veillonella spp.* varied during biofilm formation with lowest frequencies in 6-hour biofilms. Other phylogenetic groups e.g. the group of *Haemophilus/Neisseria/Actinobacillus* were detected with equal frequencies in all samples.

We conclude that characteristic signatures of microbiota can be found in saliva, interproximal dental plaque and also during oral biofilm formation. Culture-independent analysis of microbiota may provide new insights into the diversity and the relative abundance of bacterial species in various communities which was shown here for different oral compartments and growing oral biofilms.

16 New culture-independent analysis of bacterial communities modifies the current view of polymicrobial infection in patients with cystic fibrosis

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Cystic fibrosis (CF) is a recessive inherited disease caused by mutations of the CFTR gene; however, the morbidity of CF patients is severely influenced by chronic polymicrobial lung infection. Routine microbiological testing is focused on the detection of few typical pathogens using culture-based techniques. By using new culture-independent techniques unbiased semi-quantitative analysis of complex bacterial communities are now available which could help to better understand the role of microbiota in colonisation and pathogenicity of CF. In the present pilot study 51 respiratory samples of 37 CF patients in three different age groups (0 to 10, 11 to 20 and >20 years) were analyzed in parallel by culture-based and new culture-independent methods (T-RFLP). As already known, an age-dependent infection rate was found by culture-based analysis for the classical CF related pathogens with highest rates of *S. aureus* infections in the 0 to 10 year old group and rising *P. aeruginosa* infection rated in older patients (11-20 and >20 years). This finding was supported also by the culture-independent analysis of bacterial nucleic acid in respiratory samples using T-RFLP; however, we also found new aspects of respiratory infections by the culture-independent method with increasing rates of anaerobic infections in CF patients at older age which potentially contributes to clinical progression of CF lung disease; however, prospective clinical studies are required to confirm this new assumption. Culture-independent analysis also provides a quantitative view to the relative abundance of specific pathogens in bacterial communities of CF patients. High relative abundance of *Staphylococci* (24%), *Neisseria / Haemophilus* (16%), *Streptococci* (9%), *Bacteroidetes* (6%) and Veillonella (5%) genotypes were found whereas the *Pseudomonas* group was detected with surprisingly low frequencies in infected samples (1%) of all age groups.

We conclude that culture-independent analysis (e.g. T-RFLP) will fundamentally change the current view of polymicrobial lung infection in CF patients due to the unbiased detection of potentially new respiratory pathogens and due to the new quantitative aspects. We suppose that very soon culture-independent analysis of the respiratory microbiota could become a new diagnostic standard for optimized diagnostics and therapy of CF patients.

17 Molecular mechanisms underlying maturation and semi-maturation in murine dendritic cells

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Dependent on their structure and/or concentration, TLR ligands can generate different states of maturation of dendritic cells (DC). Two different states of maturation are well characterised (immature (iDC) and mature (mDC)), differing in migration behaviour, antigen processing and uptake and the ability of activating and polarizing T cells. Recently a third phenotype, termed semi-mature was discovered. It is characterised by a high capacity of antigen uptake, markedly reduced migration and an intermediate expression of co-stimulatory surface proteins and MHC class II. Particularly, these semi-mature DC fail to activate T cells in vivo and in vitro and exhibit tolerance to re-stimulation . Differentiation to this DC phenotype is thought to be mediated by Interleukin-6 (IL-6). Since the antigen presenting machinery of DC plays a crucial role in T cell activation, we focused on the differences in MHC class II transport to the DC surface between iDC, mDC and smDC as well as on differences in the expression of certain genes. Semi-maturation can be induced by stimulation of DCs with LPS^{lo} (1 ng/ml) or the commensal *Bacteroides vulgatus*.

The invariant chain (Ii) is a crucial regulator of MHC class II transport to the cell surface, as soon as Ii is degraded to a small peptide called CLIP, antigen derived peptide can be loaded on MHC class II followed by transport to the cell surface. We could prove that the intracellular amount of p10 and p21-Ii bound to MHC-II is rapidly decreasing within 24 hours in mDC, whereas iDC do not exhibit decreasing levels of uncleaved Ii even 72 hours after stimulation with LPS and smDC exhibit an intermediate reduction, assuming that the endosomal protease Cathepsin S plays a role in this regulation process. Furthermore we could show that enhanced MHC class II surface expression is mostly due to enhanced transport of MHC class II containing vesicles to the surface and not due to enhanced gene transcription. In contrast, the gene transcription of proteins like IL-12p40, IL-12p35 and Cxcl10 is enhanced in mDCs and smDCs compared to imDCs, but interestingly in smDCs this does not lead to enhanced IL-12p70 protein expression. This effect might account for the inability of sm DC to polarize T-cells into Th1 direction.

18 Accessing the secretome of the probiotic Bifidobacterium bifidum S17 strain by genomic and proteomic approaches

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Bifidobacterium bifidum S17 is a promising probiotic candidate, which strongly adheres to intestinal epithelial cells and displays potent anti-inflammatory activity both *in vitro* and *in vivo*. The molecular mechanisms responsible for the anti-inflammatory activity of this strain are unknown, however secreted and surface proteins are hypothesized to play crucial role in these processes.

The sequencing of the genome of *B. bifidum* S17 allowed us to set up theoretical secretome of this species, which is predicted to consist of at least 20 proteins with potential roles in utilization of complex oligosaccharides and peptides, pathogen exclusion in the gut and host-microbe interactions.

We were able to experimentally identify at least 26 proteins to be secreted by *B. bifidum* S17 grown in MRS broth. Contamination of the secreted proteins by cytoplasmatic contents, eventually released into the medium upon bacterial lysis, was ruled out by determination of the specific activity of glyceraldehydphosphate dehydrogenase (GAPDH) in cell-free supernatants. Moreover, at least 5 additional proteins were specifically induced in the presence of bile, human milk serum or prebiotic fructooligosaccharides. Interestingly, growth of S17 strain in FCS-supplemented DMEM medium used for the adhesion assays specifically induced expression of a 70 kDa protein. Identification of the secreted proteins of *B. bifidum* S17 by mass spectrometry will extend our knowledge on the probiotic effects of this strain.

19 The role of the host response in colicin-dependent E. coli –Salmonella competition in the gut

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Salmonella spp. has a great impact on human health as it is a major cause of bacterial food-borne disease. Probiotic *E. coli*, such as *E. coli* str. Nissle 1917 (*ECN*) has been shown to be an efficient therapy in treatment of acute, *Salmonella* spp. induced colitis. Competitive properties of this *E. coli* strain against *Salmonella* spp. are of great importance for reducing *Salmonella* spp. titers in infected patients. Here, we used the streptomycin-treated mouse model to analyze the influence of *ECN* infection on murine enteric Salmonellosis. Performing competitive infections with *ECN* and a human pathogenic *Salmonella* serovar Typhimurium strain we found that *ECN* was outgrown by the pathogen. This was dependent on the ability of the *S.* Typhimurium to produce colicin. Interestingly, the presence of concomitant gut inflammation had a major influence on colicin-dependent competition of *S.* Typhimurium and ECN. To investigate the mechanisms of inflammation-controlled colicin-killing we studied regulation of colicin expression in vivo and in vitro. Our results demonstrate that the host response is an important modulator of inter-bacterial competition in the intestinal tract. Elucidating the underlying mechanisms will eventually lead to the design of more effective therapeutics against infectious disease.

20 Deciphering the bacterial mechanisms of colonization resistance in a gnotobiotic mouse model

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It´s well established that the intestinal microbiota has a protective effect against intestinal pathogens such as non-typhoid *Salmonella*. This effect is termed „colonization resistance" (CR). The fact that the intestinal microflora excludes invaders such as enteric pathogens was described in the middle of the last century by René Dubos, Dick van der Waaij and others, but the molecular mechanism is largely unclear to date, due to the enormous complexity of the intestinal ecosystem and the lack of appropriate experimental tools. Recently, studies by Atarashi et *al*., 2011 and Fukuda et *al*., 2011 report for the first time on the mechanisms by which distinct types of commensal bacteria induce beneficial host immune responses and protect against infections.

We aim at characterizing interspecies interactions of specific members of the intestinal ecosystem and how this contributes to the establishment of CR. To this extent, we use gnotobiotic mice colonized with the Altered Schaedler Flora (ASF). In contrast to conventional mice, ASF mice are highly susceptible to infections with different pathogens (*Salmonella* spp., *E. coli*, *Clostridium difficile*). When ASF mice were co-housed with a conventional (CON) donor-mouse they re-acquired a complex microbiota and returned colonization-resistant against oral infection with *Salmonella* Typhimurium. This makes ASF mice an attractive animal model to screen different commensal bacteria with respect to their potential to colonize ASF mice and contribute to CR. Here, we report the isolation in pure culture and characterization of different members of a variety of obligate anaerobic commensals from conventional mice. We developed specific tools to detect these strains in ASF mice (FISH; qPCR) and characterized the potential of single commensal strains as well as microbial consortia to mediate CR against *Salmonella* Typhimurium infection in this mouse model.

A more detailed understanding of the biology of single members of the microbial community living in our intestine will help at elucidating their role in a complex microbial consortium. Eventually, identification of strains with beneficial functions may open up novel therapeutic options in treatment and prevention of infectious diseases.

21 Mitochondrial stress is associated with altered expression of mitochondrial creatine kinase (mtCK) in the intestinal epithelium

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The role of endoplasmic reticulum (ER) stress in various metabolically and immune-driven diseases has been extensively investigated during the last years. However, accumulating evidence suggest that other cellular stress responses such as mitochondrial signaling represent additional conserved disease mechanisms. We recently found mitochondrial unfolded protein response (mtUPR) activated in animal models of intestinal inflammation as well as in intestinal epithelial cells (IEC) from IBD patients. Interestingly, Western blot analysis of IEC showed diminished levels of mitochondrial creatine kinase (mtCK) under conditions of massive inflammation in mice and humans whereas the expression was induced in mice during the onset of colitis. Using murine MEF cells and a specific inducer of mtUPR, truncated orinithine transcarbamylase (OTCΔ), we detected mtCK-induction under non-apoptotic stress conditions whereas incubation of the murine cell line Mode-K with the ER UPR-inducer tunicamycin resulted in specific degradation of mtCK by the mitochondrial protease ClpP and apoptosis. mtCK catalyses the reversible transfer of the phosphoryl group from phospho-creatine to ADP and is implicated in the regulation of the mitochondrial permeability transition pore (mPTP). Consistent with our data, the ROS-sensitive mtCK is thought to inhibit mPTP-induction and subsequent apoptotic events. In summary, our results underscore the importance of mtCK for IEC homeostasis under stress-conditions and confirm the role of mtCK in mitochondrial live-or-death decisions.

22 Transgenic and knock-out mouse models – A tool to study inflammatory stress responses

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Cellular stress responses have been implicated in chronic diseases such as obesity, diabetes, cardiovascular and inflammatory bowel diseases (IBD). Even though phenotypically different, chronic diseases share cellular stress signaling pathways, in particular endoplasmic reticulum (ER) unfolded protein response (UPR). Eukaryotic cells have evolved to contain several specialized organelles carrying out specific cellular functions. This requires organelle signaling pathways to transmit organelle stress and regulate organelle abundance. ER UPR is one of those signaling pathways and aims to restore ER homeostasis after challenges of the ER function and to adjust organelle capacity to cellular demand. Recently, a related stress response has been identified, the mitochondrial UPR (mtUPR) which represents a distinct but interrelated signaling pathway. In mouse models of chronic intestinal inflammation as well as in IBD patients GRP78 and CPN60, hallmark proteins of ER and mitochondrial stress, respectively are induced. Although mitochondrial dysfunction and cellular stress response are key regulators of chronic metabolically-driven disorders, the role of mitochondria-related mechanisms in the pathogenesis of immune-mediated intestinal diseases such as IBD are virtually unknown. Here, we studied the impact of CPN60 and the transcription factors CHOP and ATF6α in inter-organelle signaling. Preliminary studies in cell lines bearing constructs similar to those introduced in the newly generated mouse models showed that overexpression of nATF6 and CHOP may affect mitochondria directly by inducing CPN60 gene expression. In addition, overexpression of nATF6 induces GRP78 and CHOP expression as well as XBP1 splicing. Cells overexpressing nATF6 showed decreased barrier function in response to the ER stress-inducer tunicamycin as determined by transepithelial resistance measurement.

Thus, the new mouse models generated, overexpressing CHOP or nATF6 in the intestinal epithelium, or carrying a heterozygotes knock-out for *cpn60* will provide a helpful tool to investigate the significance of ER- and mtUPR during inflammation. Future work will assess the susceptibility of these mice to DSS-induced colitis to elucidate the impact of Cpn60 expression level in the presence and absence of specific ER stress mediators.

Keywords: intestinal inflammation, transgenic mouse model, Cpn60, CHOP, nATF6

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