3rd Seeon Conference

Microbiota, Probiota and Host
Mikrobiota, Probiota und Wirt

18.- 20. June 2010
Conference Center
Monastery Seeon / Chiemsee

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

June 18, 2010

Dear Participant,

On behalf of the German Society of Hygiene and Microbiology and the Organizing Committee, welcome to the 3rd Seeon Conference “Microbiota, Probiota and Host”!

The dramatic increase of chronically degenerative diseases in the industrialized world implies a complex interaction of host genetic predispositions and environmental factors. Although the research community in Germany established a visible expertise in the identification and understanding of genetic susceptibilities as well as immune-mediated mechanisms of inflammatory processes at mucosal interfaces, there is an obvious need to integrate the search for environmental triggers of disease pathogenesis focusing on microbial and nutritional factors. The gut microbiome clearly harbors potential traits of pathogenic and beneficial bacteria, and the challenge will be to define the “detrimental or symbiotic” milieu at the level of microbe- and host-derived signals in order to develop efficacious treatment and prevention strategies for chronic inflammatory diseases at mucosal surfaces. Cutting-edge technologies such as transcriptomics, proteomics and metabolomics should be implemented in the compositional and functional analysis of the gut microbiota, host’s metabolism and immunity as well as pre- and probiotic mechanisms.

The “Seeon Conference” has been proven to become a visible platform for cross-sectional discussions bringing together basic science, genetics, and clinicial disciplines such as gastroenterology, medical microbiology and immunology, and nutritional medicine. The expectations is that a vital group of young and established scientists come together in order to establish a fundamental understanding of host-microbe interactions in health and modern pathologies including 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.

Sincerely,

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PROGRAM Friday, June 18

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

17^{15} - 18^{15}	Keynote Lecture: **J. Walter**, University of Nebraska, USA ***The Gut Microbiome in Health and Disease: An evolutionary perspective***

18^{30}	Dinner

19^{30}	DGHM Section Meeting and Elections

GUT MICROBIOME AND HOST

20^{00} – 21^{30} Chair: A. Bleich, Institute of Laboratory Animal Science, Hannover Medical School

S.J. Wagner, Biofunctionality, TU München
Impact of luminal and systemic iron on the development of chronic ileitis targeting the gut microbial composition and stress response in intestinal epithelial cells

J. F. Baines, Max-Planck-Institute for Evolutionary Biology, Plön
The influence of B4galnt2 expression on the intestinal microbiota in house mice

B. Stecher, Institute of Microbiology, ETH Zürich, CH
Commensal E.coli antagonize Salmonella Typhimurium after horizontal gene transfer in the gut

S. Ganal, Institute for Medical Microbiology+ Hygiene, University Hospital Freiburg
Role of the commensal microflora in shaping natural killer cell function at non-mucosal sites

F.-A. Heinsen, Institute for Clinical Molecular Biology, Christian-Albrechts-University Kiel
DNA-based taxonomic description of the dynamic changes of the human mucosa-associated intestinal microbiota during resilience and infection

L. Janus, Institute of Laboratory Animal Science, Hannover Medical School
Effect of murine norovirus X bacterial interaction on experimental colitis

21^{30}	Drink at the Bar – An Invitation!

PROGRAM Saturday, June 19

08^{30} - 09^{30} Keynote Lecture: **P.W. O´Toole**, University College Cork, Ireland
Molecular Mechanisms for Probiotic Function

09^{30} - 10^{00} Coffee Break / **Poster Session at the first glance**

PROBIOTIC MECHANISMS

10^{00} – 12^{00} Chair: T. Ölschläger, Institute for Molecular Infection Biology, University Würzburg

C. Jacobi, Clinic for Gastroenterology,Hepatology and Infection, University Magdeburg
Studies on quorum sensing in E.Coli Nissle 1917 (Mutaflor)

M.-A. von Schillde, Biofunctionality, TU München
Probiotic-derived Lactocepin degrades the pro-inflammatory chemokine IP-10: Impact on chronic intestinal inflammation

J. Preising, Institute for Microbiology + Biotechnology, University Ulm
Treatment with B.bifidum ameliorates colitis in two murine models of colitis

A. Troge, Institute for Molecular Infection Biology, University Würzburg
The flagellum of Escherichia coli Nissle 1917 mediates adhesion to mucin

L. von Müller, Institute of Medical Microbiology + Hygiene, University Hospital of Saarland
Oral probiotics in preterm infants and bacterial signatures in consecutive stool samples

A. Wittmann, Institute for Medical Microbiology + Hygiene, University of Tübingen
Bifidobacterium adolescentis protects from dissemination of Yersinia enterocolitica

A. Zschüttig, Institute for Medical Microbiology and Hygiene, TU Dresden
Whole genome sequencing of probiotic Escherichia coli strain G3/10

J.S. Frick, Institute for Medical Microbiology + Hygiene, University of Tübingen
E.coli Nissle 1917 inhibits T-cell induced colitis in $Rag1^{-/-}$ mice via TLR5 dependent trapping of T cells in mesenteric lymph nodes

12^{00} – 12^{45} Keynote Lecture: **F.-P. Martin**, Nestlé Research Center, Lausanne, CH
Metabolic Profiling and Chronic Inflammation

PROGRAM Saturday, June 19

12^{45} - 15^{00} Lunch and Guided Tour through the Monastery

MECHANISMS OF INFLAMMATION

15^{00} – 16^{30} Chair: A. Diefenbach, Inst. for Medical Microbiology and Hygiene, University of Freiburg

E. Rath, Biofunctionality, TU München
Mitochondrial stress in the epithelium fuels chronic intestinal inflammation via double-stranded RNA-activated protein kinase

P. Hernández, Institute for Medical Microbiology and Hygiene, University of Freiburg
Regulation of epithelial Homeostasis by LTi and NKR-LTi cells

C. Desel, Institute of Clinical Microbiology, Immunology + Hygiene, University Hospital Erlangen
MyD88 signalling is required for TDB adjuvanticity in vivo

A. Bleich, Institute of Laboratory Animal Science, Hannover Medical School
Differential host response and differential compositional changes of microflora are associated with background-determined colitis susceptibility in IL-10-deficient mice

T. Karrasch, Gastroenterology + Hepatology, University of Regensburg
PI3K-dependent GSK3ß(Ser9)-phosphorylation is implicated in the intestinal epithelial cell wound-healing response

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

16^{30} – 17^{30} Coffee Break / **Guided Poster Session** (J. Frick, Institute for Medical Microbiology + Hygiene, University of Tübingen)

17^{30} – 18^{30} Keynote Lecture: **K. Brandl**, The Scripps Research Institute, La Jolla, USA
ER stress and Chronic Inflammation

19^{30} Dinner

20^{30} Bowling at the Bar

PROGRAM Sunday, June 20

08^{30} - 09^{30} Keynote Lecture: **D. Kopecko**, FDA Center of Biologics Evaluation and Research, Maryland, USA
Campylobacter Interaction with the Human Gut Epithelium

09^{30} - 09^{35} **Poster Prices**

09^{35} - 10^{00} Coffee Break

MECHANISMS OF INFECTIONS

10^{00} – 11^{30} Chair: F. Gunzer, Inst. for Medical Microbiology and Hygiene, TU Dresden

G.A. Grassl, Institute for Experimental Medicine, University of Kiel and Research Center Borstel
Salmonella-induced intestinal fibrosis in mice is mediated by RORα and RORγ

A. Bergamo Estrela, Chemical Microbiology, Helmholtz Centre for Infection Research
Extracellular compounds from Escherichia coli after human beta-defensin-2 (hBD-2) treatment and the effects on human intestinal epithelial cells (Caco-2)

S. Steininger, Dep. of Internal Medicine II, Klinikum rechts der Isar, TU München
Tight junctions dysfunction promotes adhesion and invasion of H. hepaticus in polarized colon epithelial cells

H. Schoenen, Institute of Clinical Microbiology, Immunology + Hygiene, University Hospital Erlangen
Mincle is essential for recognition and adjuvanticity of the mycobacterial cord factor and its synthetic analog trehalose-dibehenate

E. Heupel, Institute of Microbiology and Biotechnology, University of Ulm
Tracing Bifidobacterium bifidum in different mouse models of inflammatory bowel disease

A. Parlesak, Center for Biological Sequence Analysis, Technical University of Denmark, DK
Intestinal Expression of cytochrome P450 Subclass 1A1 (CYP1A1) depends on TOLL-like receptor 2 (TLR2) functionality

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11^{30} Lunch and Departure

PROGRAM
Friday,
June 18

THE GUT MICROBIOME IN HEALTH AND DISEASE: AN EVOLUTIONARY PERSPECTIVE

J. Walter

Department of Food Science and Technology, University of Nebraska, USA

Humans harbor vast and complex microbial communities in their gastrointestinal tracts that have a major impact on their host's metabolism, immunology, and health. The ability to modulate of the gut microbiota through dietary strategies now provides exciting opportunities to prevent modern pathologies including chronic inflammatory, atopic and metabolic diseases. In this presentation I argue that the development of successful therapeutic applications that target the gut microbiome will benefit from a consideration of the biological principles that underlie microbe-host interactions in an evolutionary context. I will therefore discuss the basic characteristics of microbial symbiosis in the vertebrate gut and the potential of the development of mutualism. The phylogenetic and experimental studies that have addressed the evolution of the gut microbiome and the consequences that arise for both the microbes and the host will be reviewed. I will then explain why a mechanistic understanding of the microbial symbiosis in the vertebrate gut and its evolution will be important to determine how this partnership can go awry, and how it may reveal possibilities to redress imbalances of the gut microbiota to prevent and ameliorate disease.

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IMPACT OF LUMINAL AND SYSTEMIC IRON ON THE DEVELOPMENT OF CHRONIC ILEITIS TARGETING THE GUT MICROBIAL COMPOSITION AND STRESS RESPONSE IN INTESTINAL EPITHELIAL CELLS

Werner T [1], Wagner SJ [1], Chang S [1], Kisling S [1], Schümann K [2], Martínez I [3], Walter J [3], Haller D [1]*

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Background & Aim: Patients with inflammatory bowel disease (IBD) suffer from anaemia, but high iron concentrations can induce endoplasmic reticulum (ER) stress and protein oxidative damage. Our aim was to characterize the effects of dietary and systemic iron on microbial composition, intestinal epithelial cells (IEC) and intraepithelial lymphocytes (IEL) in chronic experimental ileitis.

Methods & Results: Histological scoring (0-12) revealed that long-term (age of 7-18 weeks) iron-deficient feeding of $TNF^{\Delta ARE/WT}$ (ARE) mice (score 2.30±0.76) resulted in almost complete protection from ileal inflammation in contrast to iron-adequately fed ARE mice (score 8.30±0.91). Short-term feeding of the iron-deficient diet in existing ileitis (age of 17-21 weeks) revealed significant reduction of the histoscore (7.15±0.38 to 3.9±1.35). Most importantly, systemic iron repletion of iron-deficiently fed ARE mice by intraperitoneal injections (90µmol iron/week) did not reverse the protective effect of iron-deficient feeding (score 1.67±0.20) suggesting a critical role for dietary but not systemic iron. 454-pyrosequencing of the 16S rRNA from cecal content revealed distinct iron-mediated and inflammation-driven changes of the microbial composition. Interestingly, the increase of Actinobacteria was associated with the protective effects of the luminal iron depletion in ARE mice independently of systemic iron repletion. Western blot analysis, immunohistochemical and TUNEL staining from inflamed (iron-adequate) versus non-inflamed (iron-deficient, iron-repleted) primary IEC and ileal tissue sections revealed down-regulation of ER stress-associated glucose regulated protein (grp-78) and pro-apoptotic mechanisms especially in ileal crypt regions. Whereas luminal iron depletion did not affect the number of pro-inflammatory $CD8\alpha\beta^+$ IEL, their cytotoxic effector function was investigated in co-culture experiments with the IEC cell line Mode-K. Activation of isolated $CD8\alpha\beta^+$ T cells from ARE mice lead to apoptosis of Mode-K cells accompanied by high levels of secreted granzyme B. This apoptotic effect occurred even faster when Mode-K cells were pre-incubated with the ER stressor tunicamycin (2h) before co-culture.

Conclusion: This study clearly demonstrates protective effects of low dietary iron and systemic iron repletion on chronic ileitis in ARE mice. Iron was shown as modulator of the microbiota as well as ER stress and apoptosis in IEC. IEL cross-talk with the epithelium and the presence of ER stress may be important factors for IEC homeostasis. These data may point to a possible therapeutic use of a combined treatment for anaemia and inflammation in IBD patients.

THE INFLUENCE OF B4GALNT2 EXPRESSION ON THE INTESTINAL MICROBIOTA IN HOUSE MICE

J.F. Baines[1,2], F. Staubach[1], J.M. Johnsen[3], D. Ginsburg[4]

[1] *Max-Planck-Institute for Evolutionary Biology, Plön, Germany*

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

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

[4] *Department of Internal Medicine, Howard Hughes Medical Institute, Department of Human Genetics, and the Life Sciences Institute, University of Michigan, Ann Arbor, MI, USA*

The glycoconjugate profile of the intestinal tract is determined by a spectrum of glycosyltransferases. GI glycosylation profiles can influence gastrointestinal pathogenic and commensal bacteria. The glycosyltranferase gene *B4galnt2* is expressed in the GI tract. Similar to other blood-group related glycosyltransferases in humans, the *B4galnt2* locus displays striking signatures of selection in natural populations of house mice. *Cis*-regulatory variation of *B4galnt2* expression in house mice leads to different tissue-specific expression patterns affecting intestinal epithelium and vascular endothelium. Blood vessel expression of *B4galnt2* leads to a phenotype in mice very similar to a common human bleeding disorder, von Willebrand disease, but the consequences of altered *B4galnt2* expression in the intestine are unknown. We hypothesize that the signatures of selection at the *B4galnt2* locus are likely the result of host – pathogen interactions. To investigate the influence of *B4galnt2* expression on the intestinal microbiota, we have applied high throughput metagenomics (16S rRNA tag pyrosequencing by 454) to a mouse knockout model and identified striking differences in commensal bacterial populations.

COMMENSAL *E.COLI* ANTAGONIZE *SALMONELLA* TYPHIMURIUM AFTER HORIZONTAL GENE TRANSFER IN THE GUT

B. Stecher, R. Denzler, N. Thompson, U. Dobrindt, F. Bernet, W.-D. Hardt

Institute of Microbiology, ETH Zürich, Wolfgang-Pauli-Strasse 10, CH-8093 Zürich, Switzerland

The intestinal tract is colonized by a complex and stable bacterial community, the microbiota. The microbiota efficiently prevents intrusion of external bacteria, such as enteric pathogens, by largely unknown mechanisms. Here, we investigated how the microbiota counteracts infection of the enteric pathogen, *Salmonella enterica* serovar Typhimurium SL1344 (*S.*Tm) in a mouse colitis model. In the majority of infected animals, *S.*Tm was the most abundant species in the gut. However, we frequently observed overgrowth of strains of commensal *E.coli*, which led to a substantial reduction in pathogen loads. Genome sequencing of one representative *E.coli* isolate revealed the presence of a conjugative plasmid encoding the genes for colicin production. We speculate that this plasmid had been acquired from *S.*Tm via horizontal gene transfer during the infection and subsequently led to increased competitiveness of the local *E.coli* population against *S.*Tm. Thus, our data reveal how the intrinsic microbiota specifically re-adapts in response to an infection and thereby mediates pathogen clearance from the gut.

ROLE OF THE COMMENSAL MICROFLORA IN SHAPING NATURAL KILLER CELL FUNCTION AT NON-MUCOSAL SITES

S. Ganal[1,3], S. Sanos[1], M. Weiß[1], C. Johner[2], A. Diefenbach[1]

[1]*Institute for Medical Microbiology and Hygiene, University Hospital Freiburg, Hermann-Herder-Str. 11, 79104 Freiburg, Germany*
[2]*Max-Planck-Institute for Immunobiology, Stübeweg 51, 79108 Freiburg, Germany*
[3]*Supported by the Boehringer-Ingelheim Fonds*

Although the mammalian intestinal mucosal surface is constantly exposed to a vast number of microorganisms, mammals coexist peacefully in a mutually beneficial relationship with the commensal microflora in the gut. The indigenous microbiota are important for the development and function of intestinal immunity. During our studies in germ-free (GF) mice, we observed that activation of an innate lymphocyte population (natural killer (NK) cells) at non-mucosal sites after injection of Toll-like receptor (TLR) agonists was strongly reduced in GF mice compared to mice hold under specific pathogen-free (SPF) conditions. NK cells are innate immune effector cells that secrete cytokines and mediate cellular cytotoxicity within hours after an infection. NK cell activation depends on the maturation/education of NK cells and on priming signals delivered by dendritic cells (DC). We investigated whether the defect in NK cell activation in GF mice was an NK cell-intrinsic maturation/education defect or rather reflects insufficiency in DC-derived priming signals. Adoptive transfer experiments of NK cells into GF or SPF mice demonstrated that defective activation of NK cells is not an NK cell-intrinsic defect but rather a consequence of the provision of ineffective priming signals by DC to NK cells in GF mice. I will report about our ongoing work to define the microbiota-dependent molecular signals that instruct the function of DC in non-mucosal organs.

DNA-BASED TAXONOMIC DESCRIPTION OF THE DYNAMIC CHANGES OF THE HUMAN MUCOSA-ASSOCIATED INTESTINAL MICROBIOTA DURING RESILIENCE AND INFECTION

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

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

The microbial community in the normal human gut is individually specific, remains stable over a period of time and usually exerts a high self-regeneratory capacity (resilience phenomenon) after external perturbation. This ability of regeneration seems to be defective in inflammatory bowel disease, which is characterized by an altered microbial composition. Similar 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. Non cultivable molecular technique (16S rRNA gene clone libraries) was applied to access the change in microbiota at five different time points over a period of six weeks. Antibiotic dosage resulted in increased *Bacteroidetes* and decreased *Firmicutes*. We observed a trend of microbiota regeneration on analyzed 10th day after antibiotic cessation in both groups (placebo/probiotic). These changes were independent of placebo or probiotic at phylum levels. At genus level a higher amount of S*treptococci* could be seen after allocation to the probiotic compared to placebo, which is likely because of S*treptococcus salivarius* be part of the probiotic. More changes at lower taxonomical levels are expected and are under investigation.

EFFECT OF MURINE NOROVIRUS X BACTERIAL INTERACTION ON EXPERIMENTAL COLITIS

L. Janus[1], M. Achard[1,2], M. Mähler[2], D. Neumann[3], N.-H. Zschemisch[1], A. Smoczek[1], H.-J. Hedrich[1], A. Bleich[1]

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[2]*BioDoc, Hannover, Germany;*
[3]*Institute of Pharmacology, Hannover Medical School, Hannover, Germany*

Murine Noroviruses are nonenveloped RNA viruses of the family *Caliciviridae*, genus Norovirus, and are highly prevalent in mouse colonies. Aim of this study was to investigate whether MNV modifies the phenotype of the IL-10-deficient (*Il10*^{tm1Cgn}, *Il10*$^{-/-}$) mouse model of IBD under A) SPF or B) germfree conditions, or C) during *Helicobacter (H.) hepaticus* infection. MNV was isolated from NOD-*Prkdc*^{scid} mice, genetically characterised, and used for oro-nasal inoculation. A) All MNV-inoculated mice seroconverted. MNV was detected in ileum, colon, mesenteric lymph nodes (MLN), and feces, but not in spleens, lungs, livers, and kidneys. Fecal shedding remained up to eight weeks after inoculation. Mild and focal inflammatory lesions were detected in large intestines of inoculated *Il10*$^{-/-}$ mice and IFNγ secretion of *in vitro* anti-CD3 stimulated MLN cells was significantly enhanced. B) Inflammatory lesions were not detected in germfree C3Bir-*Il10*$^{-/-}$ mice inoculated with MNV. IFNγ secretion levels of stimulated MLN cells did not differ between infected and control mice. C) Double-infected mice (MNV-infected mice additionally inoculated with H. hepaticus two weeks later) and mice infected with *H. hepaticus* only showed a high degree of intestinal inflammation with no significant differences between the groups and no differences in IFNγ secretion levels. In conclusion, MNV provides a colitogenic stimulus that is dependent on bacterial intestinal colonisation. Inflammation induced by a highly potent colitogenic activator, *H. hepaticus*, was not enhanced by prior infection of mice with MNV. Therefore, MNV likely plays a role in the development of inflammation by interaction with an endogenous, apathogenic intestinal flora.

PROGRAM
Saturday, June 19

MOLECULAR MECHANISMS FOR PROBIOTIC FUNCTION

P.W. O´Toole

Department of Microbiology, and the Alimentary Pharmabiotic Centre, University College Cork, Ireland, pwotoole@ucc.ie, Tel: 00 353 21 490 3997

The human gut microbiota comprises an estimated total of 10^{14} bacterial cells and several thousand bacterial phylotypes. Within this enormous diversity of intestinal commensal organisms, a relatively small number of micro-organisms have been characterized as probiotic – "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". Recent culture-independent studies have also revealed that these organisms, that are proposed to be probiotic, are typically minor members, or at best sub-dominant members, of the human gut microbiota.

Consumption of foodstuffs that contain probiotic microbes has a long history, and a diverse range of health benefits have been attributed to ingestion of probiotic bacteria. Many of these proposed benefits are controversial, and some have not been substantiated in the molecular era. Recent movements in the European regulatory environment have increased the burden of proof required for maintaining functional health claims for food products, including those marketed as being probiotic. The ways in which probiotic organisms might exert a beneficial effect upon the host are diverse, and only a few have been elucidated to a high degree of scientific clarity. They may be broadly defined as (a) direct effects upon the host and (b) effects upon the microbiota which indirectly benefit the host.

The development of genomics and post-genomic approaches has provided new leverage to explore interactions between the animal host and its probiotic commensal inhabitants. In this presentation, recent molecular analyses that provide the clearest evidence for how probiotics might exert beneficial effects will be impartially reviewed, including studies from Cork of host and microbiota interactions.

PROBIOTIC MECHANISMS

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C. Jacobi, Clinic for Gastroenterology,Hepatology and Infection, University Magdeburg
Studies on quorum sensing in E.Coli Nissle 1917 (Mutaflor)

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Probiotic-derived Lactocepin degrades the pro-inflammatory chemokine IP-10: Impact on chronic intestinal inflammation

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E.coli Nissle 1917 inhibits T-cell induced colitis in $Rag1^{-/-}$ mice via TLR5 dependent trapping of T cells in mesenteric lymph nodes

STUDIES ON QUORUM SENSING IN *E. COLI* NISSLE 1917 (MUTAFLOR)

C. Jacobi[1,3], S. Grundler[1], J.-S. Frick[2], S. Rust[3], P. Adam[4], I.B. Autenrieth[2], P. Malfertheiner[3], M. Gregor[1]

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The term „Quorum Sensing“ (QS) describes the density dependent gene regulation in bacterial cells, involving both the regulation of physiological as well as virulence determinants using signal molecules (homoserine lactones (“autoinducer-1”: AI-1) or furanosyl borate diester (AI-2)). If QS is used by *E. coli* Nissle 1917 (Mutaflor) (EcN) for gene regulation is unknown. We examined EcN for production of AI-1 and AI-2 signal molecules and constructed an AI-2 negative mutant. In addition we studied the expression of defensines, pro- and anti-inflammatory cytokines in a DSS colitis mouse model after oral challenge with the bacteria.

While AI-1 is not produced by Mutaflor, AI-2 is produced in a density dependent manner in different amounts. After constructing an AI-2 deficient mutant we showed that the bacteria are hampered in their motility. Experiments are underway to focus on the flagella. In the DSS colitis mouse model we see different expression profiles of INFgamma and IL-10 using RT PCR. While the weight curve of the mice is dependent on the bacteria (WT or mutant), the histopathology of the colon is similar. Both bacteria colonize the mice in a similar fashion. We need to elucidate the role of QS in Mutaflor further in order to get a better understanding in the biology of this important probiotic.

PROBIOTIC-DERIVED LACTOCEPIN DEGRADES THE PRO-INFLAMMATORY CHEMOKINE IP-10: IMPACT ON CHRONIC INTESTINAL INFLAMMATION

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Background and Aim. Clinical studies revealed that the probiotic mixture VSL#3 is protective in the context of inflammatory bowel disease (IBD). We previously showed that reduced cecal inflammation in VSL#3-fed Interleukin 10 deficient (IL-$10^{-/-}$) mice correlates with reduced levels of interferon-inducible protein (IP-10) in cecal epithelial cells. Mechanistically, IP-10 protein degradation in intestinal epithelial cells (IEC) was mediated by cell surface proteins of VSL#3-derived *Lactobacillus paracasei* (*L. paracasei*). The aim of the present study was to identify the active probiotic structure triggering the loss of this major pro-inflammatory chemokine.

Methods and Results. Stimulation of activated IEC (Mode-K) with *L. paracasei* conditioned media (CM) revealed that not only cell surface proteins, but also secreted compounds of *L. paracasei* mediate selective inhibition of IP-10 secretion in IEC. Size exclusion, heat treatment and ammonium sulfate precipitation assays showed that the active compound of *L. paracasei* CM is a protein exceeding a molecular mass of 100 kDa. Interestingly, phenylmethylsulfonylfluoride (PMSF), a serine protease inhibitor, abrogated the inhibitory effect of *L. paracasei* CM on secreted and IEC-surface-associated IP-10. This suggests that *L. paracasei* CM did not induce IP-10 degradation in IEC but contains a bacterial protease targeting extracellular IP-10. Consistently, cell-free assays demonstrated that recombinant IP-10 is rapidly degraded in the presence of *L. paracasei* CM. In addition to IP-10, *L. paracasei* CM was found to selectively target stromal cell-derived factor 1α (SDF-1α) and interferon-inducible T cell α-chemoattractant (I-TAC) but not interleukin 6 (IL-6), macrophage inflammatory protein-2 (MIP-2), RANTES (regulated on activation, normal T-cell expressed and secreted) and interleukin 8 (IL-8). Chromatographic fractionation of *L. paracasei* CM followed by differential liquid chromatography electrospray ionisation tandem mass spectrometry (LC-ESI-MS/MS) analysis of proteolytically active versus non-active fractions identified lactocepin, a cell-wall associated and secreted serine protease to be the active probiotic structure of *L. casei*.

Conclusion. Lactocepin was identified as a probiotic bacterial protease targeting a specific subset of pro-inflammatory chemokines including IP-10. This probiotic mechanism may contribute to a more structure-based evaluation of probiotic bacteria for therapeutical interventions in the context of chronic inflammation.

TREATMENT WITH B. BIFIDUM AMELIORATES COLITIS IN TWO MURINE MODELS OF COLITIS

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Recently we were able to demonstrate potent inhibitory effects of LPS-induced inflammatory events of a B. bifidum strain with good adhesive properties using cultured epithelial cell lines. By contrast, a non-adherent B. longum/infantis strain did not show anti-inflammatory activity in vitro. To further investigate the anti-inflammatory activity both strains were analysed in TNBS-induced colitis and the Rag 1-/- CD4+ T-cell transfer model. In a placebo-controlled set up two groups of mice received one oral dose of either the probiotic B. bifidum or the `non-probiotic´ B. longum/infantis strain followed by transfer of CD4+T-cells. Two control groups received placebo of which one group was also transfered with CD4+T-cells to induce colitis. Feeding with the probiotic and placebo was continued three times a week until the end of the trial when all animals were sacrificed. The anti-inflammatory effect of B. bifidum was assessed by measuring the weight of the animals during the trial, post mortem analysis of colonic weight and length of the dissected colons and histological scoring of colonic tissue samples. Treatment with the probiotic B. bifidum strain significantly improved colitis as measured by these parameters whereas B. longum/infantis had no effect. In the TNBS model one group of animals received one oral dose of B. bifidum or placebo daily. On day 3 animals of two groups received an intrarectal dose of TNBS or carrier. Feeding with B. bifidum or placebo was continued until sacrifice. Similar parameters as for the Rag-/- trial were analysed. Additionally, several markers of inflammation were quantified in colonic biopsies. This confirmed anti-inflammatory effect of our B. bifidum strain.

THE FLAGELLUM OF *ESCHERICHIA COLI* NISSLE 1917 MEDIATES ADHESION TO MUCIN

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The probiotic *E. coli* strain Nissle 1917 (EcN) is motile by flagellation. In addition the flagellum induces expression of human beta defensin 2. Here we report a third function for the flagellum of this probiotic strain.

To elucidate EcN´s adherence properties we used both *in vitro* and *ex vivo* studies. The results achieved showed the importance of EcN´s flagella for efficient adherence to cryosections of human gut biopsies. In contrast, adhesion to human epithelial cell-lines was also observed for a non-flagellated EcN mutant. Surprisingly, the lack of type1- or F1C-pili had no effect on adhesion efficiency of EcN, neither for the *ex vivo*- nor for the *in vitro*-experiments.

In recent studies we were able to show, that mucin is most likely the responsible receptor for flagella-mediated adherence of EcN to cryosections of human gut biopsies. The adhesion of flagellated EcN strains to these cryosections could be blocked in a dose-dependent manner by preincubating the bacteria with mucin. Compared to the wildtype, a *fliC*-deletion mutant adhered barely to mucin, whereas a hyperflagellated variant of EcN adhered twice as efficient. In addition we were able to demonstrate the direct interaction of isolated flagella with mucin by Western Blot.

These results can explain the differences between the *ex vivo* and *in vitro* studies, because the tested cell lines are not able to produce mucin. In contrast, this protein is most likely present in the cryosections. Therefore the flagellum seems to be the major adhesin of EcN *in vivo* and its receptor is mucin.

ORAL PROBIOTICS IN PRETERM INFANTS AND BACTERIAL SIGNATURES IN CONSECUTIVE STOOL SAMPLES

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Introduction. Risk of Necrotizing Enterocolitis (NEC) is reduced by oral supplementation with probiotics. The mechanisms causing protection are still elusive. On the one hand protection may be associated with changes of the gut flora (direct effects) or on the other hand by indirect effects, as e.g. by modulation of bacterial-host-interaction at the mucosal interface or by interaction with the immune system.

Material and Methods. In the present prospective randomized cross-over study we analysed the development of the bacterial community in stool samples of preterm infants for the first 6 weeks of life. 21 preterm infants at a gestational age <32 weeks and with birth-weight <1500g were included. Groups supplemented with *Lactobacillus acidophilum* and *Bifidobacterium infantis* (Infloran®) in week 1-3 (group 1) and in week 4-6 (group 2) were compared. The signatures of multiple bacterial genomes were regularly monitored in stool samples by Denaturing Gradient Gel Electrophoresis (PCR-DGGE) and the specific bands were identified by subsequent sequence typing. Bacterial signatures are actually available of >300 samples (10 patients).

Results. In general the first stool samples (meconium) were sterile, except for few patients with congenital infections. In the first weeks of life the development of an individual bacterial pattern was evolving. We could show that the development and the composition of the predominant bacterial species in infants stool were not altered by probiotic supplementation. In both groups, colonisation of infants stool flora was initiated by gram-negative bacteria, followed by gram-positive cocci, whereas the signatures of anaerobic bacteria were detected with a significant delay. Antibiotic interventions were followed by changes of bacterial signatures. Interestingly, establishment of *L. acidophilum* and *B. infantis* flora was not achieved by oral supplementation.

Conclusions. Despite the limitations due to the few cases it may be concluded that probiotic supplementation was not associated with significant changes of preterm infants stool flora pattern. Therefore we hypothesize that prevention of NEC might be associated with more indirect effects which remains still to be confirmed in future studies.

BIFIDOBACTERIUM ADOLESCENTIS PROTECTS FROM DISSEMINATION OF *YERSINIA ENTEROCOLITICA*

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The *Bifidobacterium adolescentis* is an anaerobic, gram positive bacterium and belongs to the probiotics, which are by definition “live microorganisms that, when administered in adequate amounts, confer a beneficial effect on the health of the host” (FAO/WHO 2001). To investigate a possible protective potential of *B. adolescentis* in a gastro-enteritis we chose oral *Yersinia enterocolitica* infection of mice as pathogenicity model, which leads to a gastro-enteritis in mice. Therefore four different groups of SPF BL/6 mice were investigated: first mocks, second *Bifidobacteria* fed ones, third group was orally challenged with *Yersinia* and the fourth was first *Bifidobacteria* fed and subsequently *Yersinia* infected. Body weight and fitness of *Yersinia* infected mice were controlled and five days past infection mice were sacrificed. The *Bifidobaceteria* colonized group showed less weight loss compared to control group. Whole intestines were analyzed for dendritic cell subpopulations and T-cell subsets, as well were cytokines investigated. Further were colony forming units (CFU) of *Yersinia* in faces and spleen determined. *Bifidobacteria* fed mice showed same *Yersinia* CFU in feces, but in spleens *Yersinia* were only found in mice without the probiotic strain. Colonization with *Bifidobacteria* led to an increase in plasmacytoid dendritic cells and regulatory T-cells in the intestine compared to groups without, independent of *Yersinia* infection. Next were BL/6 mice first *Yersinia* infected and then pDCs depleted using intra venous injection of an anti pDC antibody, as control was the according isotype used. Mice lacking pDCs showed stronger weight loss and a higher *Yersinia* CFU in the spleen.

Colonization with *B. adolescentis* has modulatory effects on the immune system of mice by means of pDCs and regulatory T-cell occurrence, which are probably the reason to achieve a partial protection of the hosts against *Yersinia enterocolitica* infection.

WHOLE GENOME SEQUENCING OF PROBIOTIC *ESCHERICHIA COLI* STRAIN G3/10

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Escherichia coli G3/10 is an *E. coli* genotype of the probiotic drug Symbioflor E. coli. The medicine is widely used for the treatment of functional gastrointestinal disorders in particular Colon irritabile in adults and children, functionally representing one population. However, until now only little knowledge has been gained about the contribution of each genotype to the probiotic character of the product. Furthermore it is largely unknown which molecular mechanisms are involved. In the project presented here, we performed whole genome sequencing of the different genotypes of the *E. coli* strain, Symbioflor E. coli is made of, with a special focus on *E. coli* G3/10, a genotype that attracted interest due to its ability of defensin induction in human intestinal epithelial cells. Using pyrosequencing technology we obtained about five megabases of raw sequence data which were subsequently allocated by comparative genome assembly. Automatic annotation was then performed with GenDB 2.4 annotation software followed by manual revision of each coding region using various databases. Most gaps could successfully be closed by using shotgun fosmid-library technique and PCR. Shotgun sequencing of plasmid DNA was used to identify and separate plasmids from genome data. The genome of *E. coli* G3/10 is about 4.9 megabases in size with a G/C content of 50.89% and 4682 genes. It also contains six natural plasmids with a range of size between 1.3 and 50 kilobases. Based on the completed genome of *E. coli* G3/10 it might be possible in future to identify specific genes responsible for the probiotic character of the strain and to gain insight into the strains contribution to the therapeutic effect of the probiotic drug Symbioflor E. coli.

E. COLI NISSLE 1917 INHIBITS T-CELL INDUCED COLITIS IN *RAG1*$^{-/-}$ MICE VIA TLR5 DEPENDENT TRAPPING OF T CELLS IN MESENTERIC LYMPH NODES

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Background. E. coli Nissle 1917 is used as a probiotic to maintain remission in patients with ulcerative colitis. The protective mechanisms are still not clear. In an adoptive T-cell transfer model we studied the molecular mechanisms of EcN on inflammation.

Methods. Immunodeficient *Rag1*$^{-/-}$ mice were fed orally with EcN and transplanted with naïve $CD4^+$ T cells. Weight loss and inflammatory status of the mice were monitored. Repopulation of transferred T cells in MLN and colon were analysed by FACS and mRNA cytokine expression in mucosal scrapings was measured. As EcN induces β-defensin production via TLR5 we also used an EcN strain deficient for the flagella (ΔfliC) and additionally, fed *RagxTlr5*$^{-/-}$ mice with EcN.

Results. EcN was effective in reducing the T cell mediated inflammation in *Rag1*$^{-/-}$ mice by trapping the transferred T cells in the MLN. Administration of EcN ΔfliC reduced this protective effect, suggesting a TLR5 dependent mechanism. The protective effect of EcN was also abolished in *RagxTlr5*$^{-/-}$ mice.

Conclusions. EcN seems to alter the migratory behaviour of T cells, which accumulate in the MLN in a TLR5-dependent manner. Therefore, EcN treatment limits the infiltration of T_H1 cells in the inflamed colon.

METABOLIC PROFILING AND CHRONIC INFLAMMATION

F.-P. Martin

Nestlé Research Center, Lausanne, CH

Contemporary evolution of diets and lifestyles is concomitant with the rapid progression of health issues, such as the promotion of functional digestive diseases, but underlying biochemical events are not well understood. In such a context, Nutrimetabonomics offers a novel strategy for measuring changes in the metabolic end-points of the physiological regulatory processes of an organism - that is expressed as a result of genes, environment, lifestyle, and diet. Metabolic monitoring using ^{1}H nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) analysis of biological samples is a well suited approach. Moreover, metabonomics generates multivariate information on a wide range of molecules in various biological matrices - whilst retaining the ability to extract specific biochemical information. We recently reported how such studies formed the basis of a strategy aiming to define the extent and importance of the microbiome symbiotic metabolic exchanges with the mammalian host. Deciphering the interactions between gut microbial residents and the region-specific gut physiology at the molecular level will provide new strategies for restoring and/or maintaining human health. The analysis of the complex biological profiles using sophisticated data mining tools highlights metabolic pathway interactions that can be interpreted in combination with immunological and histological outcomes. Potential biomarkers need in turn to be projected into metabolic pathway mapping to generate new biological hypothesis. In this lecture, recent applications of Nutritional Metabonomics will be introduced with special emphasis on gut metabolic health and inflammation.

MECHANISMS OF INFLAMMATION

15^{00} – 16^{30} Chair: A. Diefenbach, Inst. for Medical Microbiology and Hygiene, University of Freiburg

E. Rath, Biofunctionality, TU München
Mitochondrial stress in the epithelium fuels chronic intestinal inflammation via double-stranded RNA-activated protein kinase

P. Hernández, Institute for Medical Microbiology and Hygiene, University of Freiburg
Regulation of epithelial Homeostasis by LTi and NKR-LTi cells

C. Desel, Institute of Clinical Microbiology, Immunology + Hygiene, University Hospital Erlangen
MyD88 signalling is required for TDB adjuvanticity in vivo

A. Bleich, Institute of Laboratory Animal Science, Hannover Medical School
Differential host response and differential compositional changes of microflora are associated with background-determined colitis susceptibility in IL-10-deficient mice

T. Karrasch, Gastroenterology + Hepatology, University of Regensburg
PI3K-dependent GSK3ß(Ser9)-phosphorylation is implicated in the intestinal epithelial cell wound-healing response

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

MITOCHONDRIAL STRESS IN THE EPITHELIUM FUELS CHRONIC INTESTINAL INFLAMMATION VIA DOUBLE-STRANDED RNA-ACTIVATED PROTEIN KINASE

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Background + Aim. Inflammatory bowel diseases (IBD) are immune-mediated chronic disorders of the gastro-intestinal tract. The aim of this study was to characterize the pathological role of mitochondrial stress in the intestinal epithelium of IBD patients as well as murine models of bacteria- and T cell-mediated colitis.

Methods. Protein expression profiling in intestinal epithelial cells (IEC) from control and chronically inflamed recombination activating gene (RAG)2 and/or interleukin (IL)-10 deficient mice was performed using 2D-SDS-PAGE and MALDI-TOF mass spectrometry. The murine intestinal epithelial cell line Mode-K was transfected with the selective mitochondrial unfolded protein response (UPR)-inducer truncated ornithine transcarbamylase (OTCΔ). Pharmacological inhibitors and siRNA were used to block double-stranded RNA-activated protein kinase (PKR)-mediated signaling.

Results. Proteome analysis identified 132 differentially regulated proteins, clustering around the mitochondrial stress-associated protein chaperonin (CPN)60. Mitochondrial specific stress induction in Mode-K cells triggered the phosphorylation of eukaryotic translation initiation factor (eIF2)α through the recruitment and activation/phosphorylation of cytoplasmic PKR, exclusive of the induction of the endoplasmic reticulum (ER) stress-associated chaperone glucose-regulated protein (GRP)78. PKR inhibition completely blocked eIF2α phosphorylation and transcription factor activation including CCAAT/enhancer-binding protein homologous protein (CHOP) and cJun. Western blot and immunohistochemical analysis of colonic epithelium from IBD patients and the two murine models confirmed the increased expression levels of ER- and mitochondrial stress chaperones GRP78 and CPN60 associated with the induction of PKR.

Conclusion. These results demonstrate a novel mechanism for mitochondrial stress integration into the disease-relevant ER signaling cascade through the activation of cytoplasmic PKR, suggesting a pathological role for the mitochondrial UPR under conditions of chronic intestinal inflammation.

REGULATION OF EPITHELIAL HOMEOSTASIS BY LTI AND NKR-LTI CELLS

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Mucosal surfaces such as those of the intestinal tract are continuously exposed to both potential pathogens and beneficial commensal microorganisms. This creates a regulatory challenge to the mucosal immune system: it needs to be tolerant of the commensal microbiota while at the same time being able to mount an immune response to pathogens. It is accepted that there is dynamic cross-talk between the microflora, the epithelium and immune cells residing within the epithelium and the lamina propria. However, it remains unclear how immune cells of the lamina propria contribute to the regulation of epithelial homeostatic and/or antibacterial processes that are dependent on cues provided by the commensal microflora. Our recent data provide evidence that signals from the commensal microflora contribute to the differentiation of a lymphocyte population coexpressing stimulatory natural killer (NK) cell receptors (NKR) and the transcription factor RORgt that produce interleukin 22 (IL-22). IL-22 receptor cannot be found on hematopoeic cells but is exclusively expressed by epithelial and other non-hematopoietic cells. We now show that IL-22 is required and sufficient for epithelial expression of a group of antimicrobial and regenerative genes that play an important role in regulating epithelial homeostasis in the intestine. Our data demonstrate that IL-22-producing RORgt+ innate lymphocytes are important mediators of epithelial barrier function and regeneration processes of epithelial surfaces.

MyD88 Signalling is Required for TDB Adjuvanticity in vivo

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Successful vaccination is often dependent on the generation of strong cellular immune responses rather than antibody production. However, the most widely used adjuvant aluminiumhydroxide is only a weak inducer of Th1 responses. Thus, new adjuvants are currently being tested. Trehalose-6,6-dibehenate (TDB), the synthetic analogue of the mycobacterial cord factor trehalose-6,6-dimycolate (TDM) is a potent adjuvant not only inducing a strong Th1 but, unlike many others, also a pronounced Th17 immune response. We recently identified the C-type lectin Mincle as receptor for TDB and TDM that triggers the FcRγ-Syk-Card9 pathway for APC activation and adjuvanticity.

While APC activation in vitro was independent of TLR/MyD88-dependent signalling, in vivo data suggested the adjuvant effect of TDB was partially dependent on MyD88. In order to further dissect the MyD88-dependent pathways leading to the generation of a Th1/Th17 immune response after immunisation with a tuberculosis subunit vaccine in combination with TDB, we used several KO mouse strains and analysed adjuvant-induced immune responses in vivo. We show that development of a Th1 response required MyD88, and to some extent TLR9, but not TLR2, 3, 4 and 7. Interestingly, a Th1 immune response developed independently from IL1R signalling and inflammasome activation, whereas induction of a Th17 response by TDB required ASC.

DIFFERENTIAL HOST RESPONSE AND DIFFERENTIAL COMPOSITIONAL CHANGES OF MICROFLORA ARE ASSOCIATED WITH BACKGROUND-DETERMINED COLITIS SUSCEPTIBILITY IN IL10-DEFICIENT MICE

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Colitis susceptibility in *Il10*$^{-/-}$ (*Il10*^{tm1Cgn}) mice depends on genetic background (susceptible C3H/HeJBir vs. resistant C57BL/6J) and microflora. A major genetic locus mediating colitis-susceptibility, *Cdcs1*, was identified previously and transferred from susceptible to resistant mice. The aim of this study was to identify the impact of microflora on differential colitis susceptibility in this model system. Therefore, parental C3Bir-*Il10*$^{-/-}$, B6-*Il10*$^{-/-}$, and congenic mice on the B6-*Il10*$^{-/-}$ background carrying the C3Bir-derived *Cdcs1* locus (BC-R3), were inoculated with *H. hepaticus* and analyzed for inflammation, parental *Il10*$^{-/-}$ strains also for composition of intestinal microflora by T-RFLP. Infected C3Bir-*Il10*$^{-/-}$ and BC-R3 mice developed more severe inflammation in the cecum than B6-*Il10*$^{-/-}$ mice, but B6-*Il10*$^{-/-}$ more severe inflammation in the colon than C3Bir-*Il10*$^{-/-}$ mice. MLN cells of C3Bir-*Il10*$^{-/-}$ and BC-R3 mice secreted more proinflammatory cytokines than those of B6-*Il10*$^{-/-}$ mice, especially IFNγ and IL17. During *H. hepaticus* infection, strain specific differences in microflora composition were observed. Furthermore, *H. hepaticus* was mainly detected in the colon of C3Bir-*Il10*$^{-/-}$ mice, but in the cecum of B6-*Il10*$^{-/-}$ mice by real-time RT PCR. In conclusion, *Cdcs1* modifies the inflammatory response to *H. hepaticus* infection; however, this infection alone does not reflect the original response to a colitogenic flora as seen in conventional facilities. *H. hepaticus* distribution did not correlate with differential intestinal pathology but a strain specific differential composition of microflora was observed during *H. hepaticus* induced inflammation. Therefore, both host immune response and differential compositional changes of microflora might play a role in strain specific colitis susceptibility in *Il10*$^{-/-}$ mice.

PI3K-DEPENDENT GSK3β(SER9)-PHOSPHORYLATION IS IMPLICATED IN THE INTESTINAL EPITHELIAL CELL WOUND-HEALING RESPONSE

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Improper gastrointestinal restitution following various intestinal injuries may lead to acute and/or chronic inflammation through enhanced bacterial translocation and immune cell activation. The molecular mechanisms involved in the intestinal epithelial cells wound-healing response in the intestine are poorly understood. In order to characterize the role of GSK3β in intestinal epithelial cell restitution, confluent rat intestinal epithelial IEC18 cell monolayers were serum-starved for 16-24h and wounded by multiple scraping. Wounding rapidly induced GSK3β(Ser9) phosphorylation, which led to β-catenin accumulation as well as nuclear translocation of β-catenin. β-catenin stabilization/nuclear translocation led to enhanced LEF-TCF transcriptional activity and c-myc mRNA accumulation in wounded epithelial cell monolayers. Blocking PI3K/Akt signaling with Ly294002 prevented wound-induced GSK3β(Ser9) phosphorylation as well as β-catenin nuclear translocation and significantly attenuated restitution. Of note, inhibiting NF-κB activation via EGFR autophosphorylation with AG1478 did not prevent wound-induced GSK3β(Ser9) phosphorylation. GSK3β$^{-/-}$ cells demonstrated significantly attenuated wound-induced restitution compared to wild-type cells. We conclude that PI3K-mediated GSK3β phosphorylation is involved in the intestinal epithelial wound-healing response and operates independently of NF-κB signaling. Activation of the GSK3β pathway may be important for intestinal restitution by promoting cell motility in response to wounding.

ROLE OF EPITHELIAL NF-κB SIGNALLING IN INTESTINAL IMMUNE HOMEOSTASIS

A. Wullaert, M. Pasparakis

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Although the intestine contains trillions of bacteria that are recognized 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 activation of NF-κB 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. Germ-free 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.

ER STRESS AND INFLAMMATION

K. Brandl

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Several recent studies have recognized intestinal epithelial cells as active participants of the immune system. The epithelium can respond to a variety of immune mediators, e.g. bacterial stimuli. Moreover, dysfunction of intestinal epithelial cells due to unresolved ER stress can lead to intestinal inflammation. We have found an *N*-ethyl-*N*-nitrosourea (ENU)-induced missense error in the membrane bound transcription-factor peptidase site 1 (S1P) encoding gene (*Mbtps1*) causing enhanced susceptibility to dextran sodium sulphate (DSS) induced colitis. S1P cleaves and activates cAMP responsive binding protein (CREB)/ATF transcription factors, the sterol regulatory element binding proteins (SREBP)s, and other proteins of both endogenous and viral origin. As S1P has non-redundant function in the ATF6 dependent unfolded protein response (UPR), *Mbtps1* mutant mice show diminished levels of major endoplasmic reticulum (ER) chaperones GRP78 (BiP) and GRP94 in the colon upon DSS administration. Experiments with bone marrow chimeric mice reveal a requirement for S1P in non-hematopoietic cells, without which a diminished UPR and colitis develop. These data suggest that the ATF6-mediated UPR in the colonic epithelial compartment is critical to alleviate DSS induced colitis, and link cell-specific ER stress to the induction of organ-specific disease.

PROGRAM
Sunday,
June 20

CAMPYLOBACTER INTERACTION WITH THE HUMAN GUT EPITHELIUM

D.J. Kopecko

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Campylobacter jejuni, a zoonotic pathogen, is part of the normal gut flora in many animals, particularly birds. *Campylobacter* are the leading bacterial cause of human diarrheal disease worldwide. In addition to triggering acute diarrhea/dysentery, this pathogen also causes postdiarrheal IBS, GBS and Reiter's arthritis. Our studies have been focused on the cell biology of *C. jejuni* invasion of the terminal ileum/colon and the human immune responses to infection. Unlike *Salmonella* and *Shigella* which enter host cells via a TTSS-mediated actin-dependent process, *C. jejuni* enters human gut epithelial cells via a microtubule(Mt)-dependent mechanism that occurs independently of actin filaments, as shown by the use of biochemical inhibitors of the host cytoskeleton together with model human cell culture systems. Confocal microscopy of *Campylobacter*-infected cell lines has shown that entering *C. jejuni* colocalize with Mts and utilize the molecular motor dynein to traffick within the cell. Signal transduction studies have demonstrated that *C. jejuni* invasion requires activation of heterotrimeric G- proteins, release of Ca++ from intracellular stores, and activation of calmodulin, PKC, PI-3 kinase and ERK and p38 MAPKs. Electron microscopy of the *Campylobacter* invasion process has revealed that *C. jejuni* cells interact at the host apical cell surface via specific flagellar contacts, are internalized into endosomes that move from the apical surface to the perinuclear region and then basolaterally to release internalized *C. jejuni* via exocytosis at the basolateral host cell surface. The kinetics of entry into differentiated Caco-2 cells first suggested that M-like cells may be highly susceptible to *C. jejuni* invasion. Inhibition of this invasion process in the presence of filipin III or MβCD, together with host membrane fractionation has revealed that *Campylobacter* bind to host membrane receptors that aggregate in lipid raft fractions. Further, there are at least 7 *C. jejuni* surface proteins that bind intimately with the host plasma membrane during infection, one of which has been defined as flagellin. Further studies have shown that *C. jejuni*

internalization requires flagellar binding to a GPI-anchored receptor that then aggregates in raft fractions. Purified *C. jejuni* wildtype flagellin binds to raft fractions whereas specific glycosylation-deficient flagellin does not. Most importantly, these *C. jejuni* pseA glycosylation-deficient flagellin mutants are highly defective in invasion. Recent work has identified an M-cell associated molecule as the key receptor involved in *Campylobacter* invasion of the human intestine. *C. jejuni* interaction with this GPI-anchored receptor activates a host membrane complex of p60-dynactin-dynein-tubulin that we propose is involved in initiating endosome formation during internalization of *C. jejuni.* Finally*, C.jejuni* interaction with epithelial and dendritic cells triggers the release of proinflammatory cytokines/chemokines that result in colitis, disease symptoms, and associated inflammatory intestinal/extraintestinal sequelae.

MECHANISMS OF INFECTIONS

10^{00} – 11^{30} Chair: F. Gunzer, Inst. for Medical Microbiology and Hygiene, TU Dresden

G.A. Grassl, Institute for Experimental Medicine, University of Kiel and Research Center Borstel
Salmonella-induced intestinal fibrosis in mice is mediated by RORα and RORγ

A. Bergamo Estrela, Chemical Microbiology, Helmholtz Centre for Infection Research
Extracellular compounds from Escherichia coli after human beta-defensin-2 (hBD-2) treatment and the effects on human intestinal epithelial cells (Caco-2)

S. Steininger, Dep. of Internal Medicine II, Klinikum rechts der Isar, TU München
Tight junctions dysfunction promotes adhesion and invasion of H. hepaticus in polarized colon epithelial cells

H. Schoenen, Institute of Clinical Microbiology, Immunology + Hygiene, University Hospital Erlangen
Mincle is essential for recognition and adjuvanticity of the mycobacterial cord factor and its synthetic analog trehalose-dibehenate

E. Heupel, Institute of Microbiology and Biotechnology, University of Ulm
Tracing Bifidobacterium bifidum in different mouse models of inflammatory bowel disease

A. Parlesak, Center for Biological Sequence Analysis, Technical University of Denmark, DK
Intestinal Expression of cytochrome P450 Subclass 1A1 (CYP1A1) depends on TOLL-like receptor 2 (TLR2) functionality

SALMONELLA-INDUCED INTESTINAL FIBROSIS IN MICE IS MEDIATED BY RORα AND RORγ

G.A. Grassl[1,3], G.E. Diehl[2], E. Ma[1], Y. Valdez[1], D.R. Littman[2], B.B. Finlay[1]

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[3]*Institute of Experimental Medicine, University of Kiel and Research Center Borstel, Germany*

Intestinal fibrosis and stricture formation are serious complications of Crohn's disease, often requiring surgical intervention. Th17 cells are thought to be the primary inducers of inflammation in Crohn's patients and Th17 cell development is regulated by the transcription factors RORα and RORγ. Here we investigate the contribution of Th17 cells to the development of *Salmonella*-induced intestinal fibrosis using RORα$^{-/-}$γ$^{-/-}$ double-knockout mice. We show that oral infection of streptomycin-pretreated C57/Bl6 and RORα$^{-/-}$γ$^{-/-}$ mice leads to similar levels of chronic intestinal colonization with *S.* Typhimurium. C57/Bl6 mice displayed excessive transmural inflammation characterized by submucosal edema and destruction of crypt architecture. In contrast, we observed only mild histopathology in RORα$^{-/-}$γ$^{-/-}$ mice. Expression of fibrinogenic and pro-inflammatory cytokines was elevated and accompanied by increased numbers of fibroblasts and extensive collagen deposition in the submucosa of C57/Bl6 mice, whereas both fibrinogenic cytokine expression and collagen production were significantly reduced in RORα$^{-/-}$γ$^{-/-}$ mice. These data demonstrate that intestinal fibrosis induced by chronic *Salmonella* infection of the murine gastrointestinal tract is mediated by Th17 cells and requires both RORα and RORγ.

EXTRACELLULAR COMPOUNDS FROM *ESCHERICHIA COLI* AFTER HUMAN BETA-DEFENSIN-2 (HBD-2) TREATMENT AND THE EFFECTS ON HUMAN INTESTINAL EPITHELIAL CELLS (CACO-2)

A. Bergamo Estrela[1], M. Gutierrez[2], W.-R. Abraham[1]

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Human beta-defensins (hBD) are antimicrobial peptides expressed in the gut by epithelial cells. The inducible hBD-2 and -3 are regarded as important players in the pathophysiology of Inflammatory Bowel Diseases (IBD, comprising Crohn's Disease and Ulcerative Colitis) and present a complex regulation in the inflamed human gut. In an effort to understand the host-microbiota interactions taking place in IBD conditions, we have characterized the response of *E. coli* to hBD activity. Nucleoside-related compounds were identified by mass spectrometry as being present in culture supernatants specifically after hBD-2 treatment. No DNAse activity and no intracellular proteins were found in the supernatant, suggesting that this response is not a result of general cell lysis. Next we investigated the effects of the identified compounds upon human gut epithelial cells. Caco-2 cells were stimulated with supernatants from *E. coli* cultures, previously treated with hBD-2 or control conditions. The human cells were then analyzed for the nuclear translocation of NFkB by fluorescence microscopy and for the degradation of IkB by Western blot, to determine the level of activation of NFkB pathway. We hypothesized that the enhanced concentration of extracellular nucleosides produced when bacteria faces defensin activity may have an effect upon the host epithelia, influencing inflammatory reactions and mediating host-microbiota interactions in IBD patients.

TIGHT JUNCTION DYSFUNCTION PROMOTES ADHESION AND INVASION OF *H. HEPATICUS* IN POLARIZED COLON EPITHELIAL CELLS

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Inflammatory Bowel Disease (IBD) is caused in part by leaky intestinal barriers allowing facultative pathogens to interact with the host immune system. Thereby, an increased attachment and invasion of bacteria to intestinal epithelial cells (IEC) can be observed. To investigate if also non-invasive facultative pathogens are able to adhere and invade IEC we infected polarized mouse colon epithelial cells (PTK6 cells) *in vitro* with *Helicobacter hepaticus*.

We are able to show, that *H. hepaticus* in fact can be found intracellular. For adhesion of the bacteria to the basal-lateral surface of epithelial cells and for cell entry barrier defects are required. Electron- and immunofluorescence data suggest that *H. hepaticus* localizes to the lysosome before escaping to the cytoplasm of IEC directly. Using a gentamycin protection assay we demonstrate intracellular survival and replication of *H. hepaticus*. Endocytosis inhibitor experiments revealed that dead bacteria are internalized by caveolin- as well as clathrin-mediated endocytosis, while living bacteria shift the balance to the caveolin-mediated pathway where an escape from the lysosome is feasible. Additionally, infection of isolated primary colon epithelial cells from wildtype and caveolin-knockout mice (Cav1 -/-) showed reduced invasion of bacteria into epithelial cells lacking caveolin.

In conclusion, our data suggest, that *H. hepaticus* is able to adhere to and invade intestinal epithelial cells once the epithelial barrier is impaired. This invasion is a caveolin-mediated process and allows bacteria to proliferate in epithelial cells. Thus, caveolin-mediated invasion seems to be a promising target to prevent *H. hepaticus* induced chronic inflammation.

MINCLE IS ESSENTIAL FOR RECOGNITION AND ADJUVANTICITY OF THE MYCOBACTERIAL CORD FACTOR AND ITS SYNTHETIC ANALOG TREHALOSE-DIBEHENATE

H. Schoenen[1], B. Bodendorfer[1], K. Hitchens[2], S. Manzanero[2], K. Werninghaus[1], F. Nimmerjahn[3], E.M. Agger[4], S. Stenger[5], P. Andersen[4], J. Ruland[6], G.D. Brown[7], C. Wells[2], R. Lang[1]

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[4]*Dep. of Infectious Disease Immunology, Statens Serum Institute, Copenhagen*
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[6]*Department of Hematology, Technical University München*
[7]*Institute of Medical Sciences, University of Aberdeen*

The mycobacterial cord factor trehalose-6,6-dimycolate (TDM) and its synthetic analog trehalose-6,6-dibehenate (TDB) are potent adjuvants for Th1/Th17 vaccination that activate Syk-Card9 signaling in APCs. In this study, we have further investigated the molecular mechanism of innate immune activation by TDM and TDB. The Syk-coupling adapter protein FcRγ-chain was essential for macrophage activation and Th17 adjuvanticity. The FcRγ-associated C-type lectin receptor Mincle was expressed in macrophages and upregulated by TDM and TDB. Recombinant Mincle-Fc fusion protein specifically bound to the glycolipids. Genetic ablation of Mincle abolished TDM/TDB-induced macrophage activation and induction of T cell immune responses to a tuberculosis subunit vaccine. Macrophages lacking Mincle or FcRγ were impaired in the inflammatory response to Mycobacterium bovis bacillus Calmette-Guérin. These results establish that Mincle is a key receptor for the mycobacterial cord factor and controls the Th1/Th17 adjuvanticity of TDM and TDB.

TRACING *BIFIDOBACTERIUM BIFIDUM* IN DIFFERENT MOUSE MODELS OF INFLAMMATORY BOWEL DISEASE

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Bifidobacteria are part of the normal human gastrointestinal flora. Due to their beneficial effects on the human host, they are widely used as probiotics in dairy and pharmaceutical products. A promising application is their use in the treatment of the inflammatory bowel disease (IBD). In a previous study, we demonstrated that a strain of *B. bifidum* isolated from an infant stool sample has a strong inflammatory capacity in the $Rag^{-/-}$ transfer model of colitis. This effect was further confirmed in mice with chemically (TNBS)-induced intestinal inflammation.

At present, genetic modification of bifidobacteria is still challenging. Our group has established an efficient transformation protocol and created a range of novel *E. coli-Bifidobacterium* shuttle vectors for homologous expression of proteins in *B. bifidum*. One of these vectors, pMGS, was used to clone different fluorescent proteins (eGFP, eCFP, eYFP and mCherry) expressed under the control of P_{help}, a strong constitutive synthetic promoter. Fluorescent derivatives of our *B. bifidum* strain were generated by transformation of the resulting plasmids. These derivatives will be used to trace *B. bifidum* in different murine models of colitis.

INTESTINAL EXPRESSION OF CYTOCHROME P450 SUBCLASS 1A1 (CYP1A1) DEPENDS ON TOLL-LIKE RECEPTOR 2 (TLR2) FUNCTIONALITY

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Background. TLR2 has been so far considered to play primarily an important role in bacteria-host interaction and onset of inflammation. In previous studies, TLR-2 deficient mice showed an impaired intestinal integrity and elevated intestinal permeability.

Cytochrome P450 enzymes are an oxidoreductase enzyme group that is primarily linked to xenobiotics metabolism and formation of endogenous compounds such as bile acids and hormones. The subclass CYP1A1 plays an important role in carcinogen detoxification of polyaromatic hydrocarbons (PAH) such as benzo(a)pyrene (BAP), which in turn can induce cyp1a1 expression strongly. Moreover, CYP1A1 is contributing to eicosanoid (20-HETE and EET) formation, which contribute to intestinal epithelial homeostasis. Cyp1a1 is under control of the arylhydrocarbon receptor (AHR), which also controls expression of cyp1a2 and cyp1b1.

Aim. The current study aimed at identification of relevant gene expressions that are involved in TLR2-mediated impairment of intestinal integrity.

Methods. We screened the differential gene expressions in the intestine of mice in which the functional segment of the Toll-like receptor 2 was deleted ($Tlr2^{-/-}$) and in corresponding control (WT) animals (C57bl6/J; Agilent 42K mouse gene chip, 6 animals per genotype). In a consequent intervention study, we fed (or did not) both types of animals with 30 mg/kg/day of BAP (N=7-8) and measured both mRNA and protein expression (qRT-PCR and Western blot, resp.) in the intestine and the liver of these animals.

Results. Transcriptome analysis of intestinal tissue revealed that mRNA of Cyp1a1 was virtually absent (~ 23-fold reduction) in $Tlr2^{-/-}$ mice, while the expression of the Arh gene was moderately (~2-fold) increased compared to wild-type mice. Feeding with BAP resulted in an almost 300-fold/40-fold induction of cyp1a1/cyp1b1 mRNA expression, respectively, in the intestine of wild-type animals, but not their $Tlr2^{-/-}$ counterparts. In contrast, hepatic expression of cyp1a1 was increased moderately (4x) in $Tlr2^{-/-}$ animals, but not in WT mice. The latter finding was confirmed at the protein level.

Conclusion. TLR2 seems to be crucial for intestinal detoxification of PAH and other carcinogens that are metabolized by CYP1A1. The repeatedly predicted role for CYP1A1 in formation of eicosanoids, which might contribute to enterocyte differentiation and intestinal integrity, is seemingly dependent on the luminal presence of surface ligands from Gram-positive bacteria.

POSTER

1 IMPROVED GENETIC ACCESSIBILITY OF A POTENTIAL PROBIOTIC *BIFIDOBACTERIUM BIFIDUM* STRAIN

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We recently demonstrated the good anti-inflammatory capacity of a potential probiotic strain of *B. bifidum* in two models of murine colitis. However, studies of this and other probiotic candidate strains of bifidobacteria at the molecular level are limited by the lack of molecular tools and low transformation efficiency.

To improve the genetic accessibility of *B. bifidum*, the transformation protocol was optimized for growth medium, buffer conditions and electrical pulse settings by transforming the *E. coli-Bifidobacterium* shuttle vector pMDY23. To further improve the transformation efficiency, the restriction barrier to foreign DNA by restriction-modification (R-M) systems should be overcome. Using *in silico* analysis, one Type I and one Type II R-M system were predicted in the genome of our *B. bifidum* strain. Their presence was confirmed by PCR, Southern Blotting and Sequencing and both systems were shown to be expressed by RT-PCR. Methyltransferases were introduced for arabinose-inducible expression in methyltransferase-negative *E. coli* hosts via chromosomal integration. *E. coli-Bifidobacterium* shuttle vectors were propagated in these strains prior to transformation into *B. bifidum* in order to obtain plasmid DNA with a *B. bifidum* strain-specific methylation pattern, thereby circumventing the restriction barrier to foreign DNA in this strain.

2 CHARACTERIZATION OF THE PORCINE CAMPYLOBACTER COLI POPULATION STRUCTURE AND THE EFFECT OF PROBIOTICS ON COLONIZATION AND SHEDDING OF C. COLI

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Pigs have been demonstrated to be an important reservoir for Campylobacter (C.) coli. In contrast to C. jejuni in poultry, limited research has been focused on the population structure and the impact of probiotics to reduce the colonization and shedding of C. coli in pigs. Reducing C. coli colonization and shedding in pigs might contribute to a reduction in the incidence of C. coli infections in humans.

The aims of the first phase of the project is (i) to characterize the C. coli strain diversity in the porcine gut by genotyping faecal strains by AFLP and MLST extensively and (ii) to describe the virulence properties (e.g. motility, adherence and colonization factors, invasion capability, ability to produce toxins) of predominant strains using established virulence assays and a Campylobacter virulence microarray.

After establishing C. coli characteristics in detail, (iii) in vitro assays will be carried out to describe the potential inhibitory activities of probiotic bacteria (screening Enterococcus and Lactobacillus strains for their antagonistic effect) by co-culturing these strains with C. coli.

These results will serve as a starting point for (iv) in vivo animal experiments, carried out in a second project phase. In vivo experiments are needed to evaluate the effect of the promising probiotic bacteria on C. coli colonization and shedding in pigs.

3 *BACILLUS CEREUS* SPHINGOMYELINASE INDUCES CYTOTOXICITY IN PTK6 INTESTINAL EPITHELIAL CELLS

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Probiotic bacteria of different species are widely used and well accepted in human food industry. However, very little is known about their potential virulence profiles. *Bacillus cereus* is an opportunistic human pathogen and has been increasingly associated with food-borne gastrointestinal diseases. Nevertheless, some *B. cereus* strains are utilized as probiotics. In this study, we investigated the influence of different pathogenic strains and two probiotic strains on polarized colon epithelial cells (PTK6 cells) using an *in vitro* infection model. Fourteen out of 18 different *B. cereus* strains caused immediate cell detachment and cell death. Despite our expectations, these included two probiotic *B. cereus* strains, Bc toyoi and IP5832, which caused severe epithelial cell damage comparable to disease-associated strains. To examine the individual contribution of *B. cereus* toxins to epithelial disruption, we focussed on virulent strains because of the availability of mutants. Infections with *nheBC*[1] (non-haemolytic enterotoxin) and *plcR* (pleiotropic transcriptional regulator) deletion mutants suggest that PlcR-regulated factors other than Nhe cause cytotoxicity on PTK6 cells. Using S75 gelfiltration and a virulence *in vitro* assay we isolated a distinct cytotoxic protein within *B. cereus* culture supernatant which was identified to be sphingomyelinase (BcSMase). Its mammalian homologue is involved in cell signaling and apoptosis induction. Recombinant *Bc*SMase expressed in *E. coli* exhibited cytotoxic activity on intestinal epithelial cells (IEC). Expression in the *Bacillus plcR* deletion mutant recovered wild-type cytotoxicity. Our data strongly support sphingomyelinase as a crucial contributor to *B. cereus* virulence and as potential risk factor of bacillal probiotic strains.

[1] Fagerlund *et al.*, *Microbiology* (2008)

4 SECRETORY EXPRESSION OF VIRAL INTERLEUKIN 10 ANALOGS IN *ESCHERICHIA COLI* VIA A MODIFIED SEC-TRANSPORTER AND INDUCTION OF LYSIS PROTEINS

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Interleukin 10 is one of the cytokines with the highest antiinflammatory capability. Homologs of IL-10 are encoded by different viruses such as Cytomegalovirus (CMV) and Epstein-Barr virus (EBV), which show similar activity to cellular IL-10. As a result of selection pressure the lack of immune stimulatory effects, which are observed in human IL-10, and the higher molecular stability suggest the viral cytokines to suppress the host's immune response more efficiently than cellular IL-10. Therefore viral counterparts of human IL-10 could be a promising option for treatment of inflammatory diseases. Our aim is to apply *Escherichia coli* as chassis for secretory expression of viral IL-10, thus allowing in situ cytokine activity in patients suffering from inflammatory bowel disease. Efficent export of viral IL-10 into the *E.coli* periplasm was achieved via Sec translocase by fusing codon opimized versions of the viral genes to outer membrane protein F leader sequence. For characterization of the viral proteins, their concentration in different cell fractions was measured by ELISA and the proteins were visualized in Western Blot. Biological activity was proofed in a signal transduction pathway assay. In order to use IL-10 producing bacteria as a live drug, IL-10 needs to be delivered in a controlled fashion out of the periplasm. For this three bacterial or phage lysis systems were introduced into the recombinant IL-10 producing strains and have subsequently been tested for efficient release of the cytokine.

5 PALMITATE IN THE PLACE OF THE USUAL LAURATE IN E. COLI LIPID A PREVENTS INTESTINAL INFLAMMATION

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Background. Ulcerative colitis and Crohn's disease are chronic inflammatory disorders of the gastrointestinal tract which result from genetic factors at several susceptibility loci like *nod2*, *ibd5* or *il23R* and alterations in the interaction between resident microbes and the mucosa (Xavier et al, 2007). In an adoptive T cell transfer model we analyzed the effect of the intestinal microflora composition on the outcome of chronic inflammation. *Methods. Rag1*$^{-/-}$ mice kept under specific pathogen free (SPF) conditions or under conventional conditions (CV) were transplanted with naïve $CD4^+$ T cells and monitored for weight loss and signs of inflammation for 6 weeks. Additionally, mice were treated either with metronidazol or streptomycin to alter the intestinal flora and either received *E. coli* mpk, *B. vulgatus* or different *E. coli* strains possessing altered Lipid A structures in the drinking water. Quantitative PCR of the intestinal flora, histology of the colon, FACS staining of lamina propria T cells as well as mRNA cytokine expression of colon mucosa were performed. *Results. Rag1*$^{-/-}$ mice exhibiting an intestinal flora with higher amounts of *Enterobacteriaceae* are prone to develop colitis after T cell transfer whereas mice having high amounts of *Bacteroidetes* were protected. Furthermore we were able to change the induction or prevention of inflammation by altering the intestinal flora through gavage of specific antibiotics and either *E. coli* mpk or *B. vulgatus* to the mice. *Conclusions.* As the Lipid A in the LPS from *Enterobacteriaceae* is more immunestimulatory than the Lipid A from *Bacteroidetes* the ratio of *Bacteroidetes* to *Enterobacteriaceae* is crucial whether a genetically predisposed host develops chronic inflammation or not.

6 OVER EXPRESSION AND PURIFICATION OF A SURFACE PROTEIN OF B. BIFIDUM AND EVALUATION OF ITS ROLE IN ADHESION TO INTESTINAL EPITHELIAL CELLS

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Several beneficial effects for the host have been attributed to the presence of bifidobacteria in the intestinal tract. Adhesion of these bacteria to intestinal epithelial cells (IECs) could be an important prerequisite for their probiotic effects. Recent in vitro studies of our group showed that especially those strains of bifidobacteria that show good adhesion to IECs have a high anti-inflammatory potential. Analysis of the adhesive properties of a highly adherent *B. bifidum* strain revealed that a proteinaceous component located on the cell wall is involved in adhesion to IECs. A recent study by Guglielmetti *et. al.* (2008) suggested that this proteinaceous component could be BopA, a cell wall-associated lipoprotein. An initial *in silico* analysis of the recently sequenced genome of our *B. bifidum* strain revealed about 20 further proteins which could be implicated in adhesion and interaction with the host.

To further analyze the involvement of BopA and other proteins on adhesion a range of *E. coli-Bifidobacterium* shuttle vectors was constructed for homologous expression of proteins in *B. bifidum*. One of these vectors, pMGS, was used for over expression of BopA in *B. bifidum*. This over expressing strain was analyzed for its ability to adhere to IECs in comparison to the wild type *B. bifidum* strain.

7 METABOLIC PHENOTYPING OF CROHN´S LIKE IBD IN THE TNF$^{\Delta ARE}$ MOUSE MODEL

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Inflammatory bowel diseases (IBD) are chronically relapsing disorders where the gut acts as a communication organ between the luminal environment such as food and bacterial components and the host which is responsible for the regulation of metabolic and immune functions. The aim of this study was to characterize the metabolic changes appearing during the pathogenesis of IBD in the TNF$^{\Delta ARE}$ mouse model of Crohn´s disease like IBD.

Different tissues samples, including blood plasma, liver and ileal tissues were analysed at the age of 1, 4, 8, 12, 16 and 24 weeks using targeted and non-targeted metabonomic techniques. Chronic inflammation appeared from 8 weeks onwards according to histological scoring, and was associated with altered ileal morphologies, increased inflammatory cell infiltration and a significantly reduced body weight of inflamed animals. Computer tomographic measurements indicated a reduced amount of body fat mass, especially in the metabolically active visceral fat depot, accompanied by a significant smaller adipocyte size in this tissue under ileitis development. Application of metabonomic tools revealed a significantly different lipid metabolism in the inflamed ileum showing increased concentrations of specific sphingomyelins, glycerophospholipids and acylcarnitines, cholesterol and plasmalogens. These organ specific metabolic differences were associated with a highly significant and consistent decrease of hydroxy-sphingomyelin and plasmalogen in the plasma of inflamed mice starting already from 4 weeks onwards as revealed by targeted MS. NMR measurements could detect subtle but steady increased circulating levels of glycoproteins and decreased plasma lipids (mainly VLDL). In addition, the liver of inflamed mice showed elevated content of amino acids and differential lipid composition (e.g. unsaturated and saturated fatty acids, phospholipids, cholesterol). Apart from lipid metabolism associated changes in the tissues and plasma the inflamed mice also show pathologic changes in systemic glucose metabolism which are more pronounced after a high-fat feeding challenge.

These preliminary investigations, especially the observation of early alterations in the plasma composition even before the onset of histological changes, provide encouraging results and new insights into the IBD-related alterations of specific metabolic processes by inflammatory states.

8 IMPACT OF MATERNAL GUT INFLAMMATION ON THE GENE EXPRESSION PROFILE OF THE OFFSPRING'S INTESTINAL EPITHELIAL CELLS (IEC)

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Background and Aim. Various epidemiological and animal studies suggest that maternal stress during pregnancy can have long term consequences for disease risk of the offspring. In this study, the aim was the investigation of the impact of maternal gestational stress – in our case maternal ileal inflammation – on the gene expression profile of the offspring's IEC by using the $TNF^{\Delta ARE/+}$ mouse model for chronic Crohn's ileitis. *Methods.* The experimental setup includes two different breeding situations. Female wild type $TNF^{+/+}$ mice (WT dam) were bred with male heterozygous $TNF^{\Delta ARE/+}$ mice (ARE sire) and vice versa. Thus, 4 groups of offspring were generated: WT and ARE offspring originate from WT dams developed under normal non-inflamed conditions as well as iWT and iARE originate from ARE dams which were exposed to maternal gut inflammation during embryogenesis. Since severity of inflammation increases with the age of $TNF^{\Delta ARE/+}$ mice, the period of conception was fixed between age of 8-12 weeks for both, female and male mice. The offspring were sacrificed at embryonic day 17.5 (E17.5) as well as 1, 3 and 8 weeks postnatal. Furthermore, histological scoring was performed. Ileal samples were flash frozen and IEC were captured by laser microdissection from H/E stained 10 µm thick cryosections. Total RNA was extracted for microarray-based gene expression analysis. *Results.* Histopathological scoring (0-12) revealed no inflammation and differences in E17.5, 1 and 3 week old mice. 8 week old ARE mice showed a mild ileal inflammation compared to WT, but no changes due to maternal gut inflammation was observed within the groups of the same genotype (score WT 0.39+/-0.31; score iWT 0.50+/-0.14 and score ARE 4.08+/-0.85; score iARE 3.96+/-0.71). Nevertheless, the maternal gut inflammation during pregnancy leads to changes in the gene expression profile of the ileum in E17.5 embryos. In total 653 and 1303 genes with a threshold fold change of +/-2 were significantly (*FDR p-value* <0.05) regulated under inflammatory environment in the two WT groups and the two ARE groups, respectively. In contrast only 1 (WT/ARE) and 142 (iWT/iARE) genes were regulated within the groups of the same prenatal environment. *Conclusion.* Interestingly, a persistent maternal inflammatory exposure during embryogenesis does not influence any tissue pathology for Crohn's ileitis until an age of 8 weeks. However, there are significant changes in the gene expression profile in E17.5 embryonic mice due to the inflammatory environment.

9 Is Bacterial Communication in the Gut Highly Local? A Systems Biological Approach

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Many bacterial traits are regulated by an intercellular communication system, called quorum sensing (QS). Briefly, cells release and sense small diffusible molecules (autoinducers, AI). Dependent on the environmental concentration of the AI, genes are regulated, including many which control pathogenic or symbiotic interactions with hosts. QS seems to be relevant in the gut, although the understanding of its functionality is just beginning. QS is usually assumed to enable a cell density dependent behaviour of the whole population. This view encounters problems in the heterogeneous gut habitat. Communication is interfered by other AI producing or degrading species, by species reacting on the AI (eavesdropping), or by disruptive action by the host. How can AI transfer reliable information under such conditions?

We approach to the question by data based mathematical modelling. Our analysis indicates that the problems can be reduced within cell clusters. Cell aggregation, consisting of one (or a few) species, protects the communication system from interferences. This effect is enhanced by the positive feedback loop found within the production pathways of most AIs. We thus hypothesize that communication is highly localized. Analyzing the mean bacterial cell density or AI concentration within the gut is inadequate to understand the role of QS. Instead, spatially resolving experimental approaches are desirable. High AI concentrations in such clusters may explain the discrepancy reported between the AI threshold concentration for reactions of the bacteria (low) and the hosts (high).

10 PRODUCTION AND CHARACTERIZATION OF SIGNAL MOLECULES OF THE "QUORUM SENSING" SYSTEM IN GRAM-POSITIVE, PROBIOTIC BACTERIA

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Probiotics are defined as living microorganisms that have, when applied in adequate amounts, a beneficial effect on the host's well-being. They were used in food production e.g. milk products, as well as dragées etc. Especially in the last years an increasing commercial interest in these products was observed.
Although the beneficial effects, e.g. the stimulation of the immune system or the reduction of the development of atopic diseases in childhood, are known, the mechanisms are hardly understood. The aim of this work is to characterize signal molecules produced by the quorum sensing (QS) system of Gram-positive probiotic bacteria and to further verify the hypotheses; whether these signal molecules exhibit the ability to stimulate immune reactions.
In this study we focus on members of the genera *Lactobacillus* and *Bifidobacterium*. In a first step the QS-system regulating signaling substances will be isolated from culture supernatant, be tested on their effects on cells of the human immune system and further analyzed using high resolving FT-ICR-MS analytics. Further experiments include the development of a biosensor for the above mentioned signal molecules.
Detailed analysis of the obtained data should improve our knowledge about the involvement of signaling compounds derived from probiotic bacteria and the mechanisms behind in the positive stimulation of the human immune system.

11 INFLUENCE OF PROBIOTIC (ENTEROCOCCUS FAECIUM) TREATMENT ON INTESTINAL GENE EXPRESSION DURING SALMONELLA INFECTION

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Infections of piglets during the weaning period can be fatal and thus, lead to an increased mortality. During that period, the protective effect of colostrum is missing and piglets are exposed to new antigens, as for instance food antigens. Furthermore, due to the change to solid food, the intestine is extremely stressed. Consequently, sequelae can increase the susceptibility, especially to Salmonella infections. The involved diarrhea, low feed conversion and the reduced gain in weight affect piglets' performance. Since the EU-wide prohibition of feed antibiotics in 2006, the necessity arose to investigate alternatives which decrease piglets' susceptibility to infections. The addition of probiotics to the nutrition may be an alternative. However, the molecular principle of probiotics as treatment or prophylaxis in e.g. Salmonella infections is poorly understood. The aim of this study was to characterize the porcine intestinal gene expression during postnatal development focusing on the weaning phase. For this purpose healthy subjects but also Salmonella and Salmonella/probiotic treated groups were studied, respectively. Intestinal tissue samples (Ileum and ascending colon) from piglets were collected within 7 – 56 days after birth. In addition, samples from Salmonella typhimurium infected piglets as well as another infected group that was simultaneously treated with the probiotic strain E. faecium NCIMB 10415 were taken. Gene expression analyses were conducted by means of two color microarray experiments using the microarray-platform Pigoligoarray [1]. Clustering of genes revealed groups with differing expression in Salmonella infected piglets compared to the control and the Salmonella/probiotic treated group. Pathway analyses were performed using the web based tool DAVID [2]. Initial results point to genes with increased or decreased expression after Salmonella infection in colon. Interestingly, a restitution of these genes was observed in the probiotic treated group. Pathway analyses showed that genes with restituted expression are involved in local acute inflammatory responses (BioCarta [3]) or cell adhesion (KEGG [4]). This study represents the first characterization of the gene expression after Salmonella/E. faecium treatment during intestinal development in piglets. The presented results provide a better understanding of probiotic function in pathogen associated infection during weaning.

[1] www.pigoligoarray.org [2] http://david.abcc.ncifcrf.gov [3] http://www.biocarta.com [4] http://www.genome.jp/kegg/

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12 E-CADHERIN, A MAYOR PLAYER AT THE GASTROINTESTIANL BARRIER, IS REGULATED BY GRAM-POSITIVE PROBIOTIC BACTERIA

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The gastrointestinal tract harbours a complex microbial ecosystem that is engaged in a continuous crosstalk with the host, in this way maintaining in a balanced relationship between intestinal epithelial cells (IECs), immune responses and gut microbes. This balance can be disturbed leading to the activation of the mucosal immune system, a process that might contribute to the development of inflammatory bowel diseases (IBD). We used the transepithelial electrical resistance (TER) of a T84 cell monolayer after co-incubation with Gram-positive probiotic bacteria as read-out system to monitor barrier integrity. Based on DNA-microarray data and taking into account that defects of the intestinal barrier function facilitate the development of IBD, we focussed on genes encoding adherence junction (E-cadherin and β-catenin) proteins. Our results indicate that the barrier function is subject to modulation of different adherence junction proteins by Gram-positive probiotic bacteria. For in depth transcriptional analysis we focussed on changes in the miRNA expression patterns following *Lactobacilli* co-incubation. Upregulation of hsa-mir-92a downregulates the expression of transcription factor SNAI2 and enhances E-cadherin expression. Furthermore, we found that the phosphorylation of adherence junction proteins by different PKC isoforms (i.e. PKCδ/PKCζ) after *L. gasseri* co-incubation modulates the barrier function of epithelial cells. Accordingly the barrier function is subject not only to the presence but also to the activity of different PKC isoforms which are affected by different Gram-positive probiotic bacteria. Our study revealed cellular responses of IECs specifically induced by probiotic *Lactobacilli*. Further insight into the underlying molecular mechanisms will foster the development of new strategies for the treatment of gastrointestinal diseases (e.g. IBD).

13 DEVELOPMENT AND FUNCTION OF MUCOSAL IL-22-PRODUCING CELLS

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Cryptopatches are organized structures of approximately 1000 cells located underneath the crypts of the small intestine. The cells forming cryptopatches are RORgt (retinoic acid orphan receptor g t)-expressing lymphocytes believed to be lymphoid tissue inducer (LTi) cells. Recently, we showed that a considerable fraction of these RORγt$^+$ cells produces IL-22 and also expresses immunoreceptors often found in natural killer (NK) cells (NKRs such as NKp46, NKG2D, NKR-P1C) (Sanos, Nat Immunol 2009). We demonstrated that cues from the commensal microflora drive the differentiation of RORγt$^+$ cells into RORγt$^+$NKR$^+$ cells and the production of IL-22 but the molecular signals remain elusive. My analysis of mice lacking some of the most common genes required in pathogen recognition, such as *Myd88, Trif, Nod2, Nod1,* or mice lacking the type I or type II IFN receptors shows no difference in the proportion of RORγt$^+$NKR$^+$ cells of all RORγt$^+$ cells in comparison to wild type mice. Interestingly, MyD88-deficient mice showed a significant reduction in IL-22 production by both LTi and RORγt$^+$NKR$^+$ cells similar to our data obtained with germ-free mice. Together, these data indicate that IL-22 production by LTi and RORγt$^+$NKR$^+$ cells and RORγt$^+$NKR$^+$ cell differentiation are dependent on distinct microbiota-driven molecular signals.

14 FACTORS AFFECTING THE HOST-SPECIFIC INTESTINAL MICROBIOTA COMPOSITION

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The genetic background has been proposed to play a pivotal role in selecting intestinal bacteria colonizing a given host. However, factors that govern gut microbiota composition are largely unknown. To identify those factors, we associated germ-free mice differing in their genetic background (C3H and BL/10) with fecal bacteria from a single donor mouse. Analysis of microbiota composition 12 weeks after association revealed a strain-specific colonization pattern. Gene expression analysis with an Affymetrix chip was applied to identify genes differentially expressed in the colon mucosa of the mice. Only changes in gene expression with a high significance ($p \leq 1.85E-03$) are reported. The expression levels of the MHC gene H2-Q1 (39.7-fold), Ang4 (26.1-fold), encoding an angiogenic and antimicrobial protein, the toll-like receptor gene Tlr1 (4.2 fold) and the ß-defensin gene Defb37 (3.3-fold) were higher in BL/10. In contrast, C3H mice displayed a higher expression of Slpi (29.8-fold), Lpo (11.3-fold) and Lyzs (2.8-fold) all of which encode antimicrobial substances, Cd14 (4.2-fold) which plays a role in antigen recognition and of the mucin gene Muc1 (1.9-fold). The ~70-fold higher expression of the antimicrobial Pla2g2a in C3H indicates that this gene is defective in B10 as reported for B6 mice with a very similar genetic background. Our results support the notion that MHC genes are important for an individual microbiota composition. We identified further candidates which possibly play a role in the host-specific nature of the gut ecosystem. The relevance of the differential gene expression is currently tested with a proteomic approach.

15 FLAGELLIN OF E. COLI NISSLE 1917 MEDIATES PROTECTION IN DSS-INDUCED COLITIS BUT OTHER SECRETED FACTORS SEEM TO BE ESSENTIAL

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Background. Probiotic *E. coli* Nissle 1917 is effective as mesalazine to maintain remission in patients with ulcerative colitis. However, the protective mechanisms are still not clear. In a DSS-induced model of acute colitis we studied the molecular mechanisms of EcN on inflammation.

Methods. Wildtype C57BL/6 and TLR5$^{-/-}$ mice were fed orally with EcN and received 3,5% DSS in drinking water. Weight loss, clinical score and inflammatory status of the mice were monitored daily. Colon length and weight, cytokine production of whole colon as well as intracellular cytokines of T cells isolated from mesenteric lymph nodes and lamina propria were analyzed.

Results. EcN was effective in reducing the DSS mediated inflammation in wildtype C57BL/6 mice but only slightly in TLR5$^{-/-}$ mice suggesting a protective role for the flagella. Administration of heatkilled EcN reduced this protective effect, indicating an essential role for a secreted factor in addition to the flagella.

Conclusions. EcN prevents DSS-induced acute colitis via flagellin as well as via so far unknown secreted components. Gavage of EcN results in a reduced production of proinflammatory cytokines in the colon and thereby ameliorates diarrhoea.

16 GUT BACTERIAL GENE DIVERSITY AND COMMUNITY ANALYSIS IN PHARMACEUTICAL TREATED DIABETES MICE

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The development of Type 2 Diabetes (T2D), a highly prevalent syndrome, is commonly known to be influenced besides several genetic determinants by environmental factors such as dietary and lifestyle habitats. Recent studies depict an important role of the gut microflora in the development of T2D. In our study, we focus on analyzing the influence of compounds used in diabetes therapy on the gut microflora in a mouse model system. Eight- to 10-wk-old male diabetic-prone B6.BKS(D)-Lepr^{db}/J (db/db) mice were orally treated in the German Mouse Clinic with two different anti-diabetic compounds. The aim of the study is to analyse changes in the bacterial community monitored by fingerprinting analyses in the gastrointestinal tract in treated vs. untreated db/db mice. Further changes in microbial gene patterns and communities due to the drugs and their influence in diabetes pathophysiology will be investigated by 454 pyrosequencing. Fluorescence-in-situ-hybridization (FISH) in combination with confocal laserscanning microscopy (CLSM) will improve our knowledge about the bacterial colonization of the mouse gut system. The gut molecular community and gene pool analysis will be correlated with results from non-targeted metabolomic analyses (FTICR-MS) and mouse physiological data using bioinformatical approaches. In addition, a deterministic mathematical model will be developed.

17 RESPONSE OF INTESTINAL BACTERIA TO DIETARY FACTORS IN THE MOUSE INTESTINE

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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. Therefore we investigate to which extent dietary factors influence the protein expression of bacteria during their passage through the gut.

We used mice monoassociated with *Escherichia coli* MG1655 as a simplified model for host-microbiota interaction. The animals 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* recovered from the mouse intestinal tract.

Several of the differentially expressed bacterial proteins reflect the adaptation of *E. coli*'s metabolism to the respective diet fed to the mice. For example, the lactose-rich diet led to the induction of enzymes required for lactose utilization. This result supports previous knowledge but also demonstrates the validity of the experimental approach.

The lactose-rich diet also led to an induction of proteins involved in *E. coli*'s oxidative stress response (FUR, AHPF, DPS). The corresponding genes are under control of the OxyR transcriptional dual regulator which is activated under oxidative stress. The absence of such a stress response in *E. coli* from mice fed the protein-rich diet suggests that a carbohydrate-rich diet causes more stress in intestinal *E. coli* than a protein-rich diet. We now aim to elucidate the molecular mechanism of underlying this observation.

18 INFLUENCE OF INFLAMMATORY BOWEL DISEASE ON BACTERIAL PROTEIN EXPRESSION

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Increased numbers of *Escherichia coli* are observed in Crohn's disease and ulcerative colitis, the two major forms of inflammatory bowel disease. Aim of this project is to identify *E. coli* proteins involved in this organism's adaptation to inflammatory conditions in the gut and to find out whether these factors affect the host. Furthermore we want to elucidate the molecular basis for strain specific differences between probiotic and colitogenic *E. coli* in their response to gut inflammation.

We used mice monoassociated either with a colitogenic strain of *E. coli* (*E. coli* UNC) or with the probiotic *E. coli* Nissle (EcN) as a simplified model for host-microbe interactions. Intestinal inflammation was induced by treating the mice with 3.5% DSS. To identify differentially expressed bacterial proteins, two-dimensional difference gel electrophoresis followed by electro-spray ionization-tandem mass spectrometry was performed on samples recovered from the gut contents of the mice.

All DSS-treated mice revealed a reduction in body weight, shortening of the colon as well as histological signs of severe inflammation. However, the symptoms were less severe in the mice associated with EcN. We detected over 300 differentially expressed bacterial proteins. The proteins strain-specifically up-regulated after DSS-treatment include stress proteins, such as the chaperone proteins ClpB, DnaK and the 60 kDa chaperonin (GroEL). Interestingly tryptophanase, which converts L-tryptophan into indole was 4-fold up-regulated in EcN compared to *E. coli* UNC after DSS-treatment. Previous publications demonstrated that indole has an anti-inflammatory potential. We will therefore investigate whether the enhanced indole production by EcN helps to alleviate colitis.

19 CONSTRUCTION OF RECOMBINANT *E. COLI* NISSLE 1917 (ECN) STRAINS EXPRESSING DEFENSINS : A NOVEL THERAPY FOR TREATMENT OF INFLAMMATORY BOWEL DISEASE?

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The probiotic *Escherichia coli* strain Nissle 1917 keeps patients suffering from Ulcerative Colitis (UC) in remission. However, it is not effective in treatment of Crohn's disease (CD), most likely due to the genetically based inability of defensin production by such individuals. Therefore, we plan to restore the diminished intestinal defensin production by oral application of the recombinant EcN expressing defensin(s). In fact, several recombinant EcN derivates were constructed expressing a defensin. First of all, we designed alpha-human defensin 5 producing strains, because human defensin 5 is produced as an inactive pro-form and is proteolytically processed to the active peptide by trypsin. Next, we constructed human beta defensin 2 (HBD2) producing strains. However, HBD2 has no pro-form. Therefore, expression of HBD2 could possibly kill the HBD2 expressing bacterial cells. Optimized proHD5, mature HD5, HBD2 with and without signal sequence were expressed in *E. coli* BL21, KRX and EcN under the control of the T7-RNA polymerase. Currently, screening of antimicrobial activity *in vitro* is in progress.

20 *E. FAECALIS* GELATINASE IMPAIRS INTESTINAL BARRIER FUNCTIONS: ROLE FOR COMMENSAL-DERIVED PROTEASES IN THE PATHOLOGY OF IBD

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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 might contribute to the pathogenesis of IBD. For this purpose we focused on the zinc dependent metalloprotease Gelatinase (GelE) from *Enterococcus faecalis*. *Results.* Monoassociation studies of wild type (WT) and IL-10$^{-/-}$ mice with *E. faecalis* strain OG1RF revealed increased mRNA expression levels of GelE under conditions of experimental colitis. Further monoassociation experiments of IL-10$^{-/-}$ mice with *E. faecalis* strain OG1RF and two isogenic mutant strains that lack GelE expression (TX5264 and TX5266) demonstrated a significant reduction of proximal and distal colonic tissue pathology in the absence of GelE. E-Cadherin as important barrier and differentiation marker was completely abolished in isolated IEC of OG1RF colonized IL-10$^{-/-}$ mice indicating that GelE might impair intestinal barrier integrity. Stimulation of distal colon segments from IL-10$^{-/-}$ and TNF$^{\Delta ARE/Wt}$ mice with purified GelE supported this hypothesis by significantly reduced transepithelial electrical resistance (TER) values and higher permeability rates for sodium fluorescein. This effect could not be observed in Wt animals suggesting that GelE acts as disease modulator but remains inefficient in the healthy status. The loss of barrier function could be confirmed *in vitro* as concentrated conditioned media of *E. faecalis* OG1RF and purified GelE significantly decreased TER of cultivated IEC accompanied with a size-dependent translocation of fluorescent permeability markers. *Conclusion. E. faecalis* GelE impairs intestinal epithelial barrier function through targeting E-Cadherin protein expression. Our data clearly demonstrates the role of GelE, as a commensal-derived protease, in the development of intestinal inflammation under circumstances of genetic susceptibility.

21 BACTERIAL TRANSLOCATION IS ASSOCIATED WITH DOWNREGULATION OF PANETH CELL ANTIMICROBIAL PEPTIDES IN CIRRHOTIC RATS

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Background. Patients with liver cirrhosis show a prevalence of bacterial translocation (BT) which is defined as the migration of bacteria to lymph nodes, blood and other organs. About 70% of the translocating bacteria belong to the common intestinal flora which suggests a breakdown of mucosal barrier function. We hypothesized that BT in liver cirrhosis could be explained by diminished expression of antimicrobial peptides. *Methods.* Cirrhosis was induced in rats by inhalation of carbon tetrachloride and Phenobarbital in drinking water. Tissues were isolated from different parts of the intestinal tract from cirrhotic rats and controls. Lymph nodes, liver and spleen were tested for BT. Accordingly, rats were divided in healthy controls, liver cirrhosis with and without BT (n=15 respectively). Total RNA and protein was isolated from stomach, illeum, caecum and colon and mRNA expression of ß-actin, paneth cell α-cryptdins and inducible ß-defensins was determined by qRT-PCR using specific plasmids standards. Antimicrobial activity was assessed using a FACS assay. *Results.* In cirrhotic rats with BT, α-cryptdin expression was decreased throughout the entire GI tract, most pronounced in the proximal (p=0,02 Crypt5; p=0,01 Crypt7) and the distal ileum (p=0,02 Crypt 5; p= 0,008 Crypt7). In contrast, other antimicrobials showed no changes or an induction in case of BT at different sites. Antimicrobial activity was consistent with PCR and showed diminished activity especially in the small intestine. *Conclusion.* The lack of antimicrobial host defense via diminished Paneth cell defensin expression provides a likely explanation for bacterial translocation.

22 COMPOSITIONAL DATA ANALYSIS OF BACTERIAL METAGENOMIC DATA IN THE INTESTINE OF PIGLETS

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The use of bar coded 16S rRNA gene amplicons has been extensively used to describe bacterial communities in a large variety of different habitats. Therefore, we studied metagenomic data on intestinal bacterial communities derived from bar coded 16S rRNA PCR products extracted from 12 piglets fed a standard starter diet with low (150 ppm) or high (3000 ppm) amounts of zinc oxide (n = 6). For each piglet we derived four sets of pyrosequencing sequence data by using two DNA – extraction methods and two different PCR primers. The SEED viewer software of the MG-RAST server identified genera in each sample with results depending on the reference databases RDP, Green Gene and Silva that were used for sequence matching. As the amount of sequences differed for each piglet, it required inference based on relative proportions, which are difficult to assess due to the absence of any useful coefficient of correlation. Aitchinson [1] introduced specialized multivariate statistical methods for such data based on the multivariate log-normal distribution which we implemented using the software R to test for significant deviations between the means and dispersions derived from different samples. Furthermore, we restricted data analysis on the four most abundant genera found in each experimental group obtained by each of the 12 methods of data generation. The hypothesis of pairwise equal distributions was rejected only for data from primer 8f-534r, but not for all other data. However, when different, all pairs of distributions were found to significantly differ in dispersions rather than means. This creates a new challenge to classify multivariate observations based on different dispersions and clearly requires repeated measurements. Our contribution discusses the need of compositional data analysis methods for drawing reproducible conclusions from metagenomic data.

[1] Aitchison, J. (1986). The Statistical Analysis of Compositional Data. Monographs on Statistics and Applied Probability. Chapman and Hall Ltd., London (UK), 416p.

23 INFLUENCE OF GRAM-NEGATIVE PROBIOTICS ON THE INTESTINAL BARRIER FUNCTION – MIRNAS AS A NEW PERSPECTIVE FOR INFLAMMATORY BOWEL DISEASE TREATMENT

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The gastrointestinal tract harbours a complex microbial ecosystem, engaged in a continuous crosstalk with the host. The balanced relationship between intestinal epithelial cells (IECs) and gut microbes can be disturbed, resulting in the activation of the mucosal immune system which contributes to the development of inflammatory bowel diseases (IBD). We used a T84 cell culture model to analyze the cellular responses of IECs co-incubated with the Gram-negativ probiotic strains: *E.coli* Nissle 1917 and a colonization mutant (EcN-M1) as well as with the urogenic strain ABU (83972). Taking into account that IBD may develop after defects of barrier function and based on microarray data for co-incubated IECs we focussed on proteins (ZO-2, PAR-3, PAR-6, Occludin) of the apical junctional complex (AJC). The transepithelial resistance (TER) of the T84 monolayer was used as a read-out for monitoring epithelial barrier function. The integrity of the barrier is enforced by probiotic *E.coli* and disrupted after EPEC infection (E2348/69). Furthermore, TER indicating barrier function could be reestablished by co-incubation with Gram-negativ probiotics. To reveal the molecular mechanisms of this bacterial impact we also analyzed the expression of miRNA in IECs after co-incubation with probiotics and were able to identify miRNAs that correspond to proteins of the AJC. We confirmed the participation of miRNAs in the regulation of the AJC proteins by transfecting T84 cells with miRNAs, miRNA-inhibitors and miRNA-mimics.The results provided evidences for the involvement of the identified miRNAs in the stabalizing effects of probiotics as well as the disruptive effect of EPEC on the barrier function. This study revealed modular responses of IECs specifically induced by Gram-negativ probiotic *E.coli* strains. Further insight into the molecular mechanisms (e.g. miRNAs) of probiotic effects might foster the development of new strategies for treatment of gastrointestinal diseases.

24 COMPARISON OF GENOMES FROM PROBIOTIC *E. COLI*

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The medical probiotic product Symbioflor *E.coli* contains one type of *E. coli* with different but closely related genotypes constituting a functional unity. The genomes of these genotypes were compared in various analyses. Their annotated genes were divided into functional Cluster of Orthologous Genes (COG) categories and the fractions of represented COG categories were compared. A core-genome of gene familes conserved in all genotypes, as well as a pangenome comprising all gene families represented in Symbioflor *E.coli* were defined and the relative similarities within the different translated proteomes were analysed. The genome of Symbioflor G3/10, which was most complete at the time of analysis, was used as a reference for a BLAST atlas, which visualizes those protein-coding genes of G3/10 that are conserved in the other genotypes. Conservation in a selection of other non-pathogenic as well as pathogenic *E. coli* genomes, derived from public databases, was also assessed for comparison. The results show that the vast amount of information derived from multiple genome sequences can nevertheless be analysed and visualized in a relatively simple manner.

25 GUT BACTERIAL DIVERSITY IN THE TNF^{DELTAARE} MOUSE MODEL OF ILEITIS

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Microbes play important roles in inflammatory bowel diseases (IBD), including Crohn's disease. However, there is little data on gut bacteria involved in the onset and maintenance of chronic ileitis. Because many intestinal bacteria are still not cultured, we aimed at characterizing cultivable bacteria from the intestine of TNF^{deltaARE} mice suffering from Th1-driven ileitis. Preliminary experiments showed that antibiotic treatment attenuated inflammation in the distal ileum of TNF^{deltaARE} mice. We isolated two so far unknown bacteria from the intestine of TNF^{deltaARE} mice. The first strain was isolated from the ileal mucosa on a mucin-containing medium, hinting at possible close interactions with IEC. Based on comparative phenotypic and genetic analyses, the novel bacterium was named *Enterorhabdus mucosicola* and classified as an equol-producing risk-group-2 bacterium resistant to colistin and ciprofloxcin. The second new strain was obtained from cecal content after isolation on a selective medium containing amino acids. It was identified as a new member of the genus and was named *Bacteroides sartorii*. Molecular work indicated that the species is dominant in the mouse caecum, independent of host genotype. Finally, we isolated bacteria from the caecal content of a 25-weeks-old TNF^{deltaARE} mouse using DYNAL magnetic beads coated with mouse anti-Grp-78 antibodies. Antibody-free beads were used as control. Twenty-seven isolates were identified by 16S rRNA gene sequencing. Six strains, including four likely novel bacteria, occurred only in samples isolated on Grp-78 antibodies. They belong to the family *Coriobacteriaceae* or the genera *Enterorhabdus*, *Neisseria*, *Staphylococcus* and *Streptococcus*. Taxonomic and functional description of the isolates is underway, in particular with respect to their involvement in the regulation of immune and cell stress responses.

Keywords: Gut bacteria; TNF^{deltaARE} mice; intestinal epithelium; chronic intestinal inflammation; *Coriobacteriaceae*; *Bacteroides*

26 GENOME SEQUENCING OF A PROBIOTIC *B. BIFIDUM* STRAIN REVEALS MULTIPLE LOCI POTENTIALLY INVOLVED IN HOST-MICROBE INTERACTIONS

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Bifidobacteria belong to the key groups of beneficial intestinal bacteria. One of the reported health benefits is the suppression of intestinal inflammation in patients suffering from inflammatory bowel diseases (IBD). A *B. bifidum* strain, isolated from a breast-fed infant, was shown to adhere strongly to intestinal epithelial cells (IECs) and displayed potent anti-inflammatory activity both *in vitro* and *in vivo*. In order to identify specific genes involved in the interaction with the host, the genome of this *B. bifidum* strain was sequenced and annotated. It is comprised of single circular 2,182,470 base pair chromosome with 1,782 predicted open reading frames, two rRNA operons and 62 tRNA genes. Several genes were identified and proposed to be involved in quorum sensing, mucin degradation and bacteriocin production. Moreover, considering adhesion of S17 to the intestinal epithelium a crucial prerequisite for colonisation and thus suppression of inflammation, we have further screened S17 genome for the potential adhesins. We identified at least 12 proteins, which have complex structure and contain potential binding domains for collagen, laminin, peptidoglycans, fibrin and mucus as well as carbohydrate recognition domains. The genomes of two low-adhereing non-probiotic strains (*B. longum* biovar *infantis* and *B. breve*) have also been sequenced and annotation is currently under way. Comparative analysis of the three genomes should allow to nail down specific traits involved in the strong adhesion of the B. bifidum strain as well as and its anti-inflammatory effect.

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